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# Merosin, a tissue-specific basement membrane protein, is a laminin-like protein

(cDNA/muscle/nerve/placenta)

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**ABSTRACT** **Merosin is a basement membrane-associated protein found in placenta, striated muscle, and peripheral nerve. A 3.6-kilobase merosin cDNA clone was isolated from a placental cDNA expression library. The clone contained a 3.4-kilobase open reading frame, the 3′ portion of which includes protein sequences of proteolytic fragments of merosin. The deduced amino acid sequence of the merosin polypeptide was similar to that of the COOH-terminal region of the 400-kDa A chain of laminin. This part of laminin forms the large globule at the end of the long arm of the laminin cross and is thought to contain the neurite-promoting site and the major cell binding site(s) in laminin. The sequence identity between merosin and the laminin A chain in this region is nearly 40%. An antiserum against a synthetic peptide from the middle of the merosin cDNA sequence identified a 300-kDa polypeptide in placental extracts, indicating that the merosin polypeptide is similar in size to the laminin A chain. Intact merosin was isolated from placental extracts and shown to be covalently associated with the laminin B chains and to have a cross-like structure similar to that of laminin. The similarities between merosin and laminin show that both proteins are members of the same family of basement membrane proteins.**

Merosin is a tissue-restricted basement-membrane protein (1). It is present in the trophoblast basement membrane of the human placenta and in basement membranes of striated muscle and Schwann cells. In the mouse, merosin is not present in the fetus and only appears in muscle and nerve after birth (1). This late developmental appearance suggests a role for merosin in the maturation or differentiation of tissues. These results parallel the limited expression of merosin in human malignant tumors (2, 56) and its absence in cultured cell lines (1), which are at a lower level of differentiation than their normal tissue counterparts. The interesting spatial and temporal expression of merosin prompted us to determine its structure as a step toward elucidating its function. As shown here, merosin is structurally related to laminin.

In contrast to merosin, laminin is thought to be present in all basement membranes (3). It is a large glycoprotein composed of three polypeptide chains, a 400-kDa heavy (A) chain and two light (B) chains of about 200 kDa. The entire heavy chain of mouse laminin (4, 5) and part of the human laminin heavy chain (6) have been sequenced. The B1 and B2 light chains from mouse (7–9, 57), human (10, 11), and *Drosophila* (12, 13) have also been sequenced. The $NH_2$-terminal two-thirds of the A chain are homologous to the B1 and B2 chains but the COOH-terminal one-third has a distinct structure.

Laminin promotes attachment, spreading, motility, and growth of a variety of cell types (14–18). One of the most striking activities of laminin is its capacity to promote outgrowth of neurites from cultured neuronal cells (19–21). A major site of cell adhesion and the neurite-promoting activity appears to reside in or near the globular domain at the end of the long arm (17, 22–26). This domain of laminin corresponds to the COOH-terminal part of the A chain that has no counterpart in the B chains.

We show in this paper that the deduced amino acid sequence of the COOH-terminal portion of the merosin polypeptide chain§ is homologous to the COOH-terminal part of the laminin A chain. Furthermore, the merosin polypeptide is associated with the same light chains, B1 and B2, as laminin, and the whole molecule has a cross-like structure similar to that of laminin.

## METHODS

**Screening of cDNA Library.** A placental λgt11 cDNA library (27) was screened with affinity-purified antibodies to merosin (1, 28). The identity of the isolated cDNA clones was confirmed immunologically by the procedure described by Argraves *et al.* (29).

**Determination and Analysis of cDNA Sequences.** cDNA inserts were cleaved with various restriction enzymes, and fragments were subcloned into either M13mp19(+) (Bethesda Research Laboratories) or Bluescript SK M13(+) (Stratagene). Nucleic acid sequencing was done by the dideoxynucleotide chain-termination method of Sanger *et al.* (30) by using deoxyadenosine 5′-[α-[$^{35}$S]thio]phosphate (New England Nuclear) and a kit from United States Biochemical. The MicroGenie program (Beckman) was used to analyze the sequences. Homology searches were carried out on Bionet with EMBL, GenBank, National Biomedical Research Foundation/Protein Identification Resource, and Swiss-Prot databases (September 1988) (31).

