IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY
Volume 21, Number 1, January 1985
© 1985 Tissue Culture Association, Inc.

IMMUNOCYTOCHEMICAL LOCALIZATION OF LAMININ IN RAT ANTERIOR PITUITARY CELLS IN VIVO AND IN VITRO

C. TOUGARD, D. LOUVARD, R. PICART AND A. TIXIER-VIDAL

Groupe de Neuroendocrinologie Cellulaire, College de France, 75231 Paris Cedex 05, France, and Unité de Biologie des Membranes(D.L.), Institut Pasteur, 75724 Paris Cedex 15, France

(Received 10 December 1984; editor G.H. Sato)

SUMMARY

The distribution of laminin was investigated by immunocytochemistry in the rat anterior pituitary in vivo and in primary culture. It was localized by immunofluorescence and by immunoperoxidase in the basement membranes of the pituitary in vivo. In addition it was also found inside glandular cells both in vivo and in culture. The number of immunoreactive cells greatly varied depending on the technical approach used. It was always higher in primary cultures than in vivo. At the electron microscope level, a staining was observed on secretory granules, on rough endoplasmic reticulum cisternae as well as on the membrane of some Golgi saccules and vesicles. Such a localization, at the level of subcellular sites involved in the secretory process, suggests that these cells are able to synthesize and to export in vivo as well as in vitro this component of their basement membranes.

Keywords: laminin; anterior pituitary; primary cultures; immunocytochemistry.

INTRODUCTION

Laminin, one of the major glycoprotein components of the basement membrane, has been detected biochemically and immunocytochemically in various tissues or organs (3,4,9,11,14,17). Moreover, it has been shown to be produced by certain epithelial cell lines in culture (17), by parietal endoderm cells (8), by neuroblastoma cells (1) and by brain fetal astrocytes grown in primary culture in presence (13) or absence of serum (6).

Glandular anterior pituitary cells are organized in epithelial cords surrounded by a parenchymal basement membrane (7,16). However the components of this basement membrane have not been identified so far.

We have localized by immunocytochemistry laminin in the basement membrane of the rat anterior pituitary in vivo. In addition, laminin was detected intracellularly inside some glandular cells of the pituitary in vivo as well as in primary culture.

MATERIALS AND METHODS

Antibodies. Affinity-purified antibodies to laminin were prepared from antisera taken from rabbits immunized against pure antigens isolated from the mouse EHS sarcoma (17). We also used goat affinity-purified antibodies prepared in the laboratory of G. Martin (Bethesda) and kindly provided by M. Vigny (Paris).

Pituitaries. Pituitaries from adult male Wistar rats (180 to 200 g weight) were removed and fixed by immersion in 4% formaldehyde.

Primary cultures. Cells were obtained by enzymatic dispersion of adult male Wistar rats (180 to 200 g weight) anterior pituitaries and cultured for 6 d in serum supplemented medium (Ham's F10 solution supplemented with 10% horse serum, 2.5% fetal calf serum and antibiotics) as previously described (20).

Immunofluorescence microscopy. A. 0.5 μm frozen sections were cut from 4% formaldehyde-fixed, sucrose infiltrated pituitaries according to Tokuyasu (18) using a Sorvall MT-2B ultramicrotome equipped with an LTC 2

