Primary structure of the human laminin A chain

Limited expression in human tissues

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cDNA clones for the human laminin A chain were isolated from libraries prepared from human gestational choriocarcinoma cell line (JAR) RNA. They cover approx. 8 kb from the 5'-end of the 9.5 kb mRNA coding for this protein. Our clones contain 94 nucleotide residues for the 5'-end untranslated region and 7885 nucleotide residues of coding sequence. The complete human laminin A chain contains a 17-amino acid-residue signal peptide and a 3058-residue A chain proper. The human laminin A chain has a distinct domain structure with numerous internal cysteine-rich repeats. The large globular domain G has five repeats, which have several conserved glycine and cysteine residues. Furthermore the A chain contains 20 internal cysteine-rich repeats present in tandem arrays in three separate clusters (domains IIIa, IIIb and V). Domain I+II has a predicted continuous α -helical structure characterized by heptad repeats and three domains (IVa, IVb and VI) are predicted to contain a number of β -sheets and coiled-coil structures. Northern-blot analysis was used to study the laminin A chain expression in the JAR cell line, full-term placenta and newborn-human tissues (kidney, spleen, lung, heart muscle, psoas muscle and diaphragm muscle). The expression was detectable in newborn-human kidney and JAR cell line only. The overall amino acid sequence identity between human and mouse is 76%. The human chain has only one Arg-Gly-Asp (RGD) sequence, which is located in the long arm within domain G, whereas the single RGD sequence in the mouse chain is located in the short arm in domain IIIb. The degree of identity between the human laminin A chain sequence and the sequence available for merosin [Ehrig, Leivo, Argraves, Ruoslahti & Engvall (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 3264–3268] is about 41% and when conservative substitutions are included the degree of similarity is 54%.

INTRODUCTION

Laminin is a specific component of basement membranes that has been shown to mediate a number of biological functions such as cell adhesion, growth, migration and differentiation [1,2]. It has been shown to bind to cells through membrane receptors [3–5]. The laminin molecule is a very complex protein composed of three chains that form a cruciform structure with three short arms and one long arm [2,6]. The molecule has one heavy (A) chain (M_r approx. 400 000) and two light (B) chains (M_r approx. 200 000). To date, two related heavy chains have been described, the so called A chain [1,2] and merosin [7,8]. Furthermore, there are three types of light chains, B1, B2 [1,2] and s-laminin [9]. The complete primary structure of the mouse A chain [10] and a part of the human A chain [11] and merosin [8] have been determined. Also, the sequences of the B1 and B2 chains from man [12,13], mouse [14,15] and *Drosophila* [16,17] and the s-laminin chain from rat [9] have been reported. All the chains contain globular domains, rod-like domains with cysteine-rich repeats and an α -helical domain that participates in the triple coiled-coil structure forming the long arm of the molecule. Additionally, the A chain has a large C-terminal globule, termed domain G [2,10,18]. The existence of multiple laminin component chains enables a variety of molecular compositions whose nature, distribution and biological significance are only beginning to become unravelled. For example, the A chain has been shown to be expressed to a considerably smaller extent in adult mouse tissues than the B1 and B2 chains [19], and Schwann cells may synthesize a laminin

molecule not containing an A chain [20]. Merosin is present in human placenta and Schwann cells [7,21]. Merosin is not found in the mouse fetus but appears postnatally in muscle and nerve [7,21]. The B1 and B2 chains are expressed in most tissues that contain basement membranes, but their ratio can vary considerably [22–24]. Furthermore, the s-laminin chain is primarily found in synaptic basement membranes but is also found in the kidney [9]. A distinct 300 000- M_r laminin heavy chain in heart muscle has also been described [25].

Proteolytic-digest fragments, synthetic peptides and domain-specific antibodies have been used to dissect the biologically active regions of the mouse Engelbreth-Holm-Schwarm-tumour laminin molecule and its component chains. These studies have revealed two cell-binding domains, one in the central region of the cross (P1 fragment) and another one in the long arm [2,26–29]. It has been suggested that a Tyr-Ile-Gly-Ser-Arg (YIGSR) sequence in domain III in the B1 chain [29] or an Arg-Gly-Asp (RGD)-sequence-containing region in domain IIIb of the A chain [27] is responsible for the cell binding of the P1 fragment. According to Deutzmann *et al.* [30], a major cell-binding site is also located in end of the long arm of laminin. Also, fragments from the long arm have been shown to stimulate neural outgrowth [31,33], and a heparin-binding domain [34] has been assigned to the globular domain of the A chain. Furthermore laminin fragments from the short arm have been shown to stimulate cell growth [35], and the A chain may play a role in the polarization of epithelial cells [36].

Comparison of sequences of similar proteins from different

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X58531.

species is important to find conserved, and therefore, presumably biologically important, regions. In an attempt to elucidate the structure, function and expression of laminin, we have isolated cDNA clones for the human A chain and determined its complete structure. The results showed that the human chain has considerable sequence identity with the mouse chain, and also considerable similarity to the human merosin chain. Expression was shown to be negligible in or absent from a number of human newborn tissues except kidney.

METHODS

Isolation and identification of mouse laminin A chain cDNA clone

In order to obtain a probe to screen for the human A chain we first isolated a mouse laminin A chain cDNA clone. A λ gt11 cDNA library containing inserts derived from mouse parietal endoderm mRNA (obtained from Dr. Markku Kurkinen, U.M.D.J.N.-Robert Woods-Johnson Medical School, Piscataway, NJ, U.S.A.) was immune-screened with a rabbit polyclonal antiserum to Engelbreth-Holm-Schwarm-tumour laminin (provided by Dr. P. Brenchley, University of Manchester, Manchester, U.K.). Positive clones were screened to purity and the cDNA inserts were characterized initially on the basis of restriction mapping and the size of mRNA recognized on hybridization to Northern blots of total RNA obtained from induced F9 embryonal teratocarcinoma cells [37]. A 2.5 kb cDNA *Eco*RI insert (λ lamA1) that hybridized to an inducible 10 kb mRNA from F9 cells was subcloned into the M13mp18 vector, and sequencing revealed its origin to be an internal *Eco*RI fragment (nucleotide residues 6641–9065) of the full-length mouse laminin A chain [10].

