Virchows Arch (1997) 431:329–335

ORIGINAL ARTICLE

K. Murray · J.M. de Lera · A. Astudillo
A.M. McNicol

Organisation of basement membrane components in the human adult and fetal pituitary gland and in pituitary adenomas

Received: 17 February 1997 / Accepted: 17 May 1997

Abstract Cell–matrix interactions undoubtedly have a role in the development and maintenance of the complex nonrandom structure of the human pituitary gland. We have extended previous studies by documenting the patterns of immunoreactivity for type IV collagen, laminin and fibronectin in the fetal gland, comparing these with the adult patterns. In both we have examined the differences between the anterior lobe and intermediate zone in an attempt to elucidate the apparent differences in functional response between corticotrophs in the two areas. We have also examined expression of these proteins in a series of pituitary adenomas. Finally, we have immunolocalised β_4 integrin, a component of the $\alpha_6\beta_4$ laminin receptor, in the adult gland and in adenomas. In the anterior lobe of the adult gland, type IV collagen and laminin were present in both epithelial and vascular basement membrane. Fibronectin was related to the basement membrane but showed a less continuous distribution. β_4 Integrin was expressed on the basal aspects of pituitary cells, in association with laminin, suggesting that this did identify the $\alpha_6\beta_4$ laminin receptor. In addition, immunoreactivity was present on the lateral margins of some pituitary cells, which might indicate a role in cell–cell adhesion. None of the proteins showed specific association with any particular cell type, suggesting that these specific interactions do not regulate differentiation. This pattern of expression had developed in the fetal gland by the second trimester, with expression relating to vessels preceding that in epithelial basement membrane. Type IV collagen, laminin and fibronectin were also expressed in epithelial and vascular basement membrane in the intermediate zone of the adult gland, and around Rathke's cleft in the fetal gland. However, the organisation differed, with larger groups of cells enclosed within a single basement membrane. Possible vascular connections demonstrated between the posterior lobe and the intermediate zone would permit access of posterior lobe hormones to this zone. Our data confirmed disruption of expression in pituitary adenomas, type IV collagen, laminin and β_4 integrin having a mainly perivascular distribution, with more variable immunoreactivity for fibronectin.

Key words Pituitary gland · Anterior lobe · Intermediate zone · Adult vs fetal · Adenomas

K. Murray (✉) · A.M. McNicol
University Department of Pathology,
Glasgow Royal Infirmary University NHS Trust,
Castle Street, Glasgow G4 0SF, UK
Fax: (44) 141-211-4884

J.M. de Lera
Department of Anatomical Pathology,
Hospital Materno-Infantil, Las Palmas de Gran Canaria,
Spain

A. Astudillo
Department of Pathological Anatomy,
Hospital Central de Asturias, Oviedo, Spain

Introduction

There is increasing evidence for the importance of cell–matrix interactions in the physiological regulation of cell proliferation and differentiation, and much of the breakdown of normal structure in neoplasia is related to abnormal interactions [1–4]. Basement membrane proteins, including type IV collagen and laminin, are particularly important in this respect. Adhesion molecules are also involved [5], among them the fibronectin (FN) family. These interactions are effected via cell surface receptors, which include the integrins, a family of heterodimeric proteins composed of α- and β-subunits [6], members of which have been shown to bind type IV collagen, laminin and fibronectin.

The human pituitary gland is a complex organ, which develops from Rathke's pouch. In the adult, the hormone-secreting cells are arranged in a nonrandom manner within the anterior lobe and in the intermediate zone. What regulates the development and maintenance of these patterns is unclear, but a role for specific cell–matrix interactions is possible. Little is known of

the distribution of matrix components and whether their production is related to specific cell types. Studies in the rat have led to reports of laminin immunoreactivity. Some suggest that this is related mainly to gonadotrophs [7], while others report immunoreactivity in all cells except somatotrophs [8]. Type IV collagen has also been localised [9], and fibronectin has been reported in folliculostellate cells [10]. In the human gland, laminin, type IV collagen and fibronectin have been reported in epithelial and endothelial basement membrane [11]. A single in vitro study on hamster pituitary cells suggests that basement membrane may influence hormone secretion [12]. Even less is known of the expression of integrins. β_1 Integrins have been identified on rat anterior pituitary cells [13] and immunoreactivity for α_2, α_3, α_6, β_1, and β_4 integrin subunits in human pituitary cells [14].