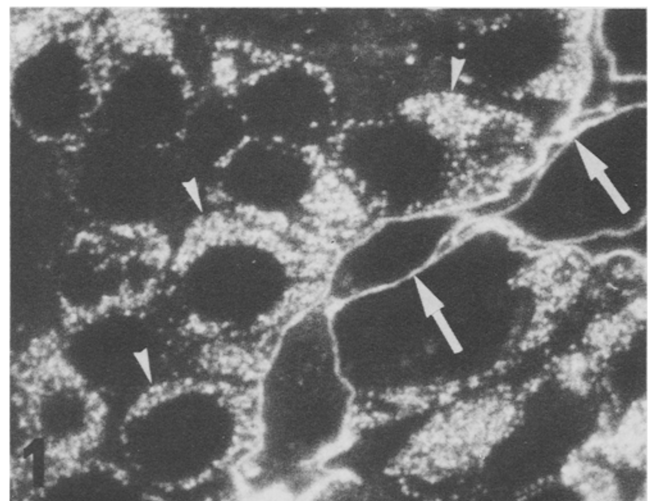

FIG. 1. Detection of laminin by immunofluorescence on 0,5 μm frozen sections of formaldehyde-fixed rat anterior pituitary. Laminin was found on the basement membranes (arrows) and within glandular cells (arrowheads) apparently concentrated on secretory granules (×1419).

adapter for ultracryomicrotomy. They were incubated with antibodies against laminin, and then with complementary second antibodies conjugated to rhodamine. *B.* Cells in primary cultures were fixed in situ with 4% formaldehyde and stained using the procedure described by Ash et al. (2).

Electron microscope immunoperoxidase labeling. A, 25-50 μm thick sections of 4% formaldehyde-fixed pituitaries were cut, without freezing, using a Sorvall TC2 sectioner and labeled with antibodies against laminin using an indirect immunoperoxidase technique followed by postfixation and embedding in araldite as previously described (19). *B,* Cells in primary cultures were fixed in situ with 4% formaldehyde, or with 0.4% glutaraldehyde in hypotonic phosphate buffer (0.01M) according to Ohtsuki et al. (15) or with a mixture of 2% formaldehyde and 0.05% glutaraldehyde as previously described (20). The immunocytochemical staining was performed direct-