Preparation of cDNA libraries from human JAR cells

cDNA libraries were made by using polyadenylated RNA from a human gestational choriocarcinoma (JAR) cell line (kindly provided by Dr. G. P. Frenette, University of Michigan Medical School, Ann Arbor, MI, U.S.A.) that secretes the laminin A chain [38,39] and whose mRNA hybridized with the mouse cDNA clone λ lamA-1 (results not shown). The polyadenylated RNA was isolated directly from cultured JAR cells with a slight modification of the Fast Track RNA Isolation Kit method (Invitrogen, San Diego, CA, U.S.A.). Briefly, the cells were lysed with a buffer containing proteinase K and incubated with oligo(dT)-cellulose. The non-polyadenylated RNA, DNA and proteins were washed off in buffer containing LiCl and the polyadenylated RNA was eluted in low-salt buffer. An oligo(dT) primer cDNA library was made from the JAR polyadenylated RNA by using cDNA synthesis and λ gt11 cloning system kits (Amersham International, Amersham, Bucks., U.K.). Briefly, the cDNA was methylated, ligated to *Eco*RI-linkers, separated from the linkers after *Eco*RI digestion on a Sephadex column, ligated into the λ gt11 vector and packaged by using Gigapack extracts (Stratagene, La Jolla, CA, U.S.A.). A first primer extension library was constructed with the JAR cell polyadenylated RNA. The cDNA was prepared with the cDNA synthesis kit (Amersham International) using a synthetic oligonucleotide primer C1 with a sequence (nucleotide residues 5540–5465) from the 5'-end of the A-1 clone, isolated from the first cDNA library. In contrast with the oligo(dT)-primed library, the cDNA was purified by using cDNA Spun Columns (Pharmacia, Uppsala, Sweden) and cloned into the λ gt11 vector by using an *Eco*RI adaptor system (Promega, Madison, WI, U.S.A.) that does not require methylation and *Eco*RI digestion of the cDNA before ligation into the λ gt11 vector. A second

primer extension cDNA library was then similarly prepared by using a synthetic primer C2 with a sequence (nucleotide residues 3422–3449) from the 5'-end region of the longest clone, C1-23, isolated from the first extension cDNA library. The cDNA was purified and cloned into a λ gt10 vector (Promega) by using *Eco*RI/*Not*I adaptors (Pharmacia).

Isolation and characterization of human laminin A chain cDNA clones

The oligo(dT)-primed JAR cell line cDNA library was screened with the 32 P-labelled mouse cDNA clone λ lamA-1 by using standard protocols [40]. The first primer extension library made with the C1 primer was screened with the longest 32 P-labelled clone (A-1) isolated from the JAR cell line cDNA library. The second primer extension library generated with the C2 primer was similarly screened with the longest 32 P-labelled clone C1-23 isolated from the first primer extension library. Purified cDNA clones were isolated, subcloned into pUC18/19 plasmids and M13mp18/19 vectors with standard methods [40] and sequenced from both strands with the dideoxynucleotide chain-termination procedure [41].

Northern-blot analysis

Human tissue samples were obtained at autopsy from a 6-day-old infant within 6 h after death (provided by Dr. Ritva Herva, University Hospital of Oulu, Oulu, Finland) and immediately frozen in liquid N₂. Total RNA was isolated [42], and about a 20 μ g sample was electrophoresed in a formaldehyde-containing gel, blotted on to nitrocellulose filters and hybridized with nick-translated cDNA inserts [40]. As probe for human laminin A chain expression the 2.5 kb A-1 clone (nucleotide residues 5320–7835; see Fig. 2) was used. For analysis of B1 chain expression an *Xba*I–*Bam*HI (2.3 kb) fragment of from the 5'-end of the cDNA sequence (nucleotide residues –180–2157) [12] was used, and for the B2 chain the 2.4 kb HL-205 cDNA containing nucleotide residues 2485–4954 [13] was used.

RESULTS

Isolation and characterization of cDNA clones

Initial screening of several commercial human cDNA libraries from placenta, fibroblasts, endothelium, HT-1080 cells and keratinocytes (Clontech Laboratories, Palo Alto, CA, U.S.A.) that were shown to contain cDNA clones coding for laminin B1 and B2 chains ([12,13]; M. Nissinen, unpublished work) did not yield any positive signals for laminin A chain clones when screened with laminin polyclonal antibodies or with the approx. 2.5 kb mouse λ lamA1 cDNA clone. However, hybridization of the mouse laminin A chain cDNA clone (λ lamA1) to an approx. 9.5 kb mRNA from the human gestational choriocarcinoma cell line JAR (results not shown) indicated significant expression of the laminin A chain gene. This cell has been shown to synthesize significant amounts of laminin containing the A chain [38,39]. Therefore an oligo(dT)-primed JAR cell line cDNA library was screened with the mouse cDNA clone, λ lamA1. This resulted in the isolation of one 2.5 kb clone, A-1 (Fig. 1), which hybridized with the expected 9.5 kb-size mRNA from the JAR cells (see Fig. 3). Initial nucleotide sequencing from both ends of the clone and comparison with the mouse [10] and 3'-end human sequence [11] revealed that the A-1 cDNA coded for the human laminin A chain. The A-1 cDNA clone overlapped 698 bp with the 5'-end of a previously reported [11] cDNA clone. The fact that the A-1 cDNA clone did not contain the 3'-end with a poly(A) tail, although the library was made with an oligo(dT) primer, is probably due to inefficient methylation of the internal *Eco*RI site

Fig. 1. cDNA clones coding the human laminin A chain, and their partial restriction map and the domain structure of the protein

ATG indicates the translation initiation signal, and TGA the 3'-end translation stop codon [11]. Restriction-enzyme sites *Eco*RI (E), *Hind*III (H) and *Pst*I (P) are shown. The domains are marked according to Sasaki *et al.* [10]. Five internal repeats in domain G are indicated by hatched boxes. Domains IIIa, IIIb and V composed of cysteine-rich internal repeats are shown by stippled boxes.

in the cDNA sequence (Fig. 1) so that the 3'-end fragment was lost during the cloning of the original cDNA into the λ gt11 library vector.

The first primer extension cDNA library was then made to obtain more 5'-end clones by using an oligonucleotide primer containing sequences from the 5'-end region of the A-1 cDNA clone. This library was screened with the A-1 cDNA, and four new cDNA clones, C1-24, C1-20, C1-29 and C1-23, were isolated and characterized (Fig. 1). The C1-23 cDNA clone was the longest one, containing an insert of 2.2 kb. A 5'-end sequence of C1-23 was similarly used to prepare the second specific cDNA library as described in the Methods section, and screening of this library with the C1-23 cDNA resulted in the isolation of cDNA clones C2-8, C2-10 and C2-12 (Fig. 1). The C2-12 cDNA was 3.6 kb, and it contained sequence coding for the 5'-end untranslated region of the mRNA, the signal peptide and the N-terminal end of the mature laminin A polypeptide chain. Altogether, the clones isolated and characterized in this study spanned 8.0 kb from the 5'-end of the 9.5 kb mRNA, and thus, together with the previously reported 2.5 kb 3'-end sequence [11], they provide the coding sequence and primary structure of the entire human laminin A chain.