**Protein Sequencing.** A 55-kDa merosin fragment was isolated from a pepsin digest of human placenta by monoclonal antibody affinity chromatography (1). Another merosin fragment was similarly isolated from a chymotrypsin digest of placenta (23). The pepsin fragment of merosin was digested further with thrombin and the fragments were electrophoretically separated on a 10–20% gradient polyacrylamide gel in the presence of $NaDodSO_4$, blotted onto polyvinylidene difluoride membranes (Millipore), and sequenced on an Applied Biosystems sequencer, as described by Matsudaira (32).

**Synthetic Peptides, Antibodies, and Immunoassays.** Two 14-amino acid peptides, Cys-Asn-Asn-Phe-Gly-Leu-Asp-

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M32076).

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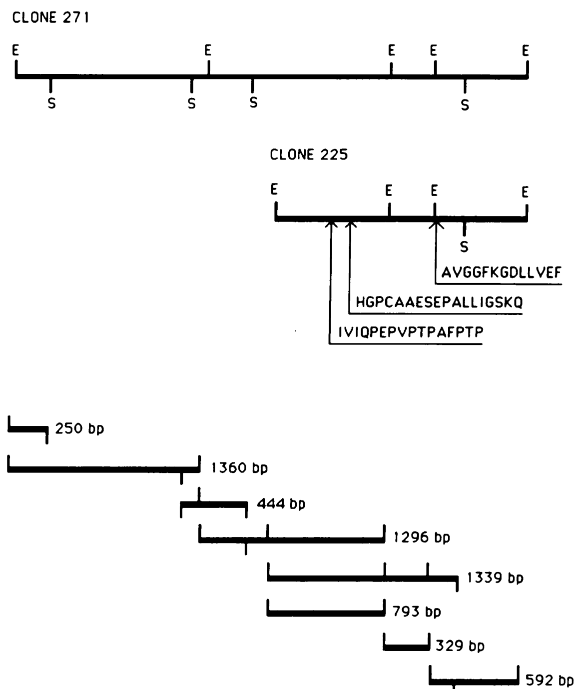


FIG. 1. Restriction maps of merosin cDNA clones. The *EcoRI* (E) and *Sst* I (S) restriction sites of the two clones, 271 and 225, which were sequenced, are shown. Fragments were cloned separately into either M13 or Bluescript and sequenced and their sizes are shown below the clones. Also shown are the relative positions of the $NH_2$-terminal sequences of three protease fragments of merosin. The single-letter amino acid code is used. bp, Base pair(s).

Leu-Lys-Ala-Asp-Asp-Lys-Ile and Cys-Ser-Ile-Val-Asp-Ile-Asp-Thr-Asn-Gln-Glu-Glu-Asn-Ile, were synthesized based on 13-residue amino acid sequences deduced from cDNA sequence. The cysteine at the $NH_2$ terminus of these peptides was added to facilitate coupling to a carrier protein. The peptides were coupled to keyhole limpet hemocyanin using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Pierce) as described by O'Sullivan *et al.* (33). The resulting conjugates were used to immunize rabbits. The antisera obtained were tested against the glutaraldehyde-crosslinked peptides in ELISAs (34) and against $NaDodSO_4$ extracts of tissue and isolated proteins in immunoblots, as described (1). The following monoclonal antibodies were used: 2E8, anti-laminin B2 (23); 4E10 and 3E5, anti-laminin B1 (23, 35); 4C7 and 11D5, anti-laminin A (ref. 23 and unpublished data); and 2G9, anti-merosin (1).

**Isolation of Merosin and Laminin from Placenta.** Intact merosin was extracted from placenta by EDTA-containing buffers and purified essentially as described by Paulsson *et al.* (36, 37) for laminin from mouse tissues. Briefly, placentas were extracted first with neutral Tris buffer and then with Tris buffer containing 10 mM EDTA. The EDTA extract was precipitated with 4 M NaCl. Proteins in the precipitate were solubilized in Tris/EDTA buffer and fractionated on a Sepharose 6B column. The void volume peak was collected and applied to a column of DEAE-cellulose. Bound proteins were eluted with a gradient of 0–0.8 M NaCl. The purification was monitored by ELISA and immunoblots with monoclonal and polyclonal antibodies against merosin and laminin. A truncated form of laminin was purified from a mild pepsin digest of placenta as described (23), except that a laminin A-chain-specific antibody was used for the antibody affinity chromatography. Rat laminin was purified from a rat yolk sac tumor (38).

