Virchows Archiv A Pathol Anat (1990) 417:89–96

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunocytochemical differential diagnosis of adrenocortical neoplasms using the monoclonal antibody D11*

Sören Schröder[1], Axel Niendorf[1], Eike Achilles[1], Manfred Dietel[1], Barbara-Christina Padberg[1], Ulrike Beisiegel[2], Henning Dralle[3], Max Bressel[4], and Günter Klöppel[5]

[1] Institute of Pathology and [2] Department of Internal Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Federal Republic of Germany
[3] Department of Surgery, Hannover School of Medicine, Hannover, Federal Republic of Germany
[4] Department of Urology, General Hospital Hamburg-Harburg, Hamburg, Federal Republic of Germany
[5] Institute of Pathology, Free University of Brussels, Brussels, Belgium

Received October 23, 1989 / Received after revision February 16, 1990 / Accepted February 21, 1990

Summary. The monoclonal antibody D11 is a valuable aid in the accurate typing of adrenal tumours as, in formalin-fixed, paraffin-embedding material, strong nuclear D11 positivity was observed only in adrenocortical cells in 190 neoplasms (including 100 adrenal tumours). This pattern was demonstrated for all zona glomerulosa cells in 27 normal adrenals and for the neoplastic cells of 15 adrenocortical adenomas derived from that zone, as judged from clinically evident hyperaldosteronism. Normal cells of zona fasciculata and reticularis also showed strong diffuse D11 immunostaining and the same nuclear plus cytoplasmic D11 reactivity was evident in 15 benign and malignant adrenocortical neoplasms derived from these zones, documented by hypercortisolism. Cytoplasmic and/or nuclear D11 staining made topohistogenetic typing possible in 15 non-functioning cortical tumours. D11 immunostaining gave negative results in 50 specimens containing normal, hyperplastic and neoplastic adrenomedullary cells. In addition, absence of D11 reactivity was recorded in 4 adrenal metastases of extra-adrenal carcinomas, 5 paragangliomas, 25 primary renal carcinomas and 59 of 60 primary thyroid carcinomas. D11 immunocytochemistry allows the accurate typing of benign and malignant adrenocortical neoplasms, irrespective of histology and function. With this method, primary adrenocortical tumours can be separated from carcinomas metastatic to the adrenal gland, including secondary tumours of similar phenotype (such as renal carcinomas). By exclusion, D11 negativity provides evidence of the medullary origin of primary adrenal tumours even in the absence of clinical, structural, histochemical and conventional immunohistochemical indicators of phaeochromocytoma.

Key words: Adrenal gland – Adrenocortical tumours – Immunocytochemistry

Offprint requests to: S. Schröder, Institut für Pathologie, Universität Hamburg (UKE), Martinistrasse 52, D-2000 Hamburg 20, FRG

* Dedicated to Prof. Dr. Dr. h.c. mult. Wilhelm Doerr on the occasion of his 75th birthday. This study has been sponsored by the Deutsche Forschungsgemeinschaft and the Hamburger Krebsgesellschaft and was presented in part at the 80th Annual Meeting of the American Association for Cancer Research, San Francisco, California, 24–27 May 1989 (Schröder et al. 1989)

Introduction

Because of their broad microscopical variety, accurate typing of adrenal tumours often poses a major diagnostic problem. Conventional histology frequently offers no conclusive evidence of the origin of individual neoplasms (cortical versus medullary, versus extra-adrenal metastatic). Electron microscopy, immunohistology (the detection of steroid hormones) and histochemistry (the detection of steroid hormone-specific enzymes) have also been found to be of little help with this problem (Page et al. 1986). There is a need for an immunohistological probe which guarantees selective staining of normal and neoplastic cortical cells in the adrenal gland. Our findings show that the antibody D11 provides us with the means with which we can solve not only differential diagnostic problems, but also some questions relating to the histogenesis of adrenocortical tumours.

Materials and methods

The monoclonal antibody D11 was produced using a 59 kDa human liver membrane protein as described elsewhere (Beisiegel et al. 1988). Proteins were analysed on SDS-polyacrylamide gel electrophoresis using the method of Neville (1971). Immunoblotting was carried out following the procedures as described by Beisiegel et al. (1981) and Hui et al. (1986). The cultivation and passage of HepG2 cells (human hepatoblastoma) and of other freshly established primary cell strains was carried out using the method of Dietel et al. (1987).

Formalin-fixed, paraffin-embedded material of the 103 adrenalectomy specimens was collected from the surgical pathology files of various Institutes of Pathology. Information relating to the preoperative symptoms were noted from the patients' medial