Nucleotide and amino acid sequences

The complete nucleotide sequence of the cDNA clones studied here is shown in Fig. 2, together with the deduced amino acid sequence. The previously reported [11] 680-residue C-terminal amino acid sequence (residues 2379–3058; Fig. 2) is also shown to provide here the complete sequence of the A chain. Differences in the amino acid sequence between the human and mouse A chains are also indicated. The sequence of the overlapping cDNA clones characterized in this study contains 95 nucleotide residues coding for the 5'-end untranslated region and 7885 nucleotide residues of open reading frame. There is a putative 17-residue signal peptide composed of the translation initiator methionine residue followed by a typical hydrophobic leucine-rich sequence that contains two cysteine residues. The signal peptide of the mouse chain is 24 residues in length and contains no cysteine residues. The N-terminal end of the mature mouse A chain, determined by amino acid sequencing [43], starts with a glutamine residue whereas the equivalent residue in the human chain is arginine.

The mature human A chain mRNA encodes 3058 amino acid residues, two less than the mouse counterpart. The A chain has

a distinct domain structure with numerous internal cysteine-rich repeats, as has been described for the mouse A chain [10]. There is a large domain G of 950 residues (residues 2109–3058) at the C-terminal end (Fig. 1). The aligned amino acid sequence of the human and mouse A chains (Fig. 2) shows that the human domain G has one extra residue (serine, residue 2523), but is missing two other residues found in the mouse (proline and glutamine at positions 2681 and 2682). The degree of sequence identity in domain G between man and mouse is 76%. The human domain I+II (residues 1539–2108) has one residue less than the mouse counterpart. Histidine-1735 in the human chain does not have a counterpart in the mouse chain, whereas valine-1658 and glutamic acid-1775 in the mouse chain do not have matches in the human chain. The degree of sequence identity between this domain in man and mouse is 63%. The A chain contains 20 internal cysteine-rich repeats present in a tandem array in three separate cluster, domains IIIa (residues 1345–1538), IIIb (residues 692–1142) and V (residues 253–495). The degrees of amino acid sequence identity of the IIIa, IIIb and V domains between man and mouse are 73%, 81% and 79% respectively. The globular domains IVa (residues 1143–1344), IVb (residues 496–691) and VI (residues 1–252) have degrees of sequence identity between man and mouse of 79%, 84% and 88% respectively.

The overall degree of amino acid sequence identity between the human and mouse chains was calculated to be 76%. All 164 cysteine residues with the exception of one (2561) are conserved between the two species. Also, 23 out of 30 potential attachment sites for asparagine-linked oligosaccharides in the human chain are conserved in the mouse. Interestingly, the human chain has only one RGD sequence, which is located in the long arm within domain G (residues 2517–2519), whereas the single RGD sequence in the mouse chain [10] is located in the short arm within domain IIIb at location residues 1123–1125.

Expression of laminin A, B1 and B2 chains in human tissues

The expression of the laminin A chain gene was compared with that of B1 and B2 chain genes in several human tissues. Total RNA isolated from newborn-human kidney, liver, spleen, lung, heart muscle, diaphragm muscle, psoas muscle and full-term placenta tissues together with RNA from the JAR cells were size-fractionated by agarose-gel electrophoresis, blotted on to nitrocellulose and hybridized with nick-translated human laminin A-chain, B1-chain and B2-chain probes. The results showed a

[illegible]