We have therefore investigated these relationships further by documenting and comparing patterns of immunoreactivity for laminin, type IV collagen and fibronectin in fetal pituitary gland and comparing these with patterns in the adult gland. We have also compared the organisation of basement membrane in the anterior lobe with that of the intermediate zone (adult) or lobe (fetal), in an attempt to elucidate the observed functional differences between corticotrophs in the two zones. We have also sought to confirm the expression of integrin β_4 subunit in the adult gland [14] and, assuming that it will localise the $\alpha_6\beta_4$ laminin receptor, to compare its distribution with laminin. Finally, we have examined a series of pituitary adenomas to confirm the published data indicating disruption of normal expression [11,15–17].

Materials and methods

Study 1

Pituitary glands were obtained at autopsy from five adults with no evidence of endocrine dysfunction and from fetuses at varying periods of gestation (less than 20 weeks, 20–25 weeks, 25–30 weeks, 30–35 weeks, over 35 weeks; four in each group). Thirty-five biopsy specimens of pituitary adenomas were also studied, comprising all the common subtypes. All were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections 5 μm thick were cut and mounted on slides coated with aminopropylethoxysilane (Sigma).

All specimens were immunostained using an indirect immunoperoxidase with primary antibodies to type IV collagen, laminin and fibronectin. Sections were also stained using antibodies to all classic pituitary hormones and to S100 protein to identify folliculostellate cells as previously described [18]. Details of staining procedures are shown in Table 1. Controls included omission of primary antibody and substitution by nonimmune serum. Specificity of the hormonal antibodies has been validated by blocking studies on normal glands.

Study 2

Five normal adult pituitary glands were obtained at autopsy, and 22 biopsy specimens of pituitary adenomas comprising all the common subtypes. All tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections 5 μm thick were cut and mounted on slides coated with aminopropyltriethoxysilane (Sigma).

Methods

All specimens were stained by an indirect immunoperoxidase method using a mouse monoclonal antibody to laminin (Sigma) and by the labelled streptavidin biotin method using mouse monoclonal antibody to the β_4 subunit of integrin (kindly donated by Oakridge National Laboratory, USA). Finally, sections of normal pituitary were double stained using an indirect alkaline phosphatase method to localise pituitary hormones, and then with a labelled streptavidin biotin method to demonstrate β_4 integrin subunit. Controls were as before.

Table 1 Antibodies used in the study

Antibody	Type	Supplier	Dilution	Method	Pretreatment
ACTH	P	Dako	1:300	Indirect	None
FSH	P	NIDDK	1:10	Indirect	None
LH	P	NIDDK	1:200	Indirect	None
βTSH	M	SAPU	1:1000	Indirect	None
Glycoprotein hormone α subunit	M	Serotec	1:2000	Indirect	None
GH	P	Dako	1:500	Indirect	None
Prolactin	M	Serotec	1:300	Indirect	None
S100 protein	P	Dako	1:300	Indirect	Trypsin, 10 min
Type IV collagen	M	Serotec	1:100	Indirect	Protease, 60 min
Laminin	M	Sigma	1:500	LSB	Protease, 5 min
Fibronectin	M	Dako	1:200	Indirect	Protease, 20 min
β4 integrin	R	Oakridge National Laboratory USA (gifted)	1:1000	LSB	Trypsin 30secs Microwave 25 min
Goat anti-rabbit Ig-HRP or AP	P	Dako	1:50	–	–
Goat anti-mouse Ig-HRP or AP	P	Dako	1:50	–	–
Biotinylated-anti-rat Ig	P	Vector	1:200	–	–
Labelled streptavidin	-	Boehringer	1:100	–	–

(ACTH adrenocorticotrophic hormone, *FSH* follicle stimulating hormone, *LH* luteinising hormone, *βTSH* thyroid stimulating hormone β subunit, *GH* growth hormone. *M* mouse monoclonal, *R* rat monoclonal, *P* rabbit polyclonal, *Ig* immunoglobulin, *HRP* horseradish peroxidase, *AP* alkaline phosphatase, *NIDDK* National Institute of Diabetes and Digestive and Kidney Diseases, *SAPU* Scottish Antibody Production Unit, *Indirect* indirect immunoperoxidase technique, *LSB* labelled streptavidin biotin technique, *Trypsin* 0.1% solution, protease 12.5 mg per 100 ml, *Microwave* in citrate buffer pH 6.0 at full power in 600-W oven)

Results

Study 1

Adult pituitary

In the anterior lobe, immunoreactivity for type IV collagen was related mainly to vascular channels, or outlined alveolar or trabecular groups of pituitary cells. These were variable in size, and some appearances suggested a complex three-dimensional arrangement (Fig. 1). At high power, a double layer could be identified in places. Laminin positivity followed a similar pattern, but was less intense and sometimes discontinuous. Fibronectin showed a more patchy distribution. No cellular staining was seen for type IV collagen or laminin, but positivity for fibronectin was identified – this was not related to any specific cell type as assessed by comparison of adjacent sections. None of the three showed preferential localisation to areas rich in particular hormone-secreting cell types.

