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ARTICLES

Heterotrimeric Configuration Is Essential to the Adhesive Function of Laminin

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Abstract Mouse PFHR9 laminin, B1B2-heterodimers, and free B1-chains were separated from one another by gel filtration on Superose 6. The cell attachment promoting activity of these species was measured after immunoprecipitation with monoclonal anti-laminin antibodies coupled to Sepharose 6MB beads. These antibodies, which did not react with the laminin E8 fragment, were directed against epitopes in the NH2-terminus of the laminin B1-chain and in the central region of laminin. After incubation with purified EHS laminin, the immunosorbents revealed efficient adhesion substrates for a rat rhabdomyosarcoma cell line which attached preferentially to the laminin E8 fragment. Although both were immunoprecipitated efficiently, B1B2-heterodimers and B1-chains, unlike PFHR9 laminin, did not support the attachment of RMS cells. On a molar basis B1B2-heterodimers were 24 times less efficient than PFHR9 laminin or EHS laminin in supporting cell attachment. These data suggest that heterotrimeric configuration is essential to the adhesive function of the laminin E8 fragment.

Key words: laminin, structure-function, adhesion

Laminin plays a major role in the control of cell movement [1–3]. In addition to promoting adhesion and migration, this structural basement membrane glycoprotein [4] facilitates cell differentiation and exhibits potent neurotropic properties [5]. The molecule produced by matrix-synthesizing cells consists of a cross-shaped heterotrimer [6] formed by the association of three subunits—i.e., A (400 kDa), B1 (220 kDa), and B2 (210 kDa) [7]. Each of these subunits is the product of its own gene [8–12].

Several adhesion sites have been identified in the laminin molecule. In particular, two distinct fragments, P1 and E8, support cell attachment [13–16]. P1, obtained after extensive digestion of laminin by various proteolytic enzymes, corresponds to the center region of the molecule [17]. P1 adhesion sites, which are cryptic in the native molecule, can be disclosed by proteolysis [18]. E8, which is obtained by mild elastase digestion, corresponds to the distal part of the long arm of the molecule [19]. It is composed of the C-terminals of the three chains, which are wound in a coiled α-helix [9,19]. The laminin domain responsible for neurotropic activity has been assigned to the E8 fragment [20].

In most of the cell lines studied so far, A-chain expression levels limit production of heterotrimeric laminin. Excess B-chains are found as heterodimers or as monomers [21–23].

In this report, the relationship between the structure and function of the adhesive domain in the laminin E8 fragment is analyzed by assessing the attachment to laminin and purified B1B2 dimers or B1 chains of a cell line exhibiting E8 dependent adhesion.

METHODS

Antibodies

The rat IgG1 monoclonal anti-laminin antibodies (MAb) 4C12 and 2D8 were obtained as previously described [16]. Affinities and specificities were determined by ELISA, dotimmunoassay, and immunoblotting, as well as by SDS-PAGE analysis of ^{35}S-labelled PFHR9 cell extract immunoprecipitates. High titer polyclonal antibodies against EHS laminin were produced in

Abbreviations used: IgGs, immunoglobulins; mAb, monoclonal antibody; PBS, phosphate buffer saline; RMS, rhabdomyosarcoma cells; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SED, standard error deviation; SEM, standard error from the mean.

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the rabbit by serial subcutaneous injections of highly purified antigens emulsified in complete Freund adjuvant. Immunoglobulins (IgGs) were prepared by affinity chromatography on protein A-Sepharose for sera and anion exchange chromatography on MonoQ (Pharmacia) for ascites. Polyclonal antilaminin IgGs were further purified on an Affigel-10 matrix (Biorad) coupled with purified laminin P1 fragments (1 mg/ml of gel). Affinity-bound IgGs were eluted with guanidine hydrochloride 6M in Tris 50 mM, pH 7.4, and extensively dialysed against phosphate buffer saline (PBS). For radioimmunoassays, laminin and antibodies were iodinated with ^{125}INa by the chloramine T method and purified from free iodine by filtration on PD 10 columns (Pharmacia). Immunosorbents were prepared by coupling purified antibodies on cyanogen-activated Sepharose 6MB beads (Pharmacia) according to the manufacturer's instructions.