**Electron Microscopy.** Electron microscopy after rotary shadowing was done as described (17, 23, 38).

## RESULTS

**Isolation and Sequencing of cDNA.** Affinity-purified antibodies against a fragment of merosin (1) were used to identify merosin cDNA clones from a placental λgt11 cDNA expression library. Two cDNA clones, designated 271 and 225 (Fig. 1), with inserts of 3.6 and 1.7 kilobases, respectively, were selected for sequencing. Multiple overlapping fragments were sequenced. Nonoverlapping fragments were sequenced in both directions. Alignment of the fragments that were cloned and sequenced is summarized in Fig. 1. The nucleic acid sequence of the cDNA revealed a 3.4-kilobase open reading frame followed by a 155-base-pair 3′ untranslated region. The deduced amino acid sequences are shown in Fig. 2. The $NH_2$-terminal amino acid sequence of fragments isolated from peptic or chymotryptic digests of placenta and the $NH_2$-terminal amino acid sequence of a 16-kDa fragment generated with thrombin (Table 1) were contained within the deduced sequence, thus defining the clones as merosin cDNA.

The deduced partial sequence of merosin comprises 1130 amino acids and contains 13 potential sites of N-glycosylation. The sequence includes five repeats of about 190 amino acids. These repeats contain a conserved 7-amino acid sequence, Leu-Phe-Val-Gly-Gly-Leu-Pro, or variations thereof (Fig. 2). This is followed 17–21 and 40–43 residues later by cysteines most of which are preceded by glycines. The

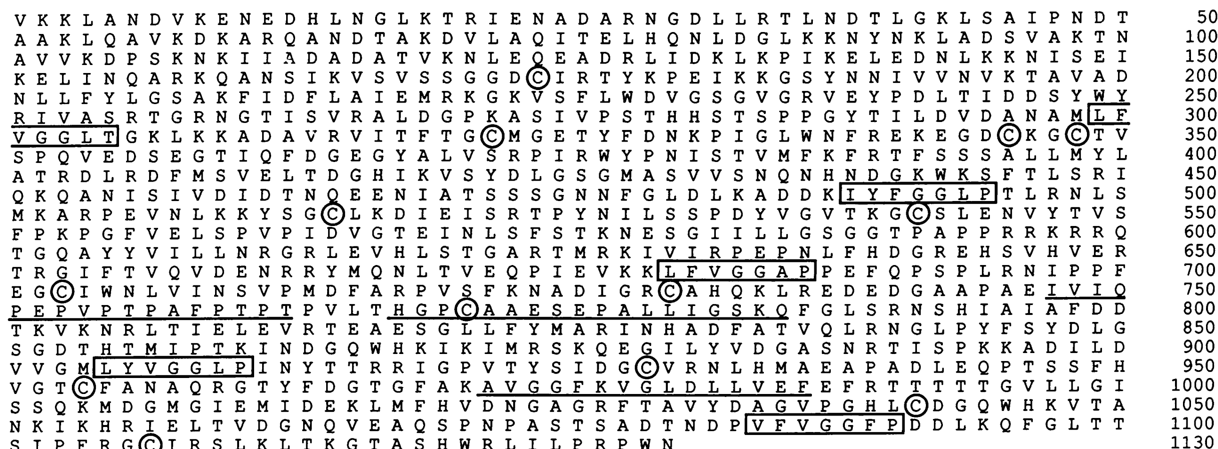


FIG. 2. Amino acid sequence of the COOH-terminal portion of merosin deduced from cDNA. Sequences obtained by amino acid sequencing are underlined. Conserved motifs of amino acid sequence are boxed and cysteines are circled. The single-letter code is used.

Table 1. $NH_2$-terminal amino acid sequences of merosin fragments

| Protease used | Fragment molecular mass, kDa | $NH_2$-terminal sequence |
|---|---|---|
| Pepsin | 55 | IVIQPEPVPTPAFPT |
| Chymotrypsin | 55 | IQPEPVPTPAFP |
| | 50 | HGPAAESEPALLIG(S)K(Q) |
| Thrombin | 16 | IVIQPEPVPXPAFP and A(T)GGFKVGLDLLVEF |

Fragments of merosin were isolated from pepsin and chymotrypsin digests of placenta by monoclonal antibody affinity chromatography. Fragments obtained from pepsin digests were further treated with thrombin. Preparations were sequenced after $NaDodSO_4$/PAGE and transferred to Millipore membranes. Amino acids in parentheses indicate tentative identification. The single-letter code is used.

average percentage of identity among the five repeats is about 25%.