In the intermediate zone, the relative intensities and localisation of all three proteins were similar to those in the anterior lobe. However, the organisation was different (Fig. 2). Cells were arranged in large trabeculae along the axis parallel to the anterior/posterior junction. There were vascular channels on the anterior and posterior margins of these groups, but little evidence of vascularisation of the main cell mass.

Fetal pituitary

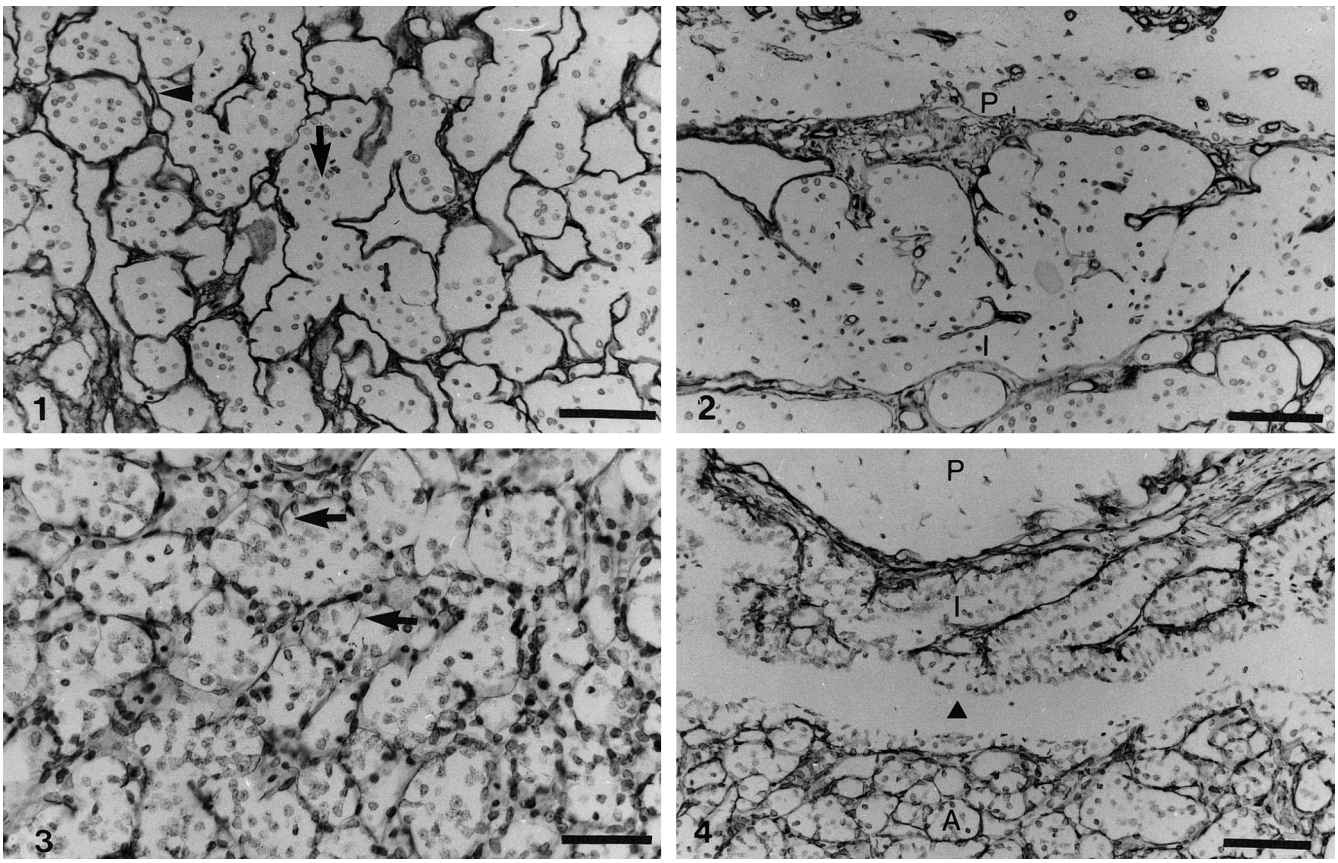
Below 20 weeks, immunoreactivity for type IV collagen was present around vascular channels, delineating small groups of pituitary cells, with projections between cells (Fig. 3). This evolved to a more adult distribution by term. Laminin immunoreactivity was similar, but the intercellular extension of immunoreactivity was not seen until around 25 weeks. Fibronectin again showed a focal distribution. Endothelial cells were immunoreactive for both laminin and fibronectin. Pituitary cells were negative.