2350	AAITTCAGCCCACTGCGCACTCAATGAT	GGAGATGAAGTGGTCTGTGACTGGTGTGCC	CGGGCTACTCTAGAGCTGTGTGAGAGA	TGGCGATGCTTACTTACTTGGAAACCCAAACA	GTGCTGGCGAATCTTGTGTCCCTGTGAC
784	N F S P T G	G D E V V G	D W G A	P G Y S G A W G	E R C
784	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
2500	TGACGGCGAACCTGGAACCCCTCGAGGCT	GGTCACTGTGACTCAGTCAAGGGGAGTGC	CTGAAGTCTCTGGGAACACAGATGGCCG	CACCTGTGAAGGTGTGCTGACGGGTCTTAT	GGGAGCGTGTGACAGCCCAAGACATGGCGC
834	C S G N V D P S E A	G H G D S V T G E C	L K C L G N T D G A	H C E R C	A D G F Y
834	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
2650	GCCTGTGAATGCATGTGAAGGCTCCGAT	TCCTGGCGTGTGCACTCTTGAGACGGGCTC	TCCTGACTGCAACCAACCTGACTGACAG	CAGTGTGACAGTGTGCTTGCATGGCTATTAT	GGGCTGCACTCAGGCGCATGGCTGGCGGCC
884	A G E G	H V K G S H	S A V C	D Q C	L H G Y Y
884	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
2800	TGCACTGCAAGCTGGCAGGCTCGGTGCA	GATGGCTGACGGATGAAGCCAGTGTCA	TGTGTGCCAGTGTGCGAGGAAGTGT	GACAGGTGTGCCATGCGCTTCTACGCCCTAC	CAGGATGTGAGCTGTACACCCCTGTGACTGC
934	C N C	S V A G S V S	D G C	T D E G Q C	H C V P G V A G K R C
934	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
2950	CCACACTCAGATACCTGCAACCCAGAA	ACTGAGAGTGTGTGCGCCCTCACACA	CAGGCTGTGAAGTGTGAAGATGTGAGAT	GGGCACCTGGGCTTACGATCGGAGTGGGG	TGCCAGGCTTGCATTTGCAAGTTCGTGGGG
984	P H T Q N T C	D P E	T G E C	V V K C	E E C
984	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3100	TCGACTCATCGGTGCGATGTGTACC	GGCATTGCGAGTGCAGTCAAAATTGGT	GGCCGGGCTCGCTTACAGTGTCTCTGGGT	TACAGAGACTTTTCCGACTGTGTCTCTGT	GACTGTGACTCAGGGGAGCGTCCGGGAC
1034	S T H R C	D V V T	G H C	Q C	K S K F G
1034	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3250	GCCTGCAACTGCAAGGCTCTCTGGCG	TGTGTGAGAGAACCGGGGCTGCGCTTGC	TGTGTGAGAGAACCGGGGCTGCGCTTGC	GAATGTGAGAGGACACTTTCCTCTCGC	GGAGACACCCCTGGCTGTGACCCGTGC
1084	A N L E Q G L G	G C V E E T G G A C	P C S	K E N V F G P Q C	N E C
1084	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3400	TTTCTGCTCGGCTGTCCACCTCTGTCTCA	GAGCTGGAGACTCAGTCAAGCCCAAGTA	AGGCTGGCTCGCATAGGCTCTCTCTGGGT	GTGCTTCTCAGATGACTGTGAGGGGACG	ACTGAGGGGTTTACTACAGGCGCCCGAC
1134	F C S G L S H L C	S E L E D Y V R T P V	L L I	- - - - -	- - - - -
1134	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3550	TTTCTGCTGGATCCCACTGCTGGGAG	CACATCCGTGCAAGCGCTTTTACTGGCG	CTGCGCAGAGCTTCCAGGACACAGCTC	ATGGCTTGTGTGCGCAACTGAGTACAGC	GTGGCTTCTTCTTGTGATGGCGCTCGC
1184	F L D A A T V R C	H I R A E P F Y W R	L P Q Q F Q G D Q L	M A Y G G K L K Y S	V A F Y S L D G V G
1184	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3700	ACCTCCAAATTGAGCTCAAGTCTCATC	AAAGTGTGCTGCAATCAGAAAGCAAGTCA	TACATGATGCAACCGCCCGCAGAGTGA	GTGACAGCAAGCAAGCAAGTCAAGTGA	GAGAAATTTTGGAAATTTTAACTCTCTT
1234	T S N F E P Q V L I	K G G R I R K Q V I	Y M D A P A P E N G	V R Q E Y E V A M R	E N F W K Y F N S V
1234	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3850	TCGTAAACCTGTACGGGAGAGATTTT	ATGCTGTCTCAGCGATGTGAGTACATC	CTCATCAAGCATGCTATGTGCAAGATTA	CAGCAGAGCAGAAATCTCAGACATTTCAATG	GAGTGTGCGAGAAAGCTCGAAAGCTGCAC
1284	S E K P V T R E D F	M S V L S D I E Y I	L I K A S Y G Q G L	Q Q S R I S D I S M	E V G R K A E K L H
1284	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4000	CCAGAGAGAGGTTCATCTTTTACAG	AAATGTGTCTGCTCTGCGACGTGGGA	TTCTCATGTCAGGACTGCGCCCTGGGTAC	CACAGAGGAAAGCTCCACAGGAGTAC	AGGGGACCGCCCTCTGCTGCTCTCTGT
1334	P E E V A S L L E N C	V C	P P G T V G	F S C	Q D C
1334	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4150	GTTCCTCGAGTTCGAACACACAGTGCAC	ACCTGTGACCCCAACACCGGAGTGTCTG	AACTGTGCGCATACACAGCAGTCAACAT	TGTGATGTGTGACTTCTGCTACTACGGG	AAAGTCACTGGCTTCAGCAAGTCACTGTCT
1384	V P C S C	N N H S D T	C D P N T G K C	L N C	G D N T A G D H
1384	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4300	CTGTGCTGCTGCTCAGAGCCCTCTGCC	AGTTTATGTCCTGCTGCTGCTGCTGCTG	GACCAAGATTTCCGCTGTGCTGCTGCTG	CTGGGCTATGAGGAAACACTGTGAAGG	TGCTCTCAAGCTTATTTATGGAAACCTCAA
1434	L C A C	P H S P P A	S F S P T C	V L E C	D H D F R C
1434	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4450	ACACAGGTGCGAGTTCGCAAGTGTGAC	TGCAACCCGACGGCTGTGCTCAGCGTGC	TGTGACCGCAGCATCTGGGAGTGGTTCG	AGGCTGGGGGCTGCGGGCTCGCGTGGCAT	GAGTGTGAACCGGAGCAGCAATTCATGATGA
1484	T P G G S C	Q K C	D C	N P H G S V H G D	C D R T S G Q C
1484	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4600	ACAGATGTGCTGCTGATGATGAGTGT	GTAGTGTGCTGCTGATGATGATGATGAG	ATGTGTGATGCGGTTCCTTCTGTAACCTC	ACTGGCATTTTCCCTGTCCATATGAAAT	TTGTCAACCTGGAATATACACTTAATAT
1534	T D C V S C	D D E C	- - - - -	- - - - -	- - - - -
1534	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4750	CTCCAGGAATCTTTTAAAGAAATATG	CAAAAGGACCTGGGAAATTAAGCTTGA	GGTGTGCAAGAAACCGGACACCTGCA	AGAAGCTCACTAGGATGTAGCGAGTACC	CAAAAGGTGAATAGGCGCACTGAGGAATC
1584	L Q E S L L K E N M	Q K D L G K I K L E	G V A E E T D N L Q	K K L T R M L A S T	Q K V N R A T E R I
1584	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4900	TTCAAGAGATCAAGCTGCGCGCTAGCC	ATTGAGAGGCTGCAATGAGTCAAGATGCT	ACACTTTTAATCAG	ACTTGTGATGAGATTTCTTACTACCCAT	TCTACTCTTCAGACATGCAACAGATGCT
1634	F K E S Q D L A V A	I E R L Q M S I T E	I M E K	T T L N Q	T L D E D F L L P N
1634	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -

5047	ACATCTTTGCTAGAAATCATGACATTAAGA	GACTTCACACAGTTGGACCAAAATGCCACC	CTTGAATCAAGCTGCTGGAAGATTATTG	TCACAAATTCAGGAATATTCAGAGCCG	CTGGAGAAATTTGGAGGTATTGAAGAGCA
1683	T S L L E I M Q I R	D F T Q L H Q N A T	L E L K A A E D L L	S Q I Q E N Y Q K P	L E E L E V L K E A
1684	S - - - G L I K E -	N - - - E M Q - -	- - - - - K - -	- R - - - K R F - -	- Q - - - K A - -
5197	GCAAGCCATGTCTTTTCAAGACACAAAT	GAACTAAAGCGGCTGAGCGCTGTGAGG	GAAGCTGAGGCAAGATGACGAGGAAGCAAC	CACCTGCTGCTCATGCTGATGCTAATCTG	AGAGAA TTCAGTGAATAAAGCTGCAT
1733	A S H V L S K H N N	E L K A A E A L V R	E A E A K M Q E S N	H L L L M V N A N L	R E F S D K L H
1734	N - - - L - - - N - S E	K - Q - - - E - L K	- - G S - T - -	- L - - - L - K - -	- R E - Q E - - R
5344	GTTTCAGAGAAACAAATCTGACCTCAG	CTCATTTGCTCAAGAGAGATGTAGAT	GCTGCTGCTCCAAACAGATCTGTACAA	GATGCTCTAGAGCTTTAGAGGTACCCAG	GATAAGCTACTTTTATGTTGCTGCAAAATC
1782	V Q E E Q N L T S E	L I V Q G R G L I D	A A A A Q T D A V Q	D A L E H L E D H Q	D K L L L W S A K I
1783	- - - - - V - - - -	- - A K - - - E W V -	- - G T H - A - A -	- T - T Q - - H - R -	- E - - - - A R - -
5494	AGGACCATATGATGACTGCTATCCAC	ATGTCCCAAGAGGACGCTGACCTGTGTC	TACAGAGCTGAGCAACCATGCCACTGAGTTC	CAGAGACTAGCAGATGTTCTGTGACGTGGC	CTTGAAACATCAGAAATGTGCTCCCTGAAT
1832	R H I D D L V M H	M S Q R N A V D L V	Y R A E D H A T E F	Q R L A D V L Y S G	L E N I R N V S L N
1833	- S - V - - - - Q - -	- - K - - R - - - -	- H - - - Q - - S - F	- S R - G A - D R G D	- - - - - V - - -
5644	GCACAGCTGCGACCTATGCTCATTAAC	ATCCAGAGCTGATTTGAAGATCGGAGGAA	CTGGCCAGAGATGCTCAGAGGACTGTGACT	GAGACGAGCTGCTCTCAGAAATCCCTTGT	TCATAAGGAAAGCGCCGCTGACGCGCAGC
1882	A T S A A Y V H Y N	I Q S L I E E S E E	L A R D A H R T V T	E T S L L S E S L V	S N G K A A V Q R S
1883	- - - - - H - - - S -	- - - T - - - A - M -	- - - A - - - K - A N	K - D - I - - - - A	- R - - - - V L - -
5794	TCAGATTTCTAAAGAGGCAACACCTC	AGCAGAGCTTCCAGGTATGCAITGGAA	CTGAGTGAATTCAGAAATAGACAAACAGA	TTTCAAGAGAAATGCTGTGTAATATACAGG	CAACCAATGAATCCTCTTGTACTTTAGA
1932	S R F L K E G E N N L	S R K L P G G I A L E	L S E L R N K T N R	F Q E N A V E I T R	Q T N E S L L I L R
1933	- - - - - S V G T R	- - - Q Q - - - T M K	- D - - K - L - S Q	- - S V D N - - K	- A - D - - A M - -
5944	GCAATTCCTGAAGTATAAGACACAGGGA	GCCAAACCAAGAGCTGGCCAGCTGTCA	AGCCAGAGCGGTGAGCAGCTGAGGAC	GTGGCGGGCTGAGCAGGAGTCTGAAC	ACATCTGCCAGCTGTCCAGGCTCAACAC
1982	A I P E G I R D K G	A K T K E L A T A A	S Q S A V S T L R D	V A G L S Q E L L N	T S A S L S R V N T
1983	E S - G - M - E - - H	- R - A R - - - A A -	N E - - - K - - E -	- L A - - - L R V F -	- - - E D - - - A
6094	ACTATGAGACACACACCTTCTGCAG	GACTCCACATGGCCACTCTGTGGCTGGA	AGAAAGTCAAGAGCTGGAATTCAGCC	AGGTTTGTGTGATTCGTTGAGAGCTTTG	AGATGTTAGAGGAGAACTGAGAGAGAAC
2032	T L R E T H Q L L Q	D S T M A T L L A G	R K V K D V E I Q A	K V L F D R L K P L	K M L E N L S R N
2033	- V Q - - - N D - - H	- - - - - T - - - -	- - M - - M - M -	- N L - L - - - -	- - - - - T - - -
6244	CTATCAGAAATTAATCTGTGATCAGCCAG	GCCTGCTGCTGAGAGCTTCTATTAAGTC	GCCTGCTGCTGAGAGAGATGATCATCGG	GCTTACGAGCTCTAGATTTCTCTTCAAC	TCATATCTTCAACATTAATGTTAGACA
2082	L S E I K L L I S Q	A R K Q A A S I K V	A V S A D R D Q	I R A Y Q P Q I S S T N	Y N T L T L N V K T
2083	- - - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
6394	CAGAACCCGATAATCTCTCTTCTTACCTC	GGTAGCAGACCGCTTCTGATTTCTTCTGCA	GTGGAGATGGCGGAGGAGTGGCTTTC	CTGTGGACCTGGGCTCGGGTCCACAGC	TTGGAGTTTCCAGACTTCCCATTTGATGAC
2132	Q E P D N L L F Y L	G S S T A S D F L A	V E M R R G R V A F	L W D L G S G S T R	L E F P D F P I D D
2133	- - - - - - - - -	- - - - - S - - - -	- - - - - K - - - -	- - - - - - - - -	- - - - - E V S - N N
6544	AACAGATGGACAGTATCATGTAGCCAGA	TTTGGAAACATGTTCTCAGTGTGTAAG	GMAATGAGCTCAATCAAAAGTACCAACA	AAAACAGTAAATCCCTGGGACAGCTAAT	GTCTGAGTGTAAACAAATTCACACTCATG
2182	N R W H S I H V I T	- - - - - M - - - -	- A - A A N Q K S P T	K T S K S P G T A N	V L D V N N S T L M
2183	- - - - - - - - -	- - - - - - - - -	- A - A A N P - V	- - - - - P S K	- - - - - I - - -
6694	TTTCTGGAGCTTGTGAGGACAAATCAAG	AAATCTCTGCTGTGAGGTTACTCATTTT	AAAGCTGCTGTGGGAGGCTTCTCTGAAT	GGAATACTCATAGGCTATGAACTATAT	GAAAGGAGGAGTGGCTGGTGGCTTC
2232	F V G G L G G Q I K	K S P A V K V T H F	K G C L G E A F L N	G K S I G L W N Y I	E R E G K C R G C F
2233	- - - - - - - - -	- - - - - - - - -	- - - - - M - - - -	- - - - - - - - -	- - - - - - - - -
6844	GGAGCTCCAGAAATGAGCCCTTCTCTC	CATTTTGAGGGAGTGGTACTCTGTGTC	GAGAAGTCACTTCCGGCTACCTGACCCAG	ATAATCATGCTTTTAAATAGCTTTTACCT	AATGAGCTTCTTCTTACCTGGGTTTCATAC
2282	G S S Q N E D P S F	H F D G S G Y S V V	E K S L P A T V T Q	I I M L F N T F S P	N G L L Y L G S Y
2283	- - - - - - - - S -	- - - - - - - - A M -	- - - - - T - R P - -	- - - - - S - - - -	- - - - - F - - - A - N
6994	GGCAAAAGACATTTTATCATCGAGCTG	TTTCTGGCAGAGTGAAGTTATGATCAG	CTGTGGTTACGAGCCATTAACCTTTTGACA	GACAGCTGTAAATGAAAGCTGTGAC	AAAATGCTTCCAGCGAAACGGAGAGAA
2332	G T K D F L S I E L	F R G R V K V M T D	L G S G P I T L L T	D R R Y N N G T W Y	K I A F Q R N R K Q
2333	- - - - - - - - -	- - - - - V - - - -	- - - - - L - - - M -	- - - - - - - - -	- - - - - - - - -
7144	GGAGTGTAGAGTTATCGAGCTTATAAC	ACCAGTAAATAAGAAACACAGAGCCGAG	ACTCCGGAGCACTTCTGACTCAACCGC	CTAGACAGGACCGGATTTATGTTGGTGA	TTACCAGGTCACAGGTGTGAAGGAGGT
2382	G V L A V I D A Y N	T S N K E T K Q G E	T P G A S S D L N R	L D K D P I Y V G G	L P R S R V V R R G
2383	- L - - - - F - - - D -	- - - - - - - - -	- - - - - A - - - -	- - - - - L - - - -	- - - - - H - K A - - K -
7294	GTCACCAACCAAGCTTTGTGGCTGCAATC	AAGAACCTGGAATPATCAGATCAACCTTT	GACTTATCAGAAATTCCTATGAGTGA	AAAGGCTGTATCTGAGGAGCCATCCGAGT	GTTAGCTTCTGAAAGAGGCTTACATTTGAA
2432	V T T K S F V G C	I K N L E I S R S T F	D L L R N S Y G V R	K G C L L L E P I R S	V S F L K G G Y I E
2433	- S S R - Y - - - -	- - - - - - - - -	- - - - - L - - - V -	- - - - - A - - - Q	- - - - - R - - - V -
7444	TTGACCAACCAATCTTTGTACCAAGATCA	GAAATGCTGTAACATTTGCCACAGAAC	AGCAGTGGCATCATCTGCTGCTGCGC	GGGAGTGTGAGAGAGGCGGCTGTGAG	GAAACACAGCTGCCCTTCTTCTCCGTCATG
2482	L P P K S L S P E S	E W L V T F A T T N	S S G I I L A A L G	G D V E K R G D R E	E A H V P F F S V M
2483	M - - - - - - - - -	- S L - A - - - - K -	- - - - - L - - - V -	- - - - - K - A - E A - G A Q	- - - - - - - - - I -
7594	CTGATCGGAGGCAACATGAGGTACATGTC	AATCTCGGGATGGGACAGCCCTGAGAAA	GCTCTCTGCAAGCTTCCCGAGTACTGTC	AGTGTGACAGGAGGCAATTCATCTCTT	GTCAGGAATCGGAGATTTATCTGTCGAA
2532	L I G G N I E V H V	N P G D G T G L R K	A L L H A P T G T C	S D G Q A H S I S L	V R N R I I T V Q
2532	- L E - R - - - -	- S - - - - - S - - -	- - - - - - - - -	- - - - - E - - - -	- - - - - V - - - I -

	TTGGATGAGAACACTCTGTGGAAATGAAG	TTGGGCACATTAGTAGAAGAGGAGCATA	AATGTCTCCANTCTTACGTCCGGCGAATT C	L D E N N P V E M K L G T L V E S R T I N V S N L Y V G G I P E G E G T S L L T M R R S F H G I -	2582
	V - - - S - - - - -	- - - P - - -	D I - - - I - - - L - - -	- - - D K A - P P M - K - -	2582
	N L I F N L E L L D F N S A V G H E Q V D L D T [C] W L S E R P K L A P D A E D S K L L R E P R A F	- - - T H - T - S - -	E - - -	- - - H G E - P P - - P T L P	2632
	- - - V V L D A Q - - -	- - -	- - -	- - -	2632
	[P] E Q [C] V V D A A L E Y V P G A H Q F G L T Q N S H F I L P F N Q S A V R K K L S V E L S I R T L F	- - - P G - - A - -	G - - - S - - - L V - - -	- - - Q - - -	2681
	Q - - - L A - - - T - - -	- - -	- - -	- - -	2682
	A S S G L I Y Y M A H Q N Q A D Y A V L Q L H G G R L H F M F D L G K G R T K V S H P A L L S D G K -	- - - V - - - M - - -	- - - Q E - - -	- - -	2730
	- - - - - - - - -	- - -	- - -	- - -	2732
	W H T V K T D Y V K R K G F I T V D G R E S P M V T V V G D G T M L D V E G L F Y L G G L P S Q Y Q	- - - I - - - A - - - M - - -	- - - S - - -	- - - R K L - - -	2780
	- - - - - E - - -	- - -	- - -	- - -	2782
	A R K I G N I T H S I P A [C] I G D V T V N S K Q L D K D S P V S A F T V N R R [C] Y A V A Q E G T Y F E	- - - - - E I M - - -	- - - G Q - - -	- - - D - - -	2830
	- - - N - - - T - - -	- - -	- - -	- - -	2832
	G S G Y A A L V K E G Y K V Q S D V N I T L E F R T S S Q N G V L G I S T A K V D A I G L E L V D -	- - - - - R L - - -	- - - T - - -	- - - S - - -	2880
	- - - - - - - - -	- - -	- - -	- - -	2882
	G K V L F H V N N G A G R I T P A Y E P K T A T V L [C] D G K W H T L Q A N K S K H R I T L I V D G N -	- - - - - A T - - - Q - - -	- - - R A - - -	- - - H - - -	2930
	- - - - - - - - -	- - -	- - -	- - -	2932
	A V G A E S P H T Q S T S V D T N N P I Y V G G Y P A G V K Q K [C] L R S Q T S F R G [C] L R K L A L I S	- - - - - A - - - D - - -	- - - H I - - -	- - - R A - - -	2980
	S - R - - - - H - - -	- - -	- - -	- - -	2982
	K S P Q V Q S L D F S R A F E L H G V F L H S [C] P G P S P * 3058	- - - - - D - - - Q - - -	- - - P - - -	- - - E - - -	3030
	R G S - - - - L - - -	- - -	- - -	- - -	3032

Fig. 2. Nucleotide sequence of laminin A chain cDNA clones and cDNA deduced amino acid sequence of entire chain

The first line shows the nucleotide sequence of cDNA clones studied in this study. The second line shows the deduced amino acid sequence from the cDNA clones together with the previously determined C-terminal amino acid sequence [11]. The third line shows the amino acid sequence of the mouse laminin A chain [10] with identical residues shown by - and differing residues by one-letter code. Missing or mismatching amino acid residues in the aligned sequence are depicted by hatched squares or rectangles. The tentative N-terminus of the mature A chains is indicated by a vertical bar. The location of cysteine residues is indicated by open boxes, and potential attachment sites for asparagine-linked oligosaccharides are underlined. RGD sequences and their counterparts in the other chain are boxed. The sequence of two oligonucleotide primers used for the preparation of primer-extended cDNA libraries are shown by arrows. The bent arrow indicates the 5'-end of the previously reported sequence [11].

Fig. 3. Northern-blot analysis of human newborn tissues hybridized with human laminin A-chain, B1-chain and B2-chain cDNA probes

Nitrocellulose filters containing size-fractionated total RNA (20 µg) were prepared and hybridized as described in the Methods section. Lane 1, JAR cells; lane 2, kidney; lane 3, liver; lane 4, spleen; lane 5, lung; lane 6, heart muscle; lane 7, diaphragm muscle; lane 8, psoas muscle; lane 9, placenta.

strong signal with the A-chain probe on 9.5 kb mRNA from the JAR cells, whereas no signal was apparent even after a long exposure in the human tissues with the exception of kidney (Fig. 3). In contrast, the B1-chain probe gave strong signals with RNA from kidney, lung, heart muscle and placenta, whereas the signals were considerably weaker for JAR cell, liver, spleen, diaphragm muscle and psoas muscle RNA. The signal pattern with the B2-chain probe was similar to that of the B1-chain probe except that RNA from JAR cells and diaphragm muscle gave somewhat more intense signals.

DISCUSSION

The present work, together with a previously reported 680-residue C-terminal sequence [11], provides the entire primary structure of the 3058-residue mature human laminin A chain, together with a 17-residue putative signal peptide sequence. Comparison with the 3060-residue mouse sequence [10] revealed a high degree of similarity between the two species. There is a 76% sequence identity at the amino acid level for the mature chains, and when conservative substitutions are included the degree of similarity is 83.7%. This degree of sequence identity is considerably less than has been noted for the B chains: for

example, human and mouse B1 chains share 93% identity at the amino acid level [12]. The conserved sequences includes 163 of 164 cysteine residues and 23 of the 30 potential attachment sites for asparagine-linked oligosaccharides present in the human chain.

The RGD triplet sequence has been shown to be involved in cell binding to several proteins such as fibronectin, vitronectin and collagens [44]. One such RGD sequence has been found located in the short arm within domain IIIb (residues 1123–1125) in the mouse laminin A chain [10]. Aumailley *et al.* [27] have reported that this region of the mouse A chain contains cell-binding activity that possibly involves the RGD sequence. However, the present study shows that the human laminin A chain does not contain an RGD (Arg-Gly-Asp) sequence in domain IIIb or any region nearby. Instead the human chain contains the sequence Arg-Ala-Asp at this position (residues 1123–1125). The human A chain does, however, contain one Arg-Gly-Asp sequence, but in domain G (residues 2517–2519), the large globular domain at the end of the long arm. The mouse A chain has the sequence Ala-Gly-Gly at this location (see Fig. 2 and ref. [10]), and in addition the human merosin chain exhibits no apparent RGD-related sequence at this or any other location in its G domain (see Fig. 4 and ref. [8]). The lack of conservation of the positions of the sole RGD sequences found in the human and mouse A chains together with the absence of such a sequence from the G-domain of human merosin in comparison with the human A chain suggests that the described RGD motifs may have arisen merely by chance and have no true biological significance. Further experiments are required to determine activities of these A chain motifs in terms of specific cell-binding.

It was of interest to compare the human laminin A chain sequence elucidated in the present study with the sequence available for human merosin, which has been shown to be similar to the laminin A chain [8]. Indeed, alignment of the two sequences (Fig. 4) demonstrates that the two proteins are closely related. The published 1130-residue merosin sequence could be easily aligned with sequence (residues 1937–3044) from the C-terminal end of the laminin A chain. The aligned sequence involved the C-terminal region (172 residues) of domain I+II of the A chain and the entire 950-residue domain G with the exception of the 14 last residues. The comparison of sequences from domain G showed that 12 out of 15 cysteine residues are conserved in the two chains in this region. A total of 414 residues were identical in this part of domain G, giving 42.5% sequence identity. Additionally there are 133 residues that represent conservative amino acid substitutions, and when they are included the total degree of sequence similarity between merosin and domain G of the A chain is 56%. It may be noted that the RGD sequence in domain G of the human A chain is not conserved in the human merosin chain (see Fig. 4). The alignment of a 172-residue sequence from domain I+II of the A chain (residues 1937–2108) with the comparable merosin sequence (173 residues) also showed a considerable degree of similarity, with 32% sequence identity and 44% sequence similarity when conservative substitutions are included. The overall sequence identity between merosin and A chain is about 41%, and when conservative substitutions are included the degree of similarity is about 54%. Consequently the merosin chain appears to be a variant of the laminin A chain, and is an integral component of the laminin molecule in certain tissues where it substitutes for the A chain.

It has become apparent that the laminin molecule exists in a variety of isoforms composed of at least five different subunit chains. The present study confirms previous data [19–24] showing differing spatial transcriptional activity of different laminin chain genes. However, the composition and biological function of laminin isoforms in different tissues and during different stages

1937 EGNL SRKLPGLALELSELRLNKTNRFFQENAVEITRQTNESLLILRAIPEGIRDKGAKTKELATSAQSASV
 1 VKKLANDVKENEDHLNGLKTRIE-ADAR-GDLLL-TL-DT-GK-S--NDTAA-LQAV-DK-RQ-NDT-K
 2007 STLRDVAGLSQELLLNTSASLSRVNTTLRETHQLLQDSTMATLLAGRKVKVDVEIQAKVLFDRLLKPLKML
 70 DV-AQITE-H-N-DGLKKKNYNKLAADVAK-NAVVK-P-KNKIIAD-DAT--NL-QE-DR-I-K--I-E-
 2075 EENLSRNLSEIKLLISQARKQAASIKVAVSADRDCIRAYQPQISSTNYNTLTNLVKTQEPPDNLLFYLGSS
 140 -D--KK-I--E--N--N--S--SGG-
 2145 TASDFLAVERMRGRVAFLLWDLGSGSTRLEFPDPIIDNRWHSIHVARFENIGSLSVKEMSSNQKSP
 210 KFI--I--K-K-S--V--V--VG-V-Y--LT--SY-YR--S-T-RN-TI--RALDGPKA-IV-S
 2211 TKTSKSPGTANVLDVNSTLMFVGGLGGQIKKSPAVKVFHFKGCLGEAFLNGKSIGLWNYIEREGKCR
 279 -HH-T--PGYTI--DA-AM--T-KL--AD--R-IT-T-M--TY-D--P--FR-K--D-K
 2279 GCFGSSQNEPDSFHFDGSGYSVVEKSLPATVTQII MLFNTFSPNGLLLYLGSYGTTKDFLSIELF
 347 -TV-P-V--SEGTIQ--E--AL--R-IRWYPN-STV-FK-R--SSA-M--ATRDLR--M-V--T
 2343 RGRVKVMTDLGSGPITLLTDRRYNNGTWYKIAFQRNRKQGVLAVIDAYNTSNKETKQGETPGASSDL
 415 D-HI--SY--MASVVSQNH-D-K-KSFTLS-IQ--ANISI-D--TNQEE-IA-SSS--NNF-L--
 2410 NRLDKDPIYVGGLPRLSRVVRRGVTTKSFVGCINKLEISRSTFDLLRNSYGVKGCILLEPIRSVS
 483 KAD--F--TL-NL-MKA-PE-NL-KYS-L-DI--TPYNI-SSPD-V--T--S--NVYT--
 2474 FLKGGYIELPPKSLSPESWLVTFAATNSSGIIAALGSDVEKRGDRREEAHVPFFSVMLIGGNI EVHVN
 551 -P-P-FV--S-VPIDVGT-INLS-S-K-E--LGS--TPAPP--RK-RQTGQAYYVIL-NR-RL--LS
 2543 PGDGTGLRKALLHAPTGTSDGQAHSISLVNRRIITVQLDENNPVEMKLGTLVESRTINVSNLVVGIP
 621 T-AR-M--IVIRPEPNLFH--RE--VHVE-T-G-F--V--RRYMQN--QP--E-KK-F--A-
 2613 EGEGLTSLTMRRSFHGCINKNLIFFNLELLDFNSAVGHEQVDLDTCWLSERPKLAPDAEDSKLLREPR
 687 PEFQP-P-RNIPP-E--W--VI-SVPM--ARP-SFKNA-IGR-AHQK-R-DEDEG-AP--IVIQPEPV-T
 2679 AFPEQCVVDAALEYVPGAHQFGLTQNSHFILPFNQSAVRKKLSVELSIRTLASSGLIYYMA
 757 P--TPTP-LTHGPC-A-SE-ALLI-SK--SR--IAIA-DDTK-KNR-TI--EV--E--LF--

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2740 H Q N Q A D Y A V L Q L H G G R L H F H E D L G K G R T K V S H P A L L S D G K W H T V K T D Y V K R K G F I T V D G R E S P M V T V V G D
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
827 R I - H - - F - T V - - R N - L P Y - S I - - S - D - H T M I - T K I N - - Q - - K I - I M R S - Q E - I L Y - - - A - - N R T I S P K K
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
2810 G T M L D V E G L F Y L G G L P S Q Y Q A R K I G N I T H S I P A C I G D V T V N S K Q L D K D S P V S A F T V N R C Y A V A Q E G T Y F D
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
896 A D I - - - V - M L - V - - - I N - T T - R - P V - Y - - D G G - V R N L H M A E A P A - L E Q - T - S - H - G T F - N - - R - - - - -
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
2880 G S G Y A A L V K E G Y K V Q S D V N I T L E F R T S S Q N G V L L G I S T A K V D A I G L E L V D G K V L F H V N N G A G R I T P A Y E P
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
966 - T - F - K A - G - - - F - - G L - L L V E F - - - T T T - - - - - S Q - M - G M - I - M I - E - L M - - - D - - - - - F - A V - D A
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
2950 K T A T V L C D G K W H T L Q A N K S K H R I T L I V D G N A V G A E S P H T Q S T S V D T N N P I Y V G G Y P A G V K Q K C L R S Q T S
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
1035 G V P G H - - - Q - - K V T - - - I - - - - E - T - - - - Q - E - Q - - - N P A - - - A - - - D - - V F - - - F - D D L - - - F G - T T - I P
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
3019 F R G C L R K L A L I K S P Q V Q S L D F S R A F E L H G V F L H S C P G P S P 3058
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
1104 - - - I - S - K - T - G T A S H W R - I L P - P W N 1130
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

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Fig. 4. Alignment of amino acid sequence of human laminin A chain with available sequence from human merosin chain.

The upper line shows the amino acid sequence of laminin A chain (residues 1937–3058). The second line shows the amino acid sequence from human merosin. Missing or mismatching amino acid residues are indicated by stippled squares or rectangles. Identical residues are shown by - and conserved amino acid substitutions by :. Cysteine residues and an RGD sequence in the A chain are highlighted by open boxes. The junction of domains G and I + II is indicated.

of development is as yet poorly understood. With regard to the A chain, the present study has demonstrated negligible expression of the A chain as compared with expression of the B1 and B2 chains in all human tissues studied. In fact, in the eight human tissues studied, we could detect expression of the A chain only in the kidney, although even in this tissue its expression was considerably lower than that of the B1 and B2 chains with the use of same-size cDNA probes with similar specific activities. It would be of interest to explore the expression of the merosin chain in the same tissues. Leivo & Engvall [7] have shown that merosin is not expressed in the mouse embryo, and after birth it is expressed only in muscle and nerves. Therefore it seems likely that in tissues other than muscle and nerves laminin molecules contain as yet unidentified variant(s) of the A chain. Another possibility is that some laminin molecules are completely devoid of a heavy (A) chain. Considering the fact that the A chain and merosin are variants of alternatively used laminin heavy chains and that other heavy laminin chains may exist [25], it would be appropriate for reasons of clarity to refer to the heavy chains collectively as A chains. Thus the 'classical' A chain could be referred to as A1, merosin as A2 and other potential A chains in a similar fashion. In addition it would be sensible to term variants of the laminin light (B) chains B1, B2 and B3 (s-laminin).

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