**Homology of Merosin with the Laminin A Chain.** Comparative analysis of the amino acid sequence of merosin with known proteins revealed a striking similarity to the mouse (Fig. 3) and human laminin A chains. No other significant similarities were found upon search of the data banks. The five repeats of merosin are also present in the COOH-terminal portion of the laminin A chain (4–6). The overall identity between the merosin sequence and the corresponding portion of the mouse laminin A chain is 39%.

**Merosin Polypeptide in Tissues.** We have demonstrated (1) the presence of an 80-kDa polypeptide in placental extracts by immunoblot analysis with antibodies to the 55-kDa peptide fragment of merosin. However, the length of the open reading frame of the merosin cDNA indicates that the mature merosin polypeptide is much larger than 80 kDa. The deduced amino acid sequence suggests that the 55-kDa fragment and the 80-kDa tissue polypeptide are COOH-terminal fragments of merosin. To identify the missing portion of the intact merosin polypeptide, we synthesized two 13-amino acid peptides from the part of the deduced amino acid presumed to be $NH_2$-terminal of the 80-kDa fragment (residues 476–488 and 457–469 in Fig. 2). Immunization of rabbits with these peptides resulted in antisera with titers of about 1:1000 in an ELISA against the immunizing peptide. By immunoblot analysis one of the peptide antisera (against residues 476–488) stained a polypeptide of about 300 kDa in $NaDodSO_4$ extracts of placenta (Fig. 4*a*, lane 1). It did not react with the 80-kDa or the 55-kDa COOH-terminal fragments of merosin (Fig. 4*a*, lanes 1 and 2). The presence of the 80-kDa fragment in the same extract was revealed by a monoclonal antibody (Fig. 4*b*, lane 1). These results suggest that the merosin polypeptide is processed into two fragments of 300 kDa and 80 kDa, respectively.

**Isolation of Intact Merosin from Placenta.** The similarity of the merosin polypeptide to the heavy chain of laminin prompted us to isolate the protein by using methods developed to isolate laminin from mouse tissues (36, 37). These methods are based on the selective solubilization of laminin from basement membranes with EDTA-containing buffers. When human placenta was sequentially extracted with a neutral buffer and with the same buffer containing EDTA, merosin antigenic activity was found mainly in the EDTA extract. Merosin could be precipitated from the extract with either 4 M NaCl or 40% saturated ammonium sulfate. When subjected to gel filtration on a Sepharose 6B column, merosin antigenic activity was eluted in the void volume peak (data not shown). Merosin bound to DEAE-cellulose and was eluted at about 0.2 M NaCl.

Fig. 5 shows $NaDodSO_4$/PAGE, electron microscopy after rotary shadowing, and ELISA analyses of the peak merosin-

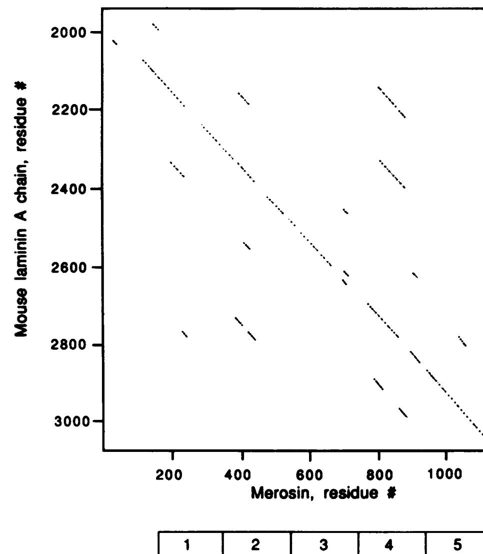


FIG. 3. Comparison of the amino acid sequences of merosin and the COOH-terminal portion of the mouse laminin A chain by dot matrix plotting. Sequences were compared using the MicroGenie matrix comparison program. The frame was set at eight amino acids with a minimal match of 40%.

containing fraction from DEAE-cellulose chromatography. The predominant component in this fraction had a molecular mass of about 700 kDa, slightly smaller than the 800-kDa rat laminin, as determined by gel electrophoresis (Fig. 5*A*). After reduction with mercaptoethanol, the merosin fraction contained polypeptides of about 600 kDa, 300 kDa, and 180–200 kDa in addition to some minor components of 60–90 kDa (Fig. 5*A*). The synthetic peptide antiserum bound to the 600-kDa and 300-kDa bands in immunoblots. Antibodies against the COOH-terminal fragment of merosin bound to an 80-kDa band (data not shown).