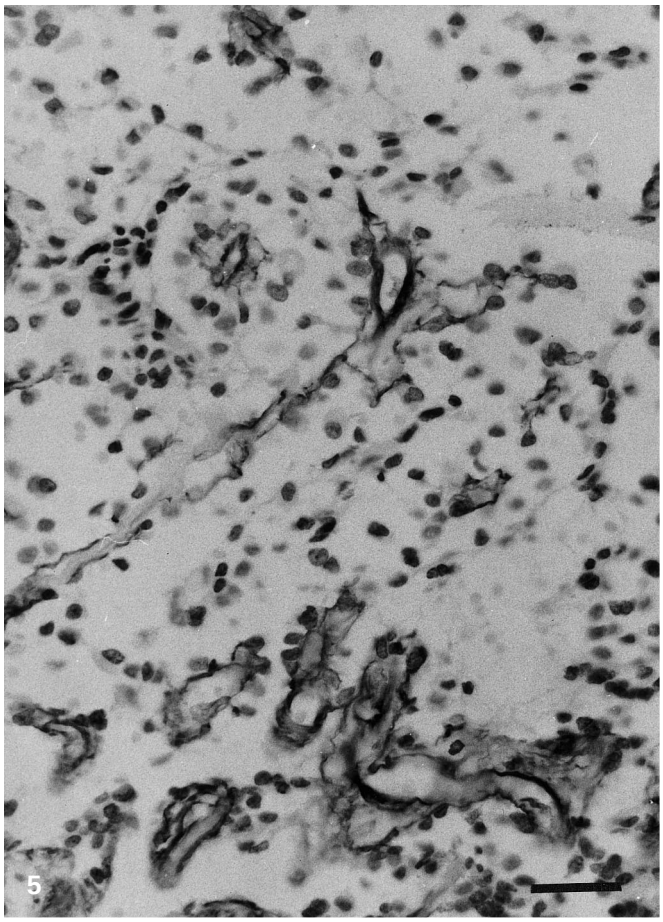
Fig. 1 Normal adult anterior pituitary immunostained for type IV collagen (TIVC). This suggests a complex three-dimensional architecture of the hormone-producing cells (*arrow*). In some places, positivity of both epithelial and vascular basement membrane can be distinguished (*arrowhead*). ×150, *bar* 67 μm

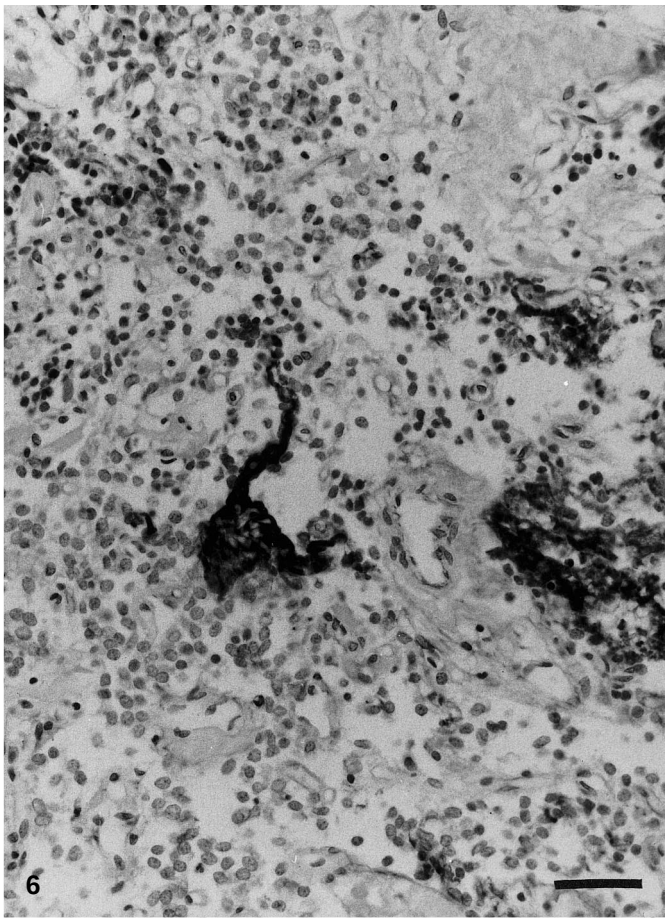
Fig. 2 Intermediate zone (*I*) of the adult pituitary immunostained for TIVC. The trabeculae are arranged parallel to the axis of the anteroposterior junction, and the size of the cell groups is larger than in the anterior lobe (*P* posterior lobe). ×150, *bar* 67 μm

