The Human Laminin Receptor Is a Member of the Integrin Family of Cell Adhesion Receptors

KURT R. GEHLSEN, LENA DILLNER, EVA ENGVALL, ERKKI RUOSLAHTI

A receptor for the adhesive basement membrane protein, laminin, was isolated from human glioblastoma cells by affinity chromatography on laminin. This receptor has a heterodimeric structure similar to that of receptors for other extracellular matrix proteins such as fibronectin and vitronectin. Incorporation of the laminin receptor into liposomal membranes makes it possible for liposomes to attach to surfaces coated with laminin. The receptor liposomes also attached to some extent to surfaces coated with fibronectin, but not with other matrix proteins. These properties identify the laminin receptor as a member of the integrin family of cell adhesion receptors.

LAMININ IS A BASEMENT MEMBRANE protein (*1*) that promotes the attachment and migration of cells and plays a role in differentiation and tumor metastasis (*2*). It has a particularly striking effect on neuronal cells promoting and guiding growth of neurites, a property that may function in the repair of nervous tissue injury (*2*). These interactions are thought to be mediated by cell surface receptors for laminin. The putative laminin receptors that have been described are a 67-kD protein (*3*), a 120-kD protein, and a 180-kD protein (*4*). There may also be a cell adhesion receptor for laminin in the integrin family of adhesion receptors (*5, 6*).

A family of transmembrane glycoprotein receptors for extracellular matrix proteins that share common structural and functional characteristics has been identified (*7*). Among the members of this family are the fibronectin and vitronectin receptors (*8, 9*), the very late antigen (VLA) proteins (*5*), chicken integrin complex (*6, 10*), the platelet receptor IIb/IIIa (*11*), and the leukocyte adhesion proteins, LFA-1, Mac-1, and p150,95 (*12*). These receptors are thought to link the cell cytoskeleton to the extracellular environment; thus they have been called integrins.

That the laminin receptor could be an integrin is suggested by the interaction of an isolated chicken integrin complex with laminin (*6*) and the finding that a monoclonal antibody that defines this complex inhibits the formation of neurites by neurons plated on laminin (*13*). Also, a monoclonal antibody to a human integrin, called VLA-3, inhibits the attachment of cells to laminin (*5*). However, whereas the binding of the chicken integrin complex to laminin is reported to be sensitive to the same RGD-containing peptides (*14*) that inhibit the fibronectin and vitronectin receptors (*7*), the attachment of cells to laminin or the extension of neurites by chicken neurons on laminin are not inhibited by such peptides (*15*). In view of these conflicting results, we have applied our affinity chromatography method, previously used to identify and isolate receptors for fibronectin and vitronectin (*8, 9*), to the analysis of the laminin receptors. We fractionated extracts of cell surface–iodinated RuGli cells, a human glioblastoma cell line that attaches well to laminin (*16*), on a laminin fragment–Sepharose affinity matrix. Elution with EDTA yielded, under nonreducing conditions, protein bands of 150 and 120 kD (Fig. 1). These proteins could not be eluted with the RGD-containing peptide having the sequence GRGDSP.