Electron microscopy after rotary shadowing was used to further characterize the merosin fraction. Cross-shaped images strongly resembling mouse and rat laminin were the predominant structures seen (Fig. 5*B*).

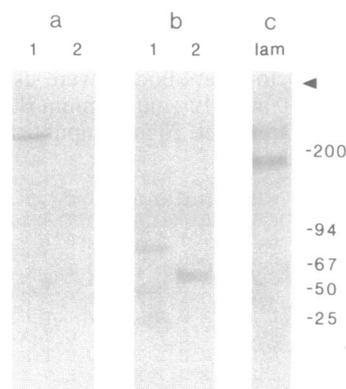


FIG. 4. Immunoblot analysis of a placental extract with peptide antiserum. $NaDodSO_4$ extract of placenta (lanes 1) and the purified fragment of merosin from a pepsin digest of placenta (lanes 2) were electrophoresed on a 2–16% polyacrylamide gradient gel in the presence of $NaDodSO_4$ and transferred to nitrocellulose. (*a*) Blot was stained with a peptide antiserum raised to a 13-amino acid peptide corresponding to residues 475–488 in Fig. 2. (*b*) Blot was stained with monoclonal antibody that recognizes COOH-terminal fragments of merosin. For comparison, a blot of mouse laminin (lam) was stained with anti-laminin (*c*). Arrowhead shows the position of the top of the separating gel and numbers (kDa) indicate the positions of molecular mass markers.

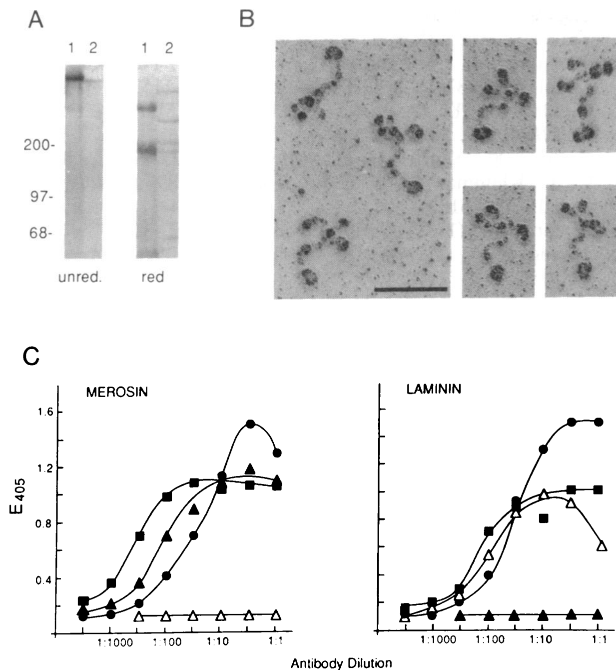


FIG. 5. Analysis of intact merosin from placenta. (*A*) NaDodSO$_4$/PAGE, under reducing (red) or nonreducing (unred) conditions, of rat laminin (lanes 1) and the merosin-containing fraction isolated from human placenta (lanes 2). Positions of molecular mass markers are shown on the left (kDa). (*B*) Electron microscopy after rotary shadowing of the merosin-containing preparation. (Bar = 100 nm.) (*C*) ELISA in microtiter wells coated with the merosin-containing preparation and in wells coated with the large pepsin fragment of laminin. The antibodies were 3E5 (■; anti-B1), 2E8 (●; anti-B2), 11D5 (△; anti-A), and 2G9 (▲; anti-merosin).

Analysis of the fraction by ELISA with merosin-specific and laminin-subunit-specific monoclonal antibodies showed that the preparation contained the merosin polypeptide and the laminin B1 and B2 light chains. No reactivity was obtained with laminin-heavy-chain-specific antibodies (Fig. 5*C*). The truncated pepsin fragment of laminin, isolated with laminin-heavy-chain-specific monoclonal antibody, reacted with antibodies specific for the heavy chain as well as with antibodies specific for the B1 and B2 chains. This laminin preparation did not react with merosin antibodies (Fig. 5*C*). These results show that the high molecular mass laminin-like molecule isolated from EDTA extracts of placenta contained no detectable laminin heavy chain but did contain laminin light chains associated with the merosin heavy chain.