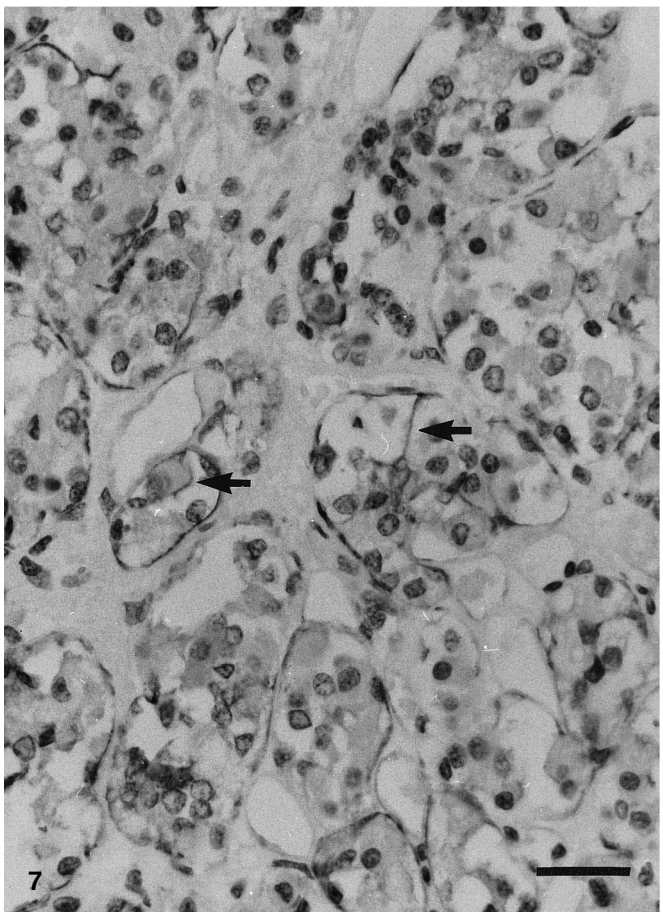
Fig. 3 Fetal pituitary (approx. 20 weeks of gestation), showing immunopositivity for TIVC around small groups of cells with short projections between cells (*arrows*). ×240, *bar* 42 μm

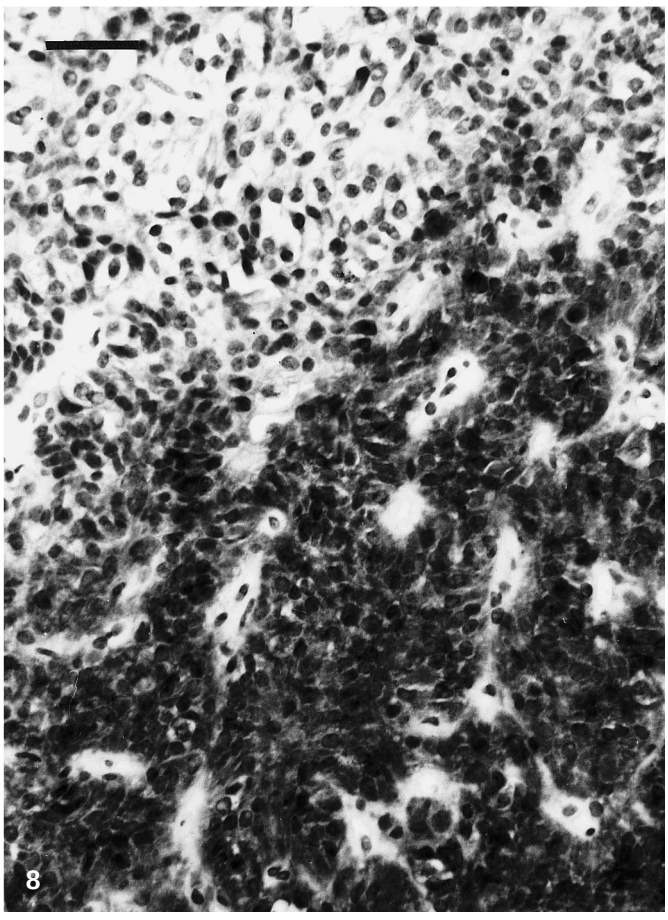
Fig. 4 Expression of TIVC in intermediate (*I*) and anterior (*A*) lobes of the fetal pituitary on the borders of Rathke's cleft (*triangle*). Positive staining is seen on the outer margins of the layers of cells comprising both anterior and intermediate lobes. Note the vascular channels extending into the intermediate lobe from the posterior lobe (*P*; *arrows*). ×150, *bar* 67 μm