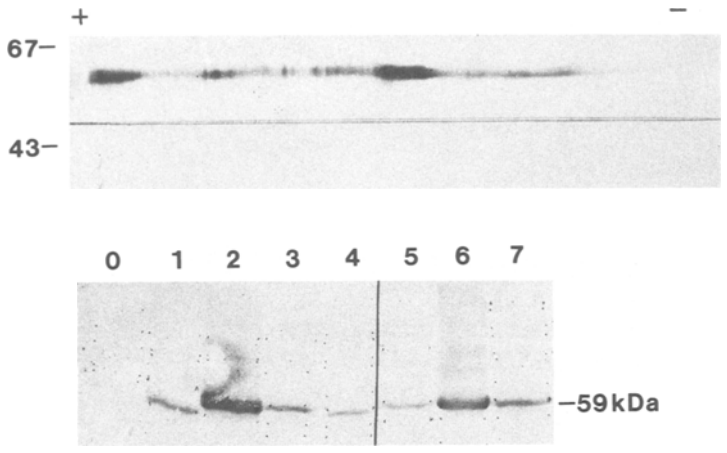

Fig. 1. Biochemical characterisation of D11-positive antigens.
Top: Immunoblot of a two-dimensional electrophoresis. Approximately 10 different dots of molecular weight 59 kDa react as antigens of D11.
Bottom: Immunoblot of an SDS gel electrophoresis using membrane homogenates of various rabbit organs: The 59 kDa antigens are detectable in all organs and in HepG2 cells, with the highest concentration in the liver and in the brain and with only a very low concentration in the adrenal gland (*1*, HepG2 cells; *2*, liver; *3*, intestine; *4*, adrenal gland; *5*, testicle; *6*, brain; *7*, adipose tissue)

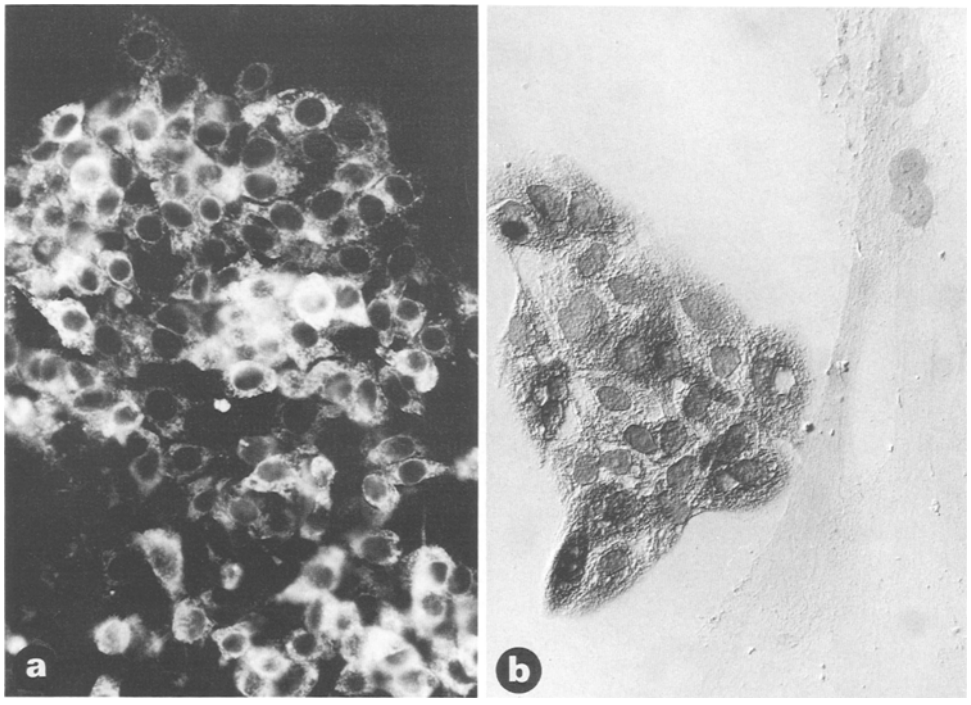

Fig. 2a, b. D11 immunocytochemistry of cultivated cells.
a Perinuclear accentuation of cytoplasmic staining of HepG2 cells. Immunofluorescence, × 300.
b Diffuse cytoplasmic staining of HepG2 cells co-cultivated with fibroblasts, fibroblasts negative. × 770

records. In each case the development of the illness following operation was documented until spring 1989. For the purpose of comparison, 25 renal cell carcinomas, 60 thyroid carcinomas, 5 paragangliomas and various normal human tissues were also brought into the study.

The immunohistological examinations used the avidin-biotin-peroxidase-complex (ABC) method (Hsu et al. 1981). The antibodies employed were directed against neuron specific enolase (NSE) (polyclonal/rabbit; Dakopatts, Copenhagen, Denmark, dilution 1:3000), chromogranin A (Chr A: monoclonal; Hybritech, San Diego, Calif., 1:5000), synaptophysin (SYN: polyclonal/rabbit; Dr. Jahn, Max-Planck-Institut, Martinsried, FRG, 1:1000). In addition, the monoclonal antibody D11 (culture supernatant; 1:5) and Grimelius' method for the demonstration of argyrophilia (Grimelius and Wilander 1984) were applied.

Results

In two-dimensional electrophoresis, approximately ten different dots of the same molecular weight showed immunoreactivity for D11 (Fig. 1a). The presence of D11-positive 59 kDa antigens were observed in the immunoblot analysis of an SDS-polyacrylamide gel electrophoresis in solubilisates of the membrane fractions of various rabbit organs as well as in cultivated HepG2 cells (Fig. 1b).

When a non cell-membrane permeabilising fixative (Bouin's solution) was used, no D11 reaction was noted in HepG2 cells, fibroblasts or in cultivated cells of different gastrointestinal carcinomas (stomach, pancreas and colon). Incubation of the primary antibody before preparation gave the same result. In contrast, a diffuse cytoplasmic fluorescence in HepG2 cells was observed after permeabilisation of the cell membrane by methanol/acetone fixation (Fig. 2a). Co-cultivated fibroblasts and other cultivated tumour cells, however, remained negative (Fig. 2b). In the liver, formalin-fixed specimens showed intense staining of hepatocytes and of occasional bile duct epithelium. In the kidney, epithelial positivity