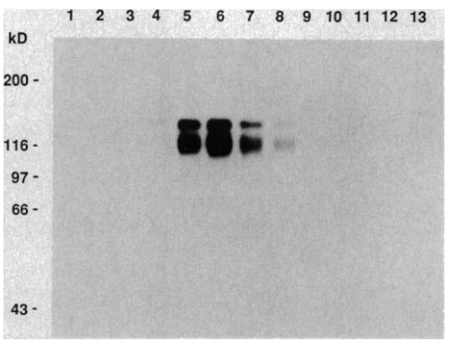

Fig. 1. Affinity chromatography of a RuGli cell extract on human laminin-Sepharose. Confluent RuGli cells were labeled with ^{125}I at the cell surface by the lactoperoxidase method, and extracted with tris-buffered saline, *p*H 7.2, 25 m*M* octyl-β-D-thioglucoside, 1 m*M* $MnCl_2$, and 1 m*M* phenylmethylsulfonylfluoride (extraction buffer). Mn^{2+} was included in the buffer because it facilitates the isolation of the fibronectin receptor by fibronectin affinity chromatography (*20*). Human laminin was isolated from a pepsin extract of placental tissue (*15*), and coupled to Sepharose at 9 mg/ml. The soluble extract from 5 ml of packed cells was applied to a 10-ml laminin-Sepharose column. The cell extract was passed through the column two times, and the column was washed with 30 ml of the extraction buffer. Fractions were eluted with 20 ml of buffer containing the synthetic peptide GRGDSP (2 mg/ml), followed by 20 ml of cation-free buffer containing 20 m*M* EDTA. Samples of each 2-ml fraction were analyzed by electrophoresis on an SDS 7.5% polyacrylamide gel. Protein bands were visualized after overnight autoradiography. Lanes 1 to 13 are fractions from the EDTA elution; no protein was seen in the GRGDSP-eluted fractions. The radioactivity in the fractions eluted with EDTA represented 2 to 3% of the total radioactivity applied to the column.

The 150-kD polypeptide of the EDTA-eluted material dissociated into a 120-kD component and one or two 30- to 35-kD components upon reduction with β-mercaptoethanol, whereas the other polypeptide migrated with an apparent molecular size of 140 kD after reduction (Fig. 2). A fibronectin receptor similar to the previously characterized fibronectin receptor from fibroblasts (*8, 17*) was isolated for comparison from the same RuGli cell extract on a fibronectin affinity matrix. No other labeled proteins were observed after prolonged autoradiography of the gels. A laminin-binding protein with subunits similar to those of the RuGli cell protein was also isolated from human placenta, indicating that this laminin-binding protein is not confined to RuGli cells. The heterodimeric structure of this laminin-binding protein, the disulfide-bonded two-chain composition of its larger subunit, and the increase in apparent molecular size of the smaller subunit upon reduction are all characteristic of the integrins (*7*).

Affinity-purified polyclonal antibodies were used to examine the relation of the laminin-binding integrin to the known integrins, the fibronectin and vitronectin receptors. The antibody to the fibronectin recep-

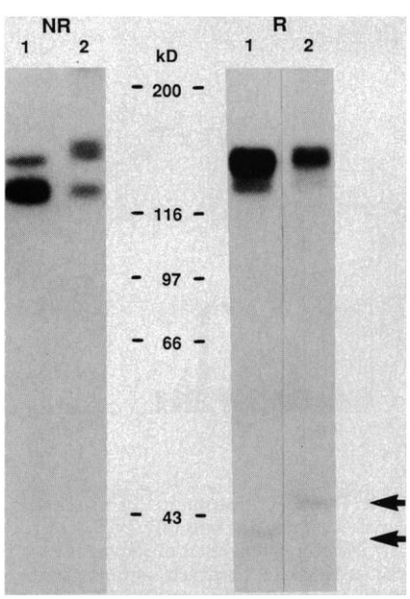

Fig. 2. Electrophoretic analysis of the laminin receptor (lane 1) and the fibronectin receptor (lane 2) under nonreducing (NR) and reducing (R) conditions. An affinity matrix consisting of the fibronectin cell attachment fragment-Sepharose was used to obtain the fibronectin receptor from the same cell extract as the laminin receptor (*8*). The conditions of the electrophoresis were the same as in Fig. 1. Arrows indicate the light chains of the alpha subunits.

Cancer Research Center, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

tor, which reacts with both the alpha and beta subunits of this receptor, cross-reacted with the smaller (beta) subunit of the laminin-binding protein on an immunoblot (Fig. 3), whereas no reactivity was seen with the antibody to the vitronectin receptor. Thus, the laminin-binding integrin is a distinct protein, one subunit of which may be related to the fibronectin receptor beta subunit.

Incorporation of laminin-binding integrin into phosphatidylcholine liposome membranes (*8*) yielded liposomes that adhered strongly to substrates coated with laminin (Fig. 4). These liposomes also bound, although to a lesser extent, to fibronectin, but not to type IV collagen (Fig. 4), and not to substrates coated with vitronectin, fibrinogen, type I collagen, or albumin. In contrast, liposomes made with fibronectin receptor from the same cells bound strongly to fibronectin, but not to laminin. The attachment of the liposomes prepared with the laminin-binding integrin was inhibited by purified chymotrypsin fragments of laminin (*15*), but not by GRGDSP (*7*), nor the laminin peptide YIGSR (*18*). These results show that the laminin-binding integrin can incorporate into the liposome membranes and that it conveys to liposomes the expected binding to laminin.