5


6


7


8

On the borders of Rathke's cleft, immunoreactivity for type IV collagen and laminin was seen on the outer margin of layers of cells on both intermediate and anterior lobes and followed the course of vascular channels extending in from both anterior and posterior lobes (Fig. 4). Again, fibronectin was more patchily distributed.

Pituitary adenomas

Immunoreactivity for type IV collagen (32 of 35 cases) and laminin (21 of 35 cases) usually related to vascular channels (Fig. 5), but occasionally pericellular staining was seen. Fibronectin immunoreactivity (25 of 35 cases) was extremely variable, ranging from occasional wispy fragments to large aggregates (Fig. 6). There was no pattern of concordance among the three. In addition, there was no correlation between tumour type or size and the pattern of staining. Tumours with a more sheet-like architecture showed less immunopositivity than those with a trabecular or papillary arrangement.

Study 2

Normal adult pituitary

The pattern of laminin immunoreactivity was as above. Immunostaining for β_4 integrin was seen on the basal aspects of cells in relation to the basement membrane. In addition, focal staining was seen on other aspects of the cell membrane (Fig. 7). Staining was seen in relation to all types of hormone-secreting cells. No convincing evidence of positivity was seen in folliculostellate cells.

Pituitary adenomas

Immunopositivity for laminin was seen in 17 of 22 adenomas, in relation to vascular basement membrane as in the previous study. Cytoplasmic β_4 integrin immunoreactivity was present in 20 of 22 cases. It was variable both within and between tumours (Fig. 8). Eight cases showed strong diffuse staining, and 2 weak diffuse staining. Strong focal staining was present in 6 cases and weak focal staining in 4 cases. There was no obvious cell membrane staining in any of the cases.

◀ **Fig. 5** Pituitary adenoma immunostained for laminin, showing a mainly perivascular localisation. ×120, *bar* 48 μm

Fig. 6 Pituitary adenoma immunostained for fibronectin, showing a variable pattern of deposition. ×210, *bar* 48 μm

Fig. 7 Normal adult pituitary showing vascular and basal cellular positivity for β_4 integrin subunit. There is also focal staining of the lateral cell margins (*arrows*). ×340, *bar* 45 μm

Fig. 8 Pituitary adenoma immunostained for β_4 integrin subunit, showing strong cytoplasmic positivity in the lower part of the field and little in the remainder. ×225, *bar* 45 μm

Discussion

Our studies have confirmed and extended previous observations on the distribution of type IV collagen, laminin and fibronectin in the adult human pituitary gland. The demonstration of type IV collagen and laminin in both parenchymal and vascular basement membranes in the anterior lobe has been reported [14, 17]. Previous descriptions [18] have indicated that anterior pituitary cells are arranged in small cell cords, with larger, more expanded cords lying in the transitional zone between normal gland and pituitary adenomas [19]. Our observations indicate that such expanded cords may be found in the absence of tumour and suggest that in some glands a more complex three-dimensional arrangement of cell cords is present than might previously have been assumed. Whether this pattern corresponds to areas described as hyperplasia in unselected autopsy pituitaries [20] is not clear. The lack of cellular staining presumably reflects a low rate of storage of these proteins.

We have for the first time documented the expression of type IV collagen and laminin in the human fetal gland. Our data indicate that both proteins are expressed by the second trimester, particularly in relation to vessels. The parenchymal basement membrane appears later, with extension between cells after 20 weeks, type IV collagen expression preceding that of laminin. This temporal relationship would fit in with the proposed role of the former in structural stability of the basement membrane [21]. The more patchy distribution of fibronectin in both adult and fetal gland suggests a separate mode of regulation of expression and a different type of role in cell–cell and cell–matrix interactions from laminin.