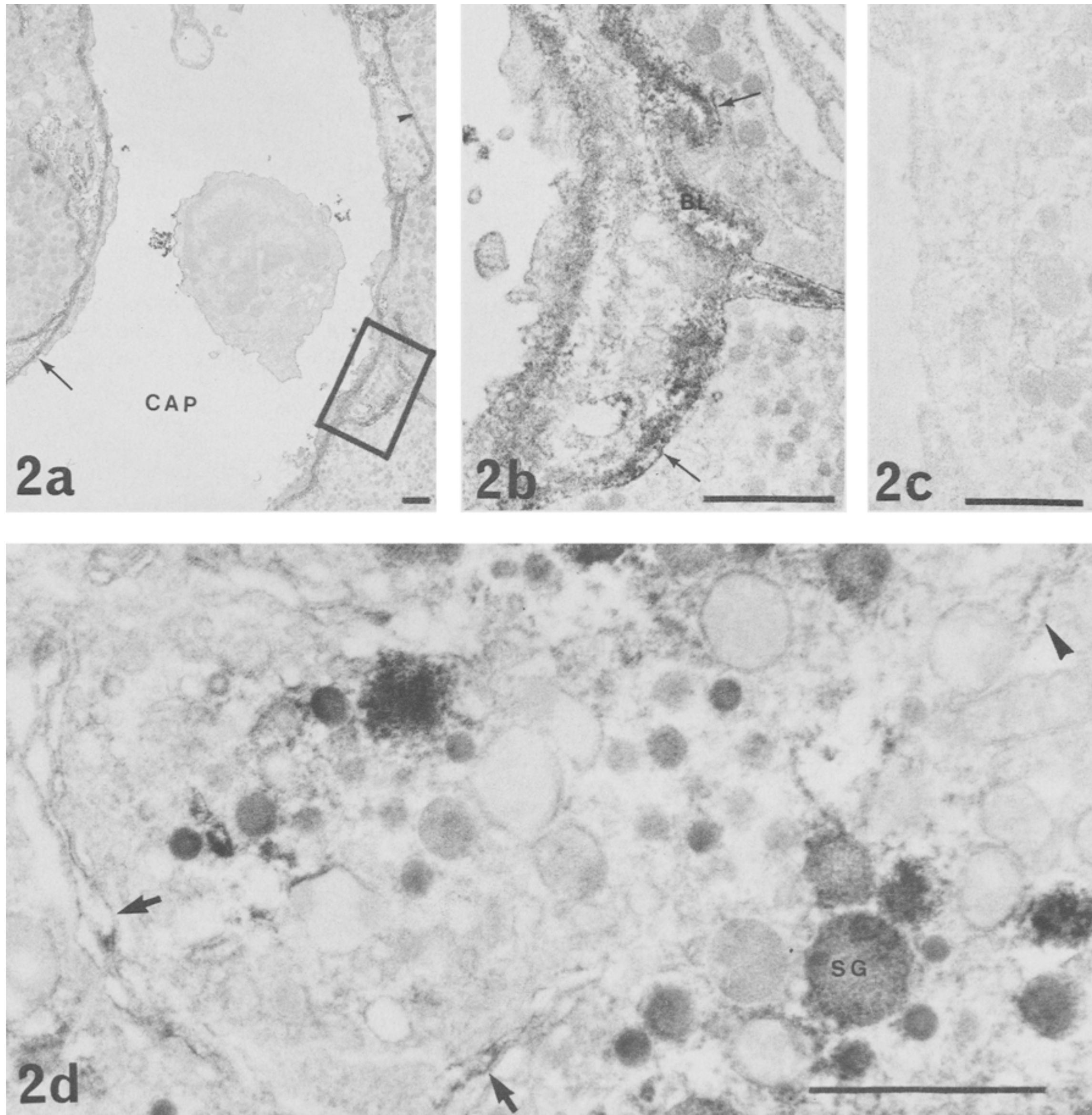

FIG. 2. Detection of laminin by electron microscope immunoperoxidase on unfrozen sections of formaldehyde-fixed rat anterior pituitary. a, At a low magnification, laminin was found on the basement membranes of both the capillary endothelium (arrows) and the glandular parenchyme (arrowheads). Cap: capillary. Bar: 1 μm (×3520). b, Detail of a. At a higher magnification, reaction product was located at the cell surface (arrows) as well as on a more external layer which may correspond to the "basal lamina" (BL) already visible by conventional electron microscopy. Bar: 1 μm (×18 150). c, In control conditions (non-immune immunoglobulins instead of antibodies against laminin) the basement membranes were unstained. Bar: 1 μm (×18 150). d, Detail of the Golgi zone of a cell which displays the ultrastructural features of a gonadotropic cell. Laminin was found on some secretory granules (SG), on the membrane of some rough endoplasmic reticulum cisternae (arrowhead) and on the membrane of some Golgi saccules (arrows). Bar: 1 μm (×31 900).

ly in the culture dishes using permeabilization with saponin and the indirect immunoperoxidase technique as previously described (20).

Controls. To test the specificity of the immunocytochemical reaction, several controls were performed: — non immune rabbit or goat immunoglobulins were used instead of antibodies against laminin — incubation with peroxidase or rhodamine conjugated second antibody only.

RESULTS AND DISCUSSION

Localization of laminin in the anterior pituitary in vivo. Whatever the technical approach, laminin was detected on the basement membranes and within glandular cells. These structures were unstained in control conditions using immunofluorescence as well as immunoperoxidase techniques at the light and electron microscope level (Fig. 2c).

The basement membranes were strongly labeled by the immunofluorescence method (Fig. 1). After immunoperoxidase staining, at the electron microscope level, laminin was detected on the basement membranes of both the capillary endothelium and the glandular parenchyme (Fig. 2a). The reaction product was located at the cell surface as well as on a more external layer which may correspond to the "lamina densa" or "basal lamina" already visible by conventional electron microscopy (7,16)(Fig. 2b). As already pointed out by other authors (5,9) for the immunocytochemical localization of extracellular matrix antigens, the labeling of cell membranes that we observed should be considered with caution.