Preparation of PFRH9 Cell Lysates

The laminin-producing mouse endodermal cell line PFHR9 [25] was grown to confluency on 150 cm^2 culture dishes in DMEM, 10% fetal calf serum (v/v). In some cases the cells were metabolically labelled with ^{35}S methionine, 200 μCi/dish in methionine deficient RPMI for 4 hours. After a wash in 50 mM phosphate buffer, 150 mM sodium chloride, pH 7.4, (PBS), cells were scraped with a rubber policeman into ice cold PBS and pelleted by centrifugation. The cell pellet was resuspended in 1.5 ml of freshly prepared ice cold lysis buffer [PBS, ethylenediamininetetraaceticacid (EDTA) 5 mM, Triton X-100 1% (v/v) and proteinase inhibitors (aprotinin 2,500 ui/ml, benzamidine 5 mM, N-ethylmaleimide 8 mM, and phenylmethanesulfonyl fluoride 2 mM)] and was gently stroked with a tight glass-glass homogenizer. After 15 minutes the ice insoluble material was pelleted by centrifugation (15,000*g*, 15 min.).

Gel Filtration of PFRH9 Cell Lysates

Lysates were filtered through 0.22 μm membranes before molecular sieving on columns packed with Superose 6 FPLC grade (1 × 50 cm) or Prep grade (1.6 × 100 cm) (Pharmacia) eluted at 4°C with PO_4HNa_2 50 mM, NaCl 75 mM, EDTA 5 mM, pH 7.4, at a flow rate of 0.1 ml a minute; 0.5 or 1 ml fractions were collected. Columns were calibrated by elution with lysates of PFHR9 cells labelled with S-35 methionine and by eluate analysis by immunoprecipitation and SDS-PAGE analysis. Then several runs were performed with unlabelled lysates. Related fractions were pooled and analyzed by radioimmunoassay and adhesion assay.

Analysis of PFHR9 Lysate Chromatograms

The eluate of gel filtered lysates labelled metabolically with ^{35}S methionine (0.1 ml of each fraction) was incubated with polyclonal antilaminin IgGs, 5 μg in 0.4 ml of lysis buffer. After 18 hours (4°C), 50 μl of protein A Sepharose 4CL (Pharmacia) were added and incubation was continued for 2 more hours on a roll-over shaker. Beads were washed by centrifugation on a cushion of Percoll [stock Percoll (see below for formulation) diluted 14% (v/v) in lysis buffer]. After aspiration of the Percoll, and washing with Tris 50 mM, pH 7.5, immunoprecipitates were boiled in SDS-PAGE sample buffer and analyzed by electrophoresis on 3–12% acrylamide gradient slab gels, followed by fixation in methanol/acetic acid/water, 40/10/50 (v/v), impregnation with Enhance and fluorography at −70°C with Hyperfilm MP (Amersham). When necessary, properly exposed autoradiograms were quantified by scanning with a Shimadzu transmission densitometer.

Attachment Assays

Purified EHS laminin or laminin material in gel filtration eluates were immunoselected overnight at 4°C with MAb 4C12 or 2D8 coupled to Sepharose 6MB beads. After washing on a Percoll cushion (see below) and with DMEM, Hepes 20 mM, pH 7.2, beads (50 to 100 μl) were incubated with rhabdomyosarcoma (RMS) S4T cells (4 × 10^4 or 1 × 10^5 cells) labelled with ^{35}S methionine, detached in PBS, 0.4 mM EDTA and resuspended as a single cell suspension (10^6 cells/ml in DMEM-Hepes). After the incubation (1 hour, 37°C), cells attached to the beads were separated from free cells on a Percoll cushion [Hepes 20 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, Sucrose 0.25 M, stock Percoll 28% (v/v), ph 7.2. Stock Percoll was 9 parts Percoll (Pharmacia) and 1 part sucrose 2.5 M]. To optimize the separation Percoll solution (2.2 ml) was dispensed in 4.5 ml tubes (Nunc, Minisorb) in which 1 ml pipette tips (Treff) cut at the narrow end were inserted. The inner chamber thus formed at the tube openings was used to decant incubates after attachment assays. After centrifugation (800*g*, 5 minutes), tips containing free cells stacked at the Percoll/medium interface were withdrawn

with a pipette. The remaining Percoll was aspirated and cells attached to the beads in the pellet were lysed with SDS 1% in water (v/v). Radioactivity in lysates was measured by liquid scintillation.

Radioimmunoassays

Laminin-like immunoactivity in gel filtration eluates was evaluated by solution competition immunoassays using ^{125}I-laminin as tracer, EHS laminin as standard competitor, and a polyclonal serum to native laminin at final dilution of 1×10^{-5}. The assay buffer was PBS, EDTA 3 mM, Triton X-100 1% (v/v), bovine serum albumin (BSA) 0.25% (w/v), pH 7.4. After incubation, antibody-bound and free ligands were separated with an anti-rabbit IgG precipitating reagent. Laminin detection sensitivity was 30 ng/ml.