In most species, the posterior wall of Rathke's pouch develops into a separate intermediate lobe (IL) comprising cells expressing the proopiomelanocortin (POMC) gene, which is also expressed in anterior pituitary corticotrophs. The lobe is relatively avascular, and the cells are regulated by direct innervation [22] and function differently from anterior corticotrophs [23]. Although the human fetal pituitary has a separate IL there is no evidence for innervation. It merges with the anterior lobe in postnatal life to form the intermediate zone. The corticotrophs in this area do not process POMC in the manner of the IL in other species [24], but they do show some differences in response from corticotrophs in the anterior lobe. They less frequently show Crooke's hyaline change [25] and maintain higher levels of immunoreactivity for adrenocorticotrophic hormone (ACTH) [26] and signal for POMC messenger RNA in the context of increased glucocorticoid negative feedback [27]. We have postulated [24] that this might be due to the effects of posterior lobe peptides, including vasopressin, an ACTH secretagogue, which reach these cells via vascular channels. Such vascular links between the lobes have been demonstrated in the pig [28].

In this study, we have shown that the intermediate lobe and posterior margin of the anterior lobe have different patterns of basement membrane proteins from the

anterior lobe in the fetal gland, and that this is mirrored in the intermediate zone of the adult gland. The cells are arranged in larger groups, and the zone is relatively avascular. Vessels from the posterior lobe appear to enter this area, thus supporting our hypothesis. Further studies mapping vessels with specific endothelial markers will be of interest.

In agreement with Farnoud et al. [17], we have demonstrated immunoreactivity for β_4 integrin in the normal adult gland; β_4 combines with α_6, functioning as a laminin receptor. The correlation of basal cellular staining with laminin immunoreactivity would be in keeping with this and support a role for this interaction in physiological cell–matrix relationships. The relevance of expression on lateral cell margins is not clear at present, but may reflect a cell–cell adhesion function; such a role has not yet been described for β_4 integrin.

Our data on expression of the various proteins in pituitary adenomas are generally in keeping with previous studies. We confirm that most TIVC and laminin immunoreactivity is seen in relation to vascular basement membrane [15–17]. We did not investigate the differential distribution of laminin subtypes. Fibronectin expression was extremely variable. The occurrence of large aggregates could reflect either increased synthesis or decreased breakdown. The latter might be related to the switch in fibronectin isoforms reported in pituitary adenomas compared with normal pituitary [29]. Fibronectin is known to stimulate angiogenesis [30]. The presence of strong focal immunoreactivity in relation to vessels seen in this study and that of Farnoud et al. [29] indicates a role in the new vessel formation which characterises these tumours [11, 19, 31]. Our findings on expression of β_4 integrin subunit fit better with those of Paulus et al. [14], who reported expression in both normal and tumorous gland. The discrepancy between our results and those of Farnoud et al. [17], who did not find immunopositivity for this subunit in adenomas, may be related to the recognition of different epitopes by the antibodies used. However, the abnormal pattern of expression with cytoplasmic staining and no membrane localisation indicates that normal interactions with laminin would be unlikely. Thus, even in this group of tumours usually classified as benign, there is evidence of significant alteration in cell–matrix interactions.

Acknowledgements We are grateful to Dr. S. J. Kennell, Oakridge National Laboratory, USA for the kind gift of anitbody to β_4 integrin and to NIDDK for antibodies to LH and FSH.