As concerns the intracellular localization of laminin in glandular cells, the number of stained cells greatly varied with the immunocytochemical procedure. By the immunofluorescence method on frozen thick sections laminin was found in a great number of glandular cells, apparently concentrated on secretory granules (Fig. 1). After immunoperoxidase staining on unfrozen sections (araldite embedded), the number of positive cells was lower. At the electron microscope level the reaction product was mainly detected within secretory granules, mostly in cells which displayed the ultrastructural features of gonadotropic cells (Fig. 2c). Moreover a light immunostaining was also observed on the membrane of the rough endoplasmic reticulum (RER) cisternae as well as on the membranes of some Golgi saccules and of some vesicles (Fig. 2c).

The localization of laminin on pituitary basement membranes is consistent with previous studies performed on various organs (4,9). The intracellular localization of laminin within glandular pituitary cells suggests that these cells are able to produce a component of their basement membrane. Such an intracellular immunostaining of laminin has been previously described in embryonic cells such as those of the parietal yolk sac where laminin was detected all along the usual secretory protein pathway (10,12).

Localization of laminin in anterior pituitary cells in primary cultures. By both immunofluorescence and immunoperoxidase staining of intact permeabilized cells an intense reaction was observed in many glandular cells (Fig. 3) whereas a less intense staining was seen on flattened fibroblasts, mainly in a juxtanuclear zone.

The subcellular localization of laminin within glandular cells varied depending on the fixation procedure. After fixation with formaldehyde, the staining could be detected on secretory granules only (not shown). After Ohtsuki fixative and formaldehyde-glutaraldehyde mixture the content of the RER cisternae and the membrane of some Golgi saccules and vesicles were immunostained (Fig. 4a,b). At least, in these conditions of culture, cells do not form a basement membrane. However the plasma membrane was punctuated with reaction product (Fig. 4a). Appropriate controls were performed on nitrocellulose filters to assure that antibodies against laminin are not cross-reacting with serum components. Thus the staining at the cell surface can be considered as a specific reaction for laminin. Such immunoreactive structures were found in several cell types including prolactin cells according to their ultrastructural organization. Similar variations in the staining of subcellular sites depending on the procedure used were previously described for a hormonal product, prolactin, in the same culture model (20).

In conclusion, these results reveal for the first time the presence of laminin not only in basement membranes of adult rat anterior pituitary, but also in numerous glandular cells, at the level of subcellular sites involved in the secretory process, suggesting that these cells are able to synthesize and to export in vivo as well as in vitro this component of basement membranes. The results obtained in vitro suggest, moreover, that laminin plays an important role in the macromolecular organization of the extracellular matrix of pituitary cells in culture.

Further studies are in progress to elucidate more precisely the nature of glandular cell types involved in the synthesis of laminin as well as to investigate the possible role of laminin in the secretory process of cultured anterior pituitary cells.

FIG. 3. Detection of laminin by immunofluorescence on intact, formaldehyde-fixed, permeabilized rat anterior pituitary cells in primary culture. An intense reaction was found in the cytoplasm of numerous glandular cells (arrows)(×929).