A sandwich immunoassay was also used for quantification of laminin material. To this MAb 4C12-Affigel 10 or Sepharose 6MB beads were incubated overnight with chromatogram eluates or known amounts of EHS laminin. After washing, beads were incubated for 2 hours more at 4°C with ^{125}I-antilaminin MAb 2D8 or affinity purified anti-P1 antibody. After washing on Percoll 14%, the amount of ^{125}I-antibody bound to the beads was measured on a γ-scintillation counter.

Another assay system was used for selection and quantification of laminin B1-chains. MAb 2D8 was on solid phase and the amount of captured ligand was quantified by binding of ^{125}I affinity purified anti-P1 IgGs.

RESULTS

Gel Filtration of the Laminin Species in PFHR9 Cell Lysates

We have previously shown that the PFHR9 cell line produces laminin, B1B2-heterodimers, and B1-chains [23]. In order to assess their individual attachment activity, it was necessary to separate these three species from one another. This was achieved by gel filtration on Superose 6. As shown in Figure 1 (A to D), laminin trailing on 400 kDa-B1B2-dimers did not exceed 10% as judged by scanning of fluorograms obtained by SDS-PAGE analysis of column fraction immunoprecipitates. Contamination of B1-chains by heterodimers or by laminin was minimal.

A major peak of immunoreactivity in the laminin position was observed when laminin material in gel filtration eluate was quantified by immunoassay with a polyclonal antibody. It was followed by minor peaks eluting at the position of B1B2-dimers and B1-chains (Fig. 1F). A two-step sandwich immunoassay using solid phase MAb 4C12 and ^{125}I affinity-purified iodinated anti-P1 IgGs provided more efficient quantification of B1B2-dimers. Two peaks of similar magnitude (Fig. 1E) were noted with this method. The first eluted in the laminin position and the second in the heterodimer position. Thus, significant amounts of B1B2-dimers were produced by the cell line.

Immunoattachment Assays

The laminin material in gel filtration eluates was immunoprecipitated with monoclonal antibodies against laminin. These antibodies were coupled to cyanogen bromide-activated Sepharose 6MB so that immunoprecipitates could serve as substrates for cell attachment assays.

The specificity of the two antilaminin monoclonal antibodies is summarized in Table I. Neither reacted with the laminin E8 fragment. MAb 4C12 immunoprecipitated laminin and B1B2-dimers by interacting with an epitope located on the laminin P1 fragment. MAb 2D8 interacted with an epitope on the laminin B1-chain. This epitope was probably located near the NH2 terminus of the chain, since the antibody did not react with laminin P1 or E8 fragments. Because they recognize different epitopes, these antibodies did not compete for laminin binding (data not shown). The antibodies did not interfere with attachment on laminin of the rat RMS cell line, which interacts preferentially with the laminin E8 fragment (data not shown).

After incubation with purified EHS laminin, MAb-Sepharose 6MB beads revealed efficient attachment substrates for RMS cells. As shown in Figure 2, cell attachment was dependent on the amount of laminin reacted with the immunosorbents. The sensitivity of the assay is demonstrated by the fact that after incubation with 200 ng/ml of laminin the amount of ligand captured was sufficient to support significant attachment. Its specificity is shown by the facts that cell attachment on laminin-immunobeads complexes was blocked by polyclonal antibodies to the laminin E8 fragment (Fig. 2) and that attachment was minimal on untreated MAb-beads (data not shown).