## DISCUSSION

The results described here show that merosin is related to laminin. The merosin polypeptide, found to be tissue-restricted and basement membrane-restricted, is homologous to the laminin heavy chain. In intact merosin this polypeptide appears to be associated with subunits similar to the light chains (B chains) of laminin. Furthermore, the merosin molecule has a cross-like shape in electron microscopy, similar to that of rodent laminins.

We determined the deduced amino acid sequence of >100 kDa from the COOH terminus of merosin and found that this sequence is 39% and 38% identical to the corresponding portion of the mouse and human laminin heavy chains, respectively. The level of similarity between merosin and laminin in this portion shows that merosin is clearly distinct from the laminin heavy chain but that the genes for these two polypeptides have derived from a common ancestral gene. The sequence of the COOH terminus of merosin indicates that it is composed of five repeats, each expected to be about 25 kDa. Similar repeats are found in the laminin heavy chain where they form the large globular domain at the end of the long arm.

The open reading frame of the merosin cDNA clone indicated that the native merosin polypeptide is much larger than the 80-kDa peptide we previously identified (1) in placental extract. A peptide antiserum, made against a deduced peptide sequence upstream from known protein sequences, reacted with a 300-kDa polypeptide. The apparent size of the intact merosin polypeptide is, therefore, 300 kDa plus 80 kDa. This is similar to the size of the laminin heavy chain, which is 400 kDa. The cleavage resulting in the 300-kDa and the 80-kDa polypeptides appears to be quantitative since antisera against the 55-kDa COOH-terminal fragment, which is contained in the 80-kDa fragment, and the antiserum against the synthetic peptide recognized only 80-kDa and 300-kDa components in placental extracts. The cleavage may, therefore, be physiological rather than artifactual. Despite the cleavage, the 80-kDa fragment is an integral part of the merosin molecule since it remained associated with the rest of the molecule through several steps of purification. The 300-kDa portion of merosin is covalently linked by disulfide bonds to polypeptides of about 200 kDa forming a complex with an apparent molecular mass of 700 kDa. The 200-kDa subunits are likely to be the B1 and B2 subunits of laminin, since B1 and B2-specific monoclonal antibodies reacted with the merosin complex in ELISA and immunoblot analyses.

In addition to being associated with laminin light chains, merosin has the cross-like structure characteristic of a laminin molecule, as shown by electron microscopy after rotary shadowing. The merosin molecules were very similar in appearance to rodent laminin.

It may seem surprising that the merosin purified from placenta contained only merosin and no detectable laminin. Laminin is definitely present in the term placenta and it can be isolated in the form of large fragments from pepsin digests of placenta. It may be that the intact laminin in placenta, in contrast to the intact merosin, is insoluble in EDTA-containing buffers. Three groups, including ours, have described isolation of laminin in intact form from placenta by using extraction with EDTA-containing buffers (39–42). It now appears that these preparations may have been merosin rather than laminin. An additional laminin subunit, M, was proposed by one of the groups (40, 41). This subunit has the same approximate molecular mass as the 300-kDa subunit of merosin but it crossreacted immunologically with laminin antisera. It is, therefore, unclear whether subunit M in fact is the 300-kDa fragment of the merosin heavy chain. It seems unlikely that the merosin heavy chain would crossreact immunologically with the heavy chain of laminin, since they are only 40% identical in their amino acid sequences. However, since the sequence of the merosin heavy chain is incomplete, it is possible that the merosin and laminin heavy chains are more similar in their $NH_2$-terminal portions.

There are other examples of laminin-like molecules with subunits that are distinct from the known A, B1, and B2 chains. One is the B1-related subunit of "s-laminin," which is mostly found in basement membranes of the synapses, glomeruli, and blood vessels (43). This subunit of s-laminin cannot be contained in a merosin molecule because merosin and s-laminin have totally different tissue distributions. Other examples of laminin-like molecules are the neurite-promoting factors made in culture by Schwann cells and muscle cells. These factors bind to but are not inhibited by anti-laminin sera, which do inhibit the neurite-promoting activity of laminin from the embryonal rodent tumors, EHS and L2 (44–49). These laminin-like factors have been reported to

lack the usual 400-kDa laminin subunit. The relationship of these factors to merosin remains to be established.

Laminin interacts with cells through a number of different receptors of the integrin family (35, 50–55) affecting the adhesion, morphology, motility, differentiation, and growth of the cells. Preliminary data indicate that merosin also interacts with cells (unpublished data). The similarity of merosin to laminin reported here gives direction to additional studies aimed at the function of merosin.

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