References

1. Von der Mark K, Von der Mark H, Goodman S (1992) Cellular responses to extracellular matrix. Kidney Int 41:632–640
2. Akiyama SK, Olden K, Yamada KM (1995) Fibronectin and integrins in invasion and metastasis. Cancer Metastasis Rev 14:173–189
3. Flug M, Kopf-Maier P (1995) The basement membrane and its involvement in carcinoma cell invasion. Acta Anat (Basel) 152:69–84
4. Rosales C, O'Brien V, Kornberg L, Juliano R (1995) Signal transduction by cell adhesion receptors. Biochim Biophys Acta 1242:77–98
5. Pignatelli M, Vessey CJ (1994) Adhesion molecules: novel molecular tools in tumor pathology. Hum Pathol 24:849–856
6. Tuckwell DS, Humphries MJ (1993) Molecular and cellular biology of integrins. Crit Rev Oncol Haematol 15:149–171
7. Holck S, Albrechtsen R, Wewer UM (1987) Laminin in the anterior pituitary gland of the rat. Lab Invest 56:481–488
8. Vila-Porcile E, Picart R, Tixier-Vidal A, Tougard C (1987) Cellular and subcellular distribution of laminin in adult rat anterior pituitary. J Histochem Cytochem 35:287–299
9. Vila-Porcile E, Picart R, Vigny M, Tixier-Vidal A, Tougard C (1992) Immunolocalization of laminin, heparan-sulfate proteoglycan, entactin and type IV collagen in the rat anterior pituitary. I. An in vivo study. Anat Rec 232:482–492
10. Liu YC, Tanaka S, Inoue K, Kurosumi K (1989) Localization of fibronectin in folliculostellate cells of the rat anterior pituitary by the double bridge peroxidase-antiperoxidase method. Histochemistry 92:43–45
11. Farnoud MR, Lissak B, Kujas M, Peillon F, Racadot J, Li JY (1992) Specific alterations of the basement membrane and stroma antigens in human pituitary tumours in comparison with the normal anterior pituitary. An immunocytochemical study. Virchows Arch [A] 421:449–455
12. Horacek MJ, Dada MO, Terracio L (1992) Reconstituted basement membrane influences prolactin, LH and FSH secretion from adult and fetal adenohypophyseal cells in vitro. J Cell Physiol 151:180–189
13. Horacek MJ, Kawaguchi T, Terracio L (1994) Adult adenohypophysial cells express beta 1 integrins and prefer laminin during cell-substratum adhesion. In Vitro Cell Dev Biol [A] 30:35–40
14. Paulus W, Baur I, Schuppan D, Roggendorf W (1993) Characterization of integrin receptors in normal and neoplastic human brain. Am J Pathol 143:154–163
15. Holck S, Wewer VM, Albrechtsen R (1986) Intercellular deposits of basement membrane material in active human pituitary adenomas detected by immunostaining for laminin and electron microscopy. Acta Neuropathol (Berl) 71:326–331
16. Farnoud MR, Derome P, Peillon F, Li JY (1994) Immunohistochemical localization of different laminin isoforms in human normal and adenomatous anterior pituitary. Lab Invest 70:399–406
17. Farnoud MR, Li JY, Peillon F (1996) Alterations of cell–matrix interactions in human pituitary adenomas. Endocr Pathol 6:364
18. McNicol AM, Smith S, Richmond JA, Graham DI, Teasdale GM (1990) Immunohistochemical demonstration of alpha$_1$-antichymotrypsin and alpha$_1$-antitrypsin in the normal pituitary gland and its tumors. Endocr Pathol 1:109–115
19. Farnoud MR, Kujas M, Derome P, Racadot J, Peillon F, Li JY (1994) Interactions between normal and tumoral tissues at the boundary of human anterior pituitary adenomas. An immunohistochemical study. Virchows Arch [A] 424:75–82
20. Saeger W (1996) Latent hyperplasias and adenomas in postmortem pituitaries. Endocr Pathol [3]:379
21. Kuhn K (1995) Basement membrane (type IV) collagen. Matrix Biol 14:439–445
22. Kurosumi K, Matsuzawa T, Shibasaki K (1961) Electron microscope studies on the fine structures of the pars nervosa and pars intermedia, and their morphological interrelation in the normal rat hypophysis. Gen Comp Endocrinol 1:433–452
23. Mains RE, Eipper BA (1979) Synthesis and secretion of corticotropins, melanotropins and endorphin by rat pituitary. J Biol Chem 254:7885–7894
24. McNicol AM (1986) A study of intermediate lobe differentiation in the human pituitary gland. J Pathol (Lond) 150:169–173
25. Halmi NS, McCormick WF, Decker JF Jr (1971) The natural history of hyalinisation of ACTH-MSH cells in man. Arch Pathol 91:319–326

26. McNicol AM (1981) Patterns of corticotropic cells in the adult human pituitary in Cushing's disease. Diagn Histopathol 4:335–341
27. Fehn M, Farquharson MA, Sautner D, Saeger W, Ludecke DK, McNicol AM (1993) Demonstration of pro-opiomelanocortin mRNA in pituitary adenomas and para-adenomatous gland in Cushing's disease and Nelson's syndrome. J Pathol (Lond) 169:335–339
28. Page RB (1982) Pituitary blood flow. Am J Physiol 243:E427–E442
29. Farnoud MR, Farhadian F, Samuel JL, Derome P, Peillon F, Li JY (1995) Fibronectin isoforms are differentially expressed in normal and adenomatous human anterior pituitaries. Int J Cancer 61:27–34
30. Paku S, Paweletz N (1991) First steps of tumor-related angiogenesis. Lab Invest 65:334–346
31. Racadot J, Gremain J, Kujas M, Drouet Y, Olivier L (1986) La participation de vasseaux artériels à l'irrigation sanguine des adénomes de l'hypophyse humaine, implication fonctionnelle. Bull Assoc Anat (Nancy) 70:5–12