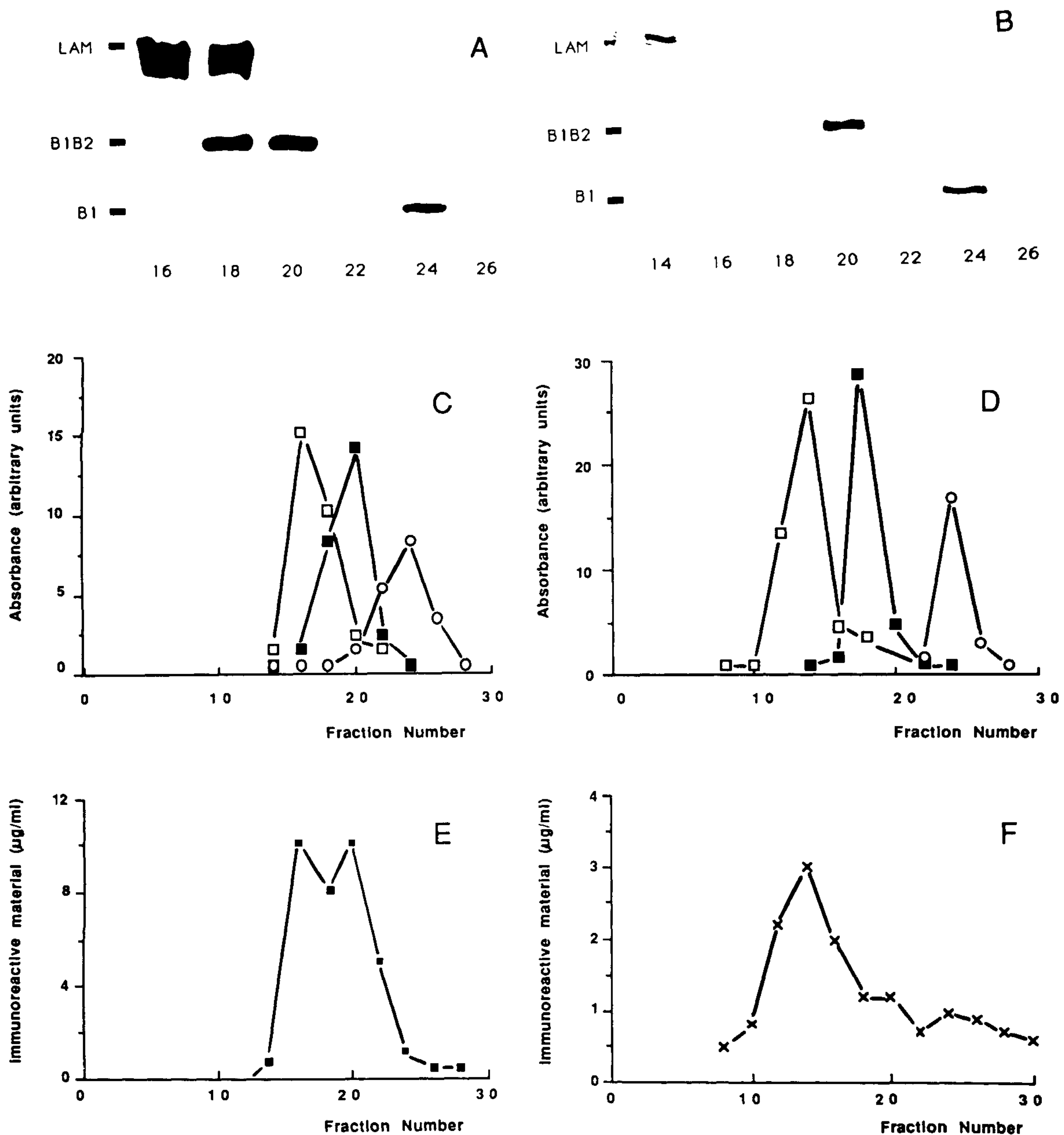

Fig. 1. Gel filtration purification of the laminin species from the PFHR9 cell line. Lysates of PFHR9 cells metabolically labelled with ^{35}S methionine were gel filtered on a Superose 6 column FPLC grade (0.9 × 28 cm), (left panel; **A, C, E**) or on Superose 6 Prep grade (1.5 × 50 cm), (right panel; **B, D, F**). Columns were eluted in PBS-EDTA (0.1 ml/min.) and 0.5 or 1 ml fractions were collected. A and B: An aliquot of each fraction was immunoprecipitated with polyclonal antilaminin IgGs. The unreduced precipitates were electrophoresed on 3–12% polyacrylamide SDS gels. Fluorograms of the gels are shown. Fraction numbers are indicated under the lanes. The migration position of laminin species (laminin: LAM, B1B2-heterodimers: B1B2, and B1 chain: B1) are indicated in margins. C and D: Autoradiograms in A and B were scanned by transmission densitometry. Absorbance related to laminin (open squares), B1B2-heterodimers (closed squares), and B1 chains (open circles) is plotted as a function of fraction number. E and F: Laminin-like-immunoreactive content in columns fractions determined by two step sandwich immunoassay using solid phase mab4C12, EHS laminin as standard and iodinated immunopurified antiP1 immunoglobulins (E) or by solution competition immunoassay using polyclonal antilaminin IgGs and EHS laminin as tracer and standard (F).

TABLE I. Laminin MAb Epitope Mapping

	2D8	4C12	
Lam	+	+	1, 2, 3, 4[a]
Lam (Red and Alk)[b]	0	0	3
P1	0	+	1, 2, 3
E8	0	0	1, 2, 3
B1B2	+	+	3, 4
B1	+	0	4

[a]Reaction (+, positive; 0, negative) in 1, dotimmunoassay; 2, enzyme linked immunoassay; 3, immunoblotting; 4, immunoprecipitation.
[b]Laminin reduced and alkylated.