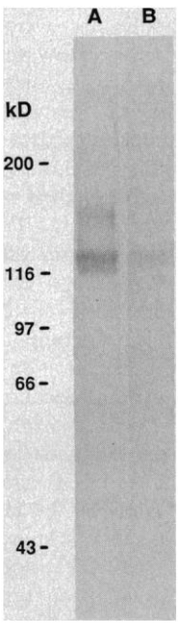

Fig. 3. Immunoblot analysis of laminin receptor. The fibronectin (**A**) and laminin (**B**) receptors isolated from RuGli cells were subjected to electrophoresis under nonreducing conditions and transferred to nitrocellulose filters. The filters were incubated with rabbit antibodies to the fibronectin receptor (*17*), and the bound antibodies were detected with goat antibodies to rabbit immunoglobulin G that were conjugated to horseradish peroxidase.

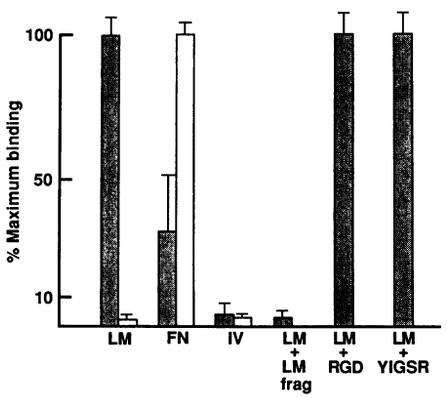

Fig. 4. Binding of laminin receptor-containing liposomes to substrates. Microtiter wells were coated with various proteins (20 μg/ml, in phosphate buffered saline) overnight at 4°C (*15*). Unoccupied binding sites on the plastic were blocked by incubating the wells with bovine serum albumin (BSA) at 5 mg/ml, and the binding or inhibition of binding of receptor liposomes to the wells was tested (*8, 9*). The results of six experiments are expressed as a percentage of maximal binding. The mean plus standard error after subtraction of background from BSA attachment is shown: LM, human laminin; FN, fibronectin; IV, type IV collagen; LM + LM frag., LM + 20 μg of chymotryptic laminin fragments (*15*); LM + RGD, LM + 1 mg/ml GRGDSP; LM + YIGSR, LM + 1 mg/ml YIGSR; fibronectin receptor liposomes (open bars); LMR, laminin receptor liposomes (shaded bars).

The laminin-binding integrin described above is probably the receptor that mediates the adhesion of the RuGli cells to laminin. The specificity of this receptor agrees with the specificity of the attachment of the intact RuGli cells to laminin. Both the cell attachment and the binding of the receptor to laminin require divalent cations, and neither is inhibited by RGD-containing peptides (*15*). The inability of RGD peptides to inhibit binding suggests that the cell-binding site in laminin may be different from the main cell attachment site in fibronectin and in the other RGD-containing adhesive proteins. The laminin receptor is thus similar to the leukocyte adhesion receptors, which are a subfamily of integrins that also bind to their ligands in the presence of RGD-containing peptides (*7, 12*).

That the integrin described above is the laminin receptor is supported by the fact that the laminin fragments used in the receptor isolation appear to contain only one cell attachment and neurite-promoting site (*15*). This site is located in a different region of the laminin molecule from that of the YIGSR site, which is thought to bind the 67-kD receptor (*18*). Accordingly, we found no indication of a surface-labeled 67-kD protein in our affinity chromatography fractions, and the YIGSR peptide did not inhibit the binding of this receptor to laminin.