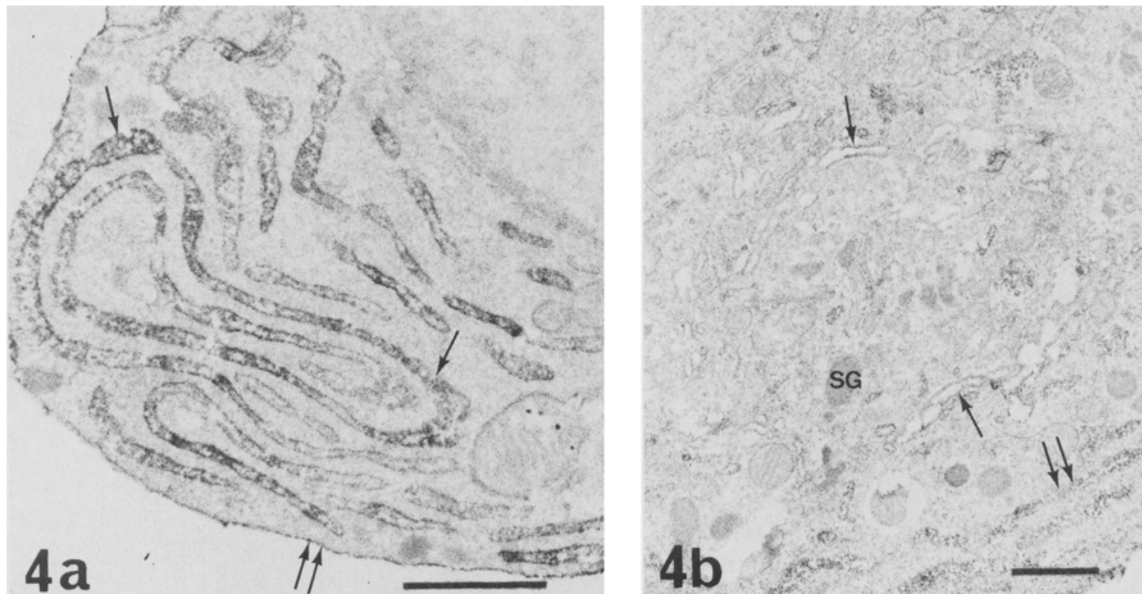

FIG. 4. Detection of laminin by electron microscope immunoperoxidase on intact and permeabilized rat glandular pituitary cells in primary culture. a, After fixation with 2% formaldehyde-0,05% glutaraldehyde the membrane and the content of the rough endoplasmic reticulum cisternae (arrows) were immunostained. Moreover, the plasma membrane (double arrows) was punctuated with reaction product. Bar: 1 μm (×18 810). b, Detail of a Golgi zone of a presumptive prolactin cell fixed with 0,4% glutaraldehyde in hypotonic buffer. A light reaction product was found on the membrane of some Golgi saccules (arrows). The content of the rough endoplasmic reticulum cisternae (double arrows) was immunostained. With these fixatives secretory granules (SG) were not stained. Bar: 1 μm (×11 400).

REFERENCES

1. Alitalo, K.; Kurkinen, M.; Vaheri, A.; Virtanen, I.; Rohde, H.; Timpl, R. Basal lamina glycoproteins are produced by neuroblastoma cells. Nature 287: 465–466; 1980.

2. Ash, J. F.; Louvard, D.; Singer, S. J. Antibody-induced linkages of plasma membrane proteins to intracellular actomyosin-containing filaments in cultured fibroblasts. Proc. Natl. Acad. Sci. USA 74: 5584–5588; 1977.

3. Chung, A. E.; Jaffe, R.; Freeman, L. L.; Vergnes, J. P.; Braginski, J. E.; Carlin, B. Properties of a basement membrane-related glycoprotein synthesized in culture by a mouse embryonal carcinoma derived cell line. Cell 16: 277–287; 1979.

4. Courtoy, P. J.; Timpl, R.; Farquhar, M. G. Comparative distribution of laminin, type IV collagen, and fibronectin in the rat glomerulus. J. Histochem. Cytochem. 30: 874–886; 1982.

5. Courtoy, P. J.; Picton, D. H.; Farquhar, M. G. Resolution and limitations of the immunoperoxidase procedure in the localization of extracellular matrix antigens. J. Histochem. Cytochem. 31: 945–951; 1983.

6. Faivre-Bauman, A.; Puymirat, J.; Loudes, C.; Barret, A.; Tixier-Vidal, A. Laminin promotes attachment and neurite elongation of fetal hypothalamic neurons grown in serum-free medium. Neurosci. Letters 44: 83–89; 1984.

7. Farquhar, M. G. Fine structure and function in capillaries of the anterior pituitary gland. Angiology 12: 270–292; 1961.