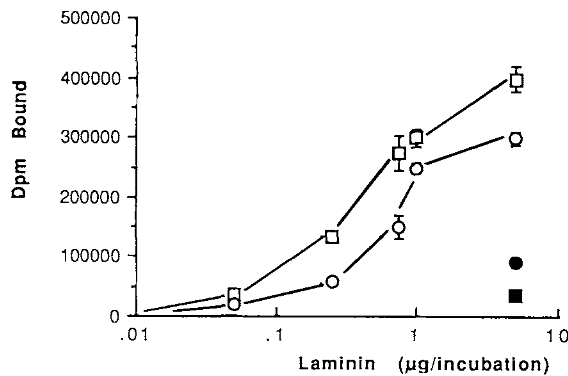

Fig. 2. Immunoattachment assay. Sepharose 6MB beads coupled with antilaminin MAbs-4C12 (squares) or-2D8 (circles) were reacted (18 h, 4°C) with various amounts of purified EHS laminin (µg/incubation). After washing, 50 µl of beads were incubated with 3 × 10^4 RMS S4T cells previously labelled with ^{35}S methionine (1 × 10^6 dpm) (open symbols) or cells plus anti-E8 antibody (5 µg) (closed symbols). After 1 h at 37°C, incubates were centrifuged on a 28% Percoll cushion to separate free cells from cells attached to the beads. Radioactivity bound to the beads was measured (Dpm Bound). Assays were run in triplicate and mean ± SED is shown.

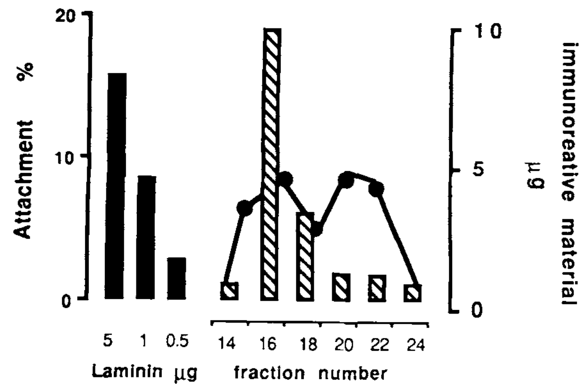

Fig. 3. Attachment of RMS cells to MAb4C12 immunoprecipitates from a gel filtration eluate of PFHR9 cell lysate. A PFHR9 cell extract was gel filtered on Superose 6 (0.9 × 28 cm). Fractions or EHS laminin standards were immunoprecipitated with MAb4C12-Sepharose 6MB beads (0.5 ml). After washing, beads (0.1 ml) were incubated with RMS cells labelled with ^{35}S methionine (1 h, 37°C) or with ^{125}I affinity purified anti-P1 antibodies (2 h, 4°C). After washing on Percoll beads bound radioactivity was measured. Attachment on column eluate (striped bars) or laminin (closed bars) immunoprecipitates is expressed as a percentage of input cells. Binding values of anti-P1 antibody to EHS laminin immunoprecipitates were used as references to calculate the amount of laminin-like material in column fraction immunoprecipitates (curve with closed circles). Experiments were performed in duplicate and standard error to the mean (SEM) (not shown) was less than 10%.

Attachment Properties of the PFHR9 Laminin Species

The attachment rates of RMS cells to MAb 4C12-Sepharose 6MB beads reacted with known amounts of EHS laminin or with gel filtration eluate of PFHR9 cell lysates were compared.

As shown in Figure 3 the immunoprecipitates from fractions eluting at the laminin position supported significant attachment. No attachment was observed with fractions at the B1B2-dimer position, despite the large quantity of immunoreactive material precipitated as shown by binding of ^{125}I-antiP1 IgGs.

A similar experiment was performed with lysates of PFHR9 cells metabolically labelled with ^{35}S methionine. This label enabled the identification of adhesion substrates by SDS-PAGE. MAb 4C12-Sepharose 6MB and MAb 2D8-Sepharose 6MB beads were used to select B1B2-dimers and B1-chains respectively, (fraction 21 and 25, Fig. 1A,C,E). Significant amounts of S35-material was precipitated, which showed a typical migration pattern for B1B2 dimers (Fig. 4C) and B1 chain (Fig. 4F) by SDS-PAGE under reducing and non-reducing conditions. In contrast to EHS laminin, neither B1B2 or B1 immunoprecipitates supported cell attachment (Fig. 4B,E). This was not due to the amount of immunoreactive ligands present, since binding of ^{125}I-MAb 2D8 and ^{125}I-affinity purified anti-P1 IgGs showed respectively that large quantities of B1B2 dimers and B1 chains were captured (Fig. 4A,D). These data, which were confirmed in an independent experiment, showed that the adhesive to immunoreactive activity was far superior for EHS laminin than for B1B2 dimers or B1 chains.

To ascertain these findings, the specific activity for cell attachment of PFHR9 laminin and B1B2-heterodimers was evaluated under dose-response conditions.

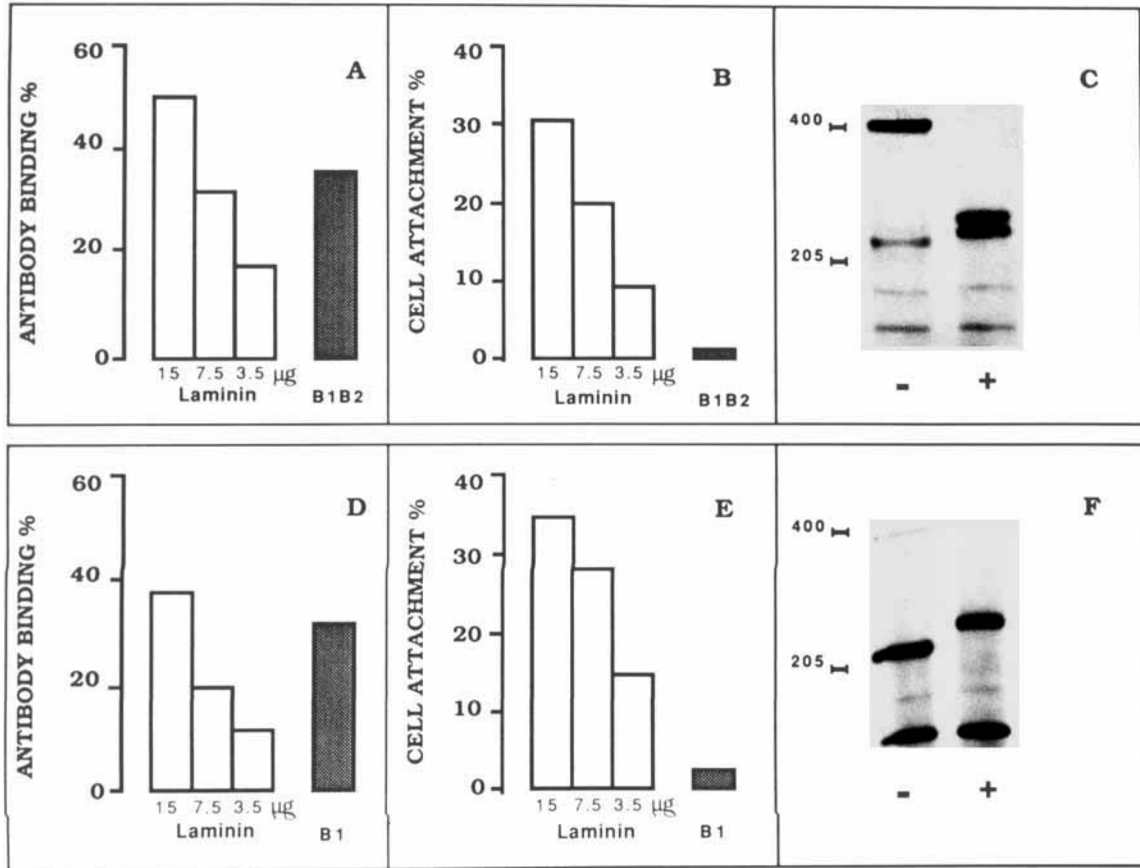

Fig. 4. Antigenic and cell adhesion properties of B1B2 dimers or B1 chains compared with those of purified EHS laminin. Sepharose 6MB beads coupled with Mab 4C12 (**A, B, C**) or with Mab 2D8 (**D, E, F**) were incubated (18h, 4°C), respectively, with B1B2 dimers or B1 chains (fraction 21 and fraction 25 in Fig. 1, panels A, C, E). For comparative purposes each of the sorbents was also incubated with EHS laminin at various concentration (15, 7.5, and 3.5 µg). After the incubation beads were shared in three parts to analyse the antigenic, the cell attachment properties, and the SDS-PAGE migration pattern of captured ligands. A and D show the antigenic properties of the immunoprecipitates analysed by binding of complementary ^{125}I antibodies. Along with this ^{125}I Mab 2D8 (A) and ^{125}I anti P1 IgGs (D) were incubated (2h, 4°C) with Mab 4C12 and Mab 2D8 immunoprecipitates, respectively. After the incubation and washing, the amount of antibody bound to the beads was evaluated by γ scintillation counting and is expressed as a percentage of input material. B and E: The cell attachment properties of the immunoprecipitates was evaluated by incubating the immunoprecipitates with RMS cells (5×10^4 cells, 1 h at 37°C) as indicated in Figure 2. After washing on a Percoll cushion, the amount of cells attached to the beads was evaluated by β scintillation counting and is expressed as a percentage of the radioactivity contained in input cells. Open bars and closed bars in panels A, B, D, and E correspond to the antibody or cell attachment levels to EHS laminin and B1B2 dimers or B1 chain immunoprecipitates, respectively (mean value of duplicate experiments with a standard error less than 5%). C and F: Electrophoretograms of the B1B2 and B1 chain immunoprecipitates analysed by SDS PAGE under nonreducing (−) or reducing (+) conditions and fluorography [numbers in margins correspond to the molecular weight (kDa) and they point at the migration position of the laminin A chain (400) and myosin (205)].

The binding curves obtained in radioimmunoassays between ^{125}I-MAb 2D8 and MAb 4C12 beads incubated with varying amounts of EHS laminin, PFHR9 laminin, or B1B2-dimers were dose dependent and parallel (Fig. 5A). This result was in agreement with the data in Table I showing that the antibodies were directed towards different epitopes present in all three molecules. Precise quantification of the material assayed was therefore possible. As shown in Figure 5B, a same specific activity was found for PFHR9 laminin and EHS laminin when immunoreactivity of the substrates was related to attachment activity. Attachment between RMS and B1B2 heterodimers was significant only at the highest compound dose. In terms of weight,

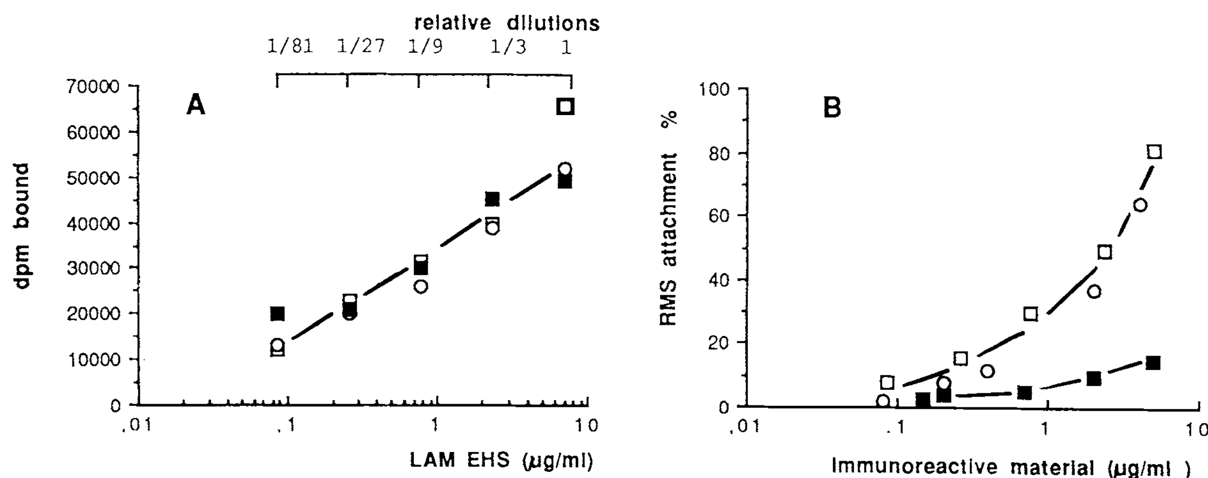

Fig. 5. Specific activity for RMS cell attachment to PFHR9 laminin and B1B2-heterodimers. Serial dilutions of EHS laminin 7 μg (white squares), PFHR9 laminin (white circles) and B1B2-heterodimers (black squares) were reacted with MAb 4C12 Sepharose 6MB beads. After washing, beads were incubated (1h, 37°C) with RMS cells labeled with ^{35}S methionine (15 × 10^{4} cells) or with ^{125}I-MAb 2D8 (2h, 4°C). After incubation and washing on Percoll, bead-bound radioactivity was counted. **A:** Binding levels of ^{125}I-MAb 2D8 to MAb4C12 immunoprecipitates. **B:** Cell attachment specific activity. Experiment was run in triplicate and standard error deviation were less than 10%.

adhesion between RMS and B1B2-heterodimers was 12 times less than between RMS and PFHR9 or EHS laminin.

DISCUSSION

We compared the cell attachment properties of PFHR9 laminin, B1B2-heterodimers, and B1-chains after purification by gel filtration and immunoprecipitation with antilaminin monoclonal antibodies coupled to Sepharose 6MB beads. Immunoprecipitated ligands, quantified by binding with complementary anti-laminin antibodies, were used as substrates for cell attachment.

A high cell attachment was consistently observed with PFHR9 laminin immunoprecipitates. The specific activity for attachment to EHS and PFHR9 laminins did not differ significantly. In contrast, hardly any attachment to B1B2-dimers or B1-chains occurred. The specific activity for attachment between RMS and B1B2-heterodimers was at least 24 times lower on a molar basis than between RMS and laminin. However, attachment to B1B2-dimers was probably overestimated, since gel filtration was only 90% efficient in separating dimers from laminin. Thus, contamination by laminin was sufficient to explain the low attachment levels, despite large quantities of heterodimers. Altogether these data suggest that B1B2-dimers lack the adhesive function of laminin.

Pulse-chase analysis of laminin biosynthesis in the PFHR9 cell line and in other laminin producing cell lines as well indicated that B-chain incorporation in laminin proceeds via the formation of heterodimers which rapidly associate with available A-chains [21–23]. The process is thought to be stochiometric and excess dimers remain free. Therefore, heterodimers may be considered as unassembled intermediates of laminin biosynthesis. Their lack of adhesive activity implies that the heterotrimeric configuration is essential to the adhesive function of laminin.

Our study was conducted with a rat rhabdomyosarcoma cell line highly sensitive to laminin for cell attachment. This attachment did not involve homotypic interaction with cell surface laminin [16] or heterotypic association with cell surface heparansulfate proteoglycans (it was not modified when laminin substrates or substrates and cells were incubated with heparin up to 50 mg/ml [J.C. Lissitzky, unpublished data]). Furthermore, the cells attached 20 times more efficiently to E8 substrates than to P1 coatings and attachment on EHS laminin was blocked by anti-E8 IgGs, but not by anti-P1 antibodies [16]. Thus, laminin mediated adhesion of these cells was mainly dependent on interaction with a domain in the E8 fragment. Therefore, it is likely that the heterotrimeric requirement for laminin adhesive function applies to the attachment domain in the E8 fragment.

Expression of laminin genes is non-coordinated. It has been shown that B-chain genes are expressed earlier during development than A-chain genes [25]. This observation has been particularly well documented in the developing kidney, where B-chain expression predominates until differentiation occurs [26–28]. A-chain expression is triggered when kidney is induced to differentiate and it is contemporaneous with epithelium polarization and basement membrane production. Klein et al. [27] showed that polarization can be blocked by antibodies to the laminin E8 fragment. These findings suggest that the morphological events associated with differentiation implicate the long arm of laminin, which becomes functional the moment A-chains are expressed. Our finding that the heterotrimeric configuration is essential to the adhesive function of E8 is consonant with these observations, since the morphological function of laminin could conceivably involve adhesion receptors and cytoskeleton remodeling to some extent.

Two non-exclusive hypothesis may be put forward to explain the lack of adhesion with B1B2-dimers.

A first, straightforward one would be that one or more A-chain sequences are critical for cell attachment. This hypothesis is corroborated by the recent discovery that a C-terminal A-chain peptide sequence IKVAV supports attachment and spreading of a number of cell lines [29]. Also relevant in this regard are the facts that the P22-3 form of the peptide was found highly adhesive with RMS cells and that the cell lines most reactive with E8, such as RMS, were the most responsive to IKVAV as shown by attachment assays under dose-response conditions (J.C. Lissitzky, unpublished data). Furthermore, the laminin E8 fragment may contain A-chain related attachment sites other than the IKVAV sequence. Indeed, two A-chain dependent adhesion sites have been located in the rod and the terminal globular domain of E8 respectively [30]. Also, the findings that IKVAV binds to a 68,000 Dalton protein in hepatocytes [31] and that E8 mediated attachment of epithelial cells is blocked by monoclonal antibodies to the integrin α6 chain [32] may be an indirect indication that they are multiple attachment sites in the laminin long arm.

Alternatively, however, it is possible that B-dimers or A-chain exhibit latent adhesion sites which become functional due to conformational changes occurring when heterodimers associate with the A-chain. This explanation would fit with the fact that denaturating treatment abolishes the adhesive function of both laminin and E8 [13,30,33], an observation suggesting that attachment domains are conformationally positioned in native molecules. In support with this hypothesis Deutzmann et al. have been able to obtain a highly adhesive complex by recombining two biologically inactive polypeptides purified from trypsin digests of the laminin E8 fragment which corresponded, respectively, to the C-terminus of the A chain and to disulfid linked C-terminals of the B1 and B2 chain [30]. These data converge with our finding to indicate that the heterotrimeric configuration (i.e., dimer to A-chain association) is essential to the adhesive function of laminin.

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