Furthermore, that the laminin receptor is an integrin related to the fibronectin receptor agrees with the previous results that antibodies to integrins inhibit cell attachment and neurite outgrowth on laminin (*5, 13*). It also agrees with the demonstrated direct binding of a chicken integrin complex to laminin (*6*), although that binding could be inhibited by concentrations of RGD-containing peptides that do not affect the binding of the human integrin to laminin. The reason for this difference may be that our receptor is not the human homologue of the laminin-binding integrin in the chicken integrin complex. The human counterpart is thought to be VLA-3 (*5*), but VLA-3 appears to be different from the laminin receptor we describe here by the criteria of alpha subunit NH_2-terminal amino acid sequence (*19*) and receptor binding specificity. Antibody inhibition of cell attachment suggests that VLA-3 binds to fibronectin, type IV collagen and laminin (*5*), whereas our laminin receptor fails to bind to type IV collagen and binds only marginally to fibronectin. Taken together, these results show that the laminin adhesion receptor is an integrin and that possibly two laminin receptors, one specific for laminin and another capable of recognizing laminin along with other proteins may exist. The effects of laminin on the adhesion and differentiation of a variety of cells, especially neuronal cells, make laminin and its receptor a particularly interesting adhesion system.

REFERENCES

1. R. H. Timpl *et al.*, *J. Biol. Chem.* **254**, 9933 (1979); A. E. Chung *et al.*, *Biochem. Biophys. Res. Commun.* **79**, 859 (1977); G. R. Martin and R. Timpl, *Annu. Rev. Cell. Biol.* **3**, 57 (1987).
2. V. P. Terranova *et al.*, *Cancer Res.* **42**, 2265 (1982); M. Manthorpe *et al.*, *J. Cell Biol.* **97**, 1882 (1983); S. L. Rodgers *et al.*, *ibid.* **98**, 212 (1983); A. Baron von Evercooren *et al.*, *J. Neurosci. Res.* **8**, 179 (1982); D. Edgar, R. Timpl, H. Thoenen, *EMBO J.* **3**, 1463 (1984); G. E. Davis *et al.*, *Science* **236**, 1106 (1987); S. L. Goodman *et al.*, *Biochem. Biophys. Res. Commun.* **111**, 804 (1983).
3. M. L. Malinoff and M. S. Wicha, *J. Cell Biol.* **96**, 1475 (1983); V. P. Terranova *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 444 (1983); U. M. Wewer *et al.*, *ibid.* **83**, 7137 (1986).
4. H. K. Kleinman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1282 (1988); N. R. Smalheiser and N. B. Schwartz, *ibid.* **84**, 6457 (1987).
5. M. E. Hemler *et al.*, *J. Biol. Chem.* **262**, 3300 (1987); Y. Takada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3239 (1987).
6. A. F. Horwitz *et al.*, *J. Cell Biol.* **101**, 2134 (1985).
7. E. Ruoslahti and M. D. Pierschbacher, *Science* **238**, 491 (1987); R. O. Hynes, *Cell* **48**, 549 (1987).
8. R. Pytela *et al.*, *Cell* **40**, 191 (1985).
9. R. Pytela, M. D. Pierschbacher, E. Ruoslahti, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5766 (1985).
10. C. A. Buck *et al.*, *J. Cell Biol.* **103**, 2421 (1986).
11. L. A. Fitzgerald, B. Steiner, S. C. Rall, S. S. Lo, D. R. Phillips, *J. Biol. Chem.* **262**, 3936 (1987).
12. D. E. Staunton *et al.*, *Cell* **52**, 925 (1988); T. K. Kishimoto *et al.*, *ibid.* **48**, 681 (1987).
13. D. E. Hall *et al.*, *J. Cell Biol.* **104**, 623 (1987); K. J. Tomaselli *et al.*, *ibid.* **105**, 2347 (1987).
14. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E. Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
15. E. Engvall *et al.*, *J. Cell Biol.* **103**, 2457 (1986); L. Dillner *et al.*, *Exp. Cell Res.*, in press.
16. S. L. Goodman and D. Newgreen, *EMBO J.* **4**, 2769 (1985).
17. W. S. Argraves *et al.*, *J. Cell Biol.* **105**, 1183 (1987).
18. J. Graf *et al.*, *Cell* **48**, 989 (1987).
19. K. R. Gehlsen, E. Ruoslahti, unpublished results.
20. J. Gailit and E. Ruoslahti, *J. Biol. Chem.*, in press.

21. Supported by grants CA42507, CA28896, DK30051, the Cancer Center Support grant CA30199 and Fellowship GM12228 to K.R.G. from the NIH, DHHS.

12 May 1988; accepted 21 July 1988