8. Hogan, B. L. M.; Cooper, A. R.; Kurkinen, M. Incorporation into Reichert's membrane of laminin-like extracellular proteins synthesized by parietal endoderm cells of the mouse embryo. Developm. Biol. 80: 289–300; 1980.

9. Laurie, G. W.; Leblond, C. P.; Martin, G. R. Localization of type IV collagen, laminin, heparan sulfate proteoglycan and fibronectin to the basal lamina of basement membranes. J. Cell Biol. 95: 340–344; 1982.

10. Laurie, G. W.; Leblond, C. P.; Martin, G. R.; Silver, M. H. Intracellular localization of basement membrane precursors in the endodermal cells of the rat parietal yolk sac. III. Immunostaining for laminin and its precursors. J. Histochem. Cytochem. 30: 991–998; 1982.

11. Laurie, G. W.; Leblond, C. P.; Martin, G. R. Light microscopic immunolocalization of type IV collagen, laminin, heparan sulfate proteoglycan, and fibronectin in the basement membranes of a variety of rat organs. Amer. J. Anat. 167: 71–82; 1983.

12. Laurie, G. W.; Leblond, C. P. What is known of the production of basement membrane components. J. Histochem. Cytochem. 31: 159–163; 1983.

13. Liesi, P.; Dahl, D.; Vaheri, A. Laminin is produced by early rat astrocytes in primary culture. J. Cell Biol. 96: 920–924; 1983.

14. Madri, J. A.; Roll, F. J.; Furthmayr, H.; Foidart, J. M. Ultrastructural localization of fibronectin and laminin in the basement membranes of the murine kidney. J. Cell Biol. 86: 682–687; 1980.

15. Ohtsuki, I.; Manzi, R. M.; Palade, G. E.; Jamieson, J. D. Entry of macro-molecular tracers into cells fixed with low concentrations of aldehydes. Biol. Cell. 31: 119–126; 1978.

16. Olivier, L.; Vila-Porcile, E. Les lames basales des cordons épithéliaux du lobe antérieur de l'hypophyse chez le Rat. C. R. Soc. Biol. 166: 1413–1416; 1972.

17. Timpl, R.; Rohde, H.; Robey, P. G.; Rennard, S. I.; Foidart, J. M.; Martin, G. R. Laminin-A glycoprotein from basement membranes. J. Biol. Chem. 254: 9933-9937; 1979.
18. Tokuyasu, K. T. A technique for ultracryotomy of cell suspension and tissues. J. Cell biol. 57: 551-565; 1973.
19. Tougard, C.; Kerdelhué, B.; Tixier-Vidal, A.; Jutisz, M. Light and electron microscope localization of binding sites of antibodies against ovine luteinizing hormone and its two subunits in rat adenohypophysis using peroxidase labeled antibody technique. J. Cell Biol. 58: 503-521; 1973.
20. Tougard, C.; Picart, R.; Tixier-Vidal, A. Electron-microscopic cytochemical studies on the secretory process in rat prolactin cells in primary culture. Amer. J. Anat. 158: 471-490; 1980.

We thank Drs. M. Vigny and G. R. Martin for the gift of goat affinity-purified antibodies to laminin, Dr. H. Reggio for his helpful advice in ultracryomicrotomy, E. Rosenbaum for her technical assistance, C. Pennarun for the micrographs and A. Bayon for typing the manuscript.

This work was supported by grants from CNRS (Grant E.R. 89 and ATP "Pharmacologie des Récepteurs des Neuromediateurs"). Part of this work was performed at the EMBL (Heidelberg) during a short stay of C. Tougard (supported by an EMBO short term fellowship).

EDITOR'S STATEMENT

This paper documents the interesting observation that glandular cells from anterior pituitary contain laminin in their basement membranes and also apparently synthesize and secrete this extracellular matrix component.

Gordon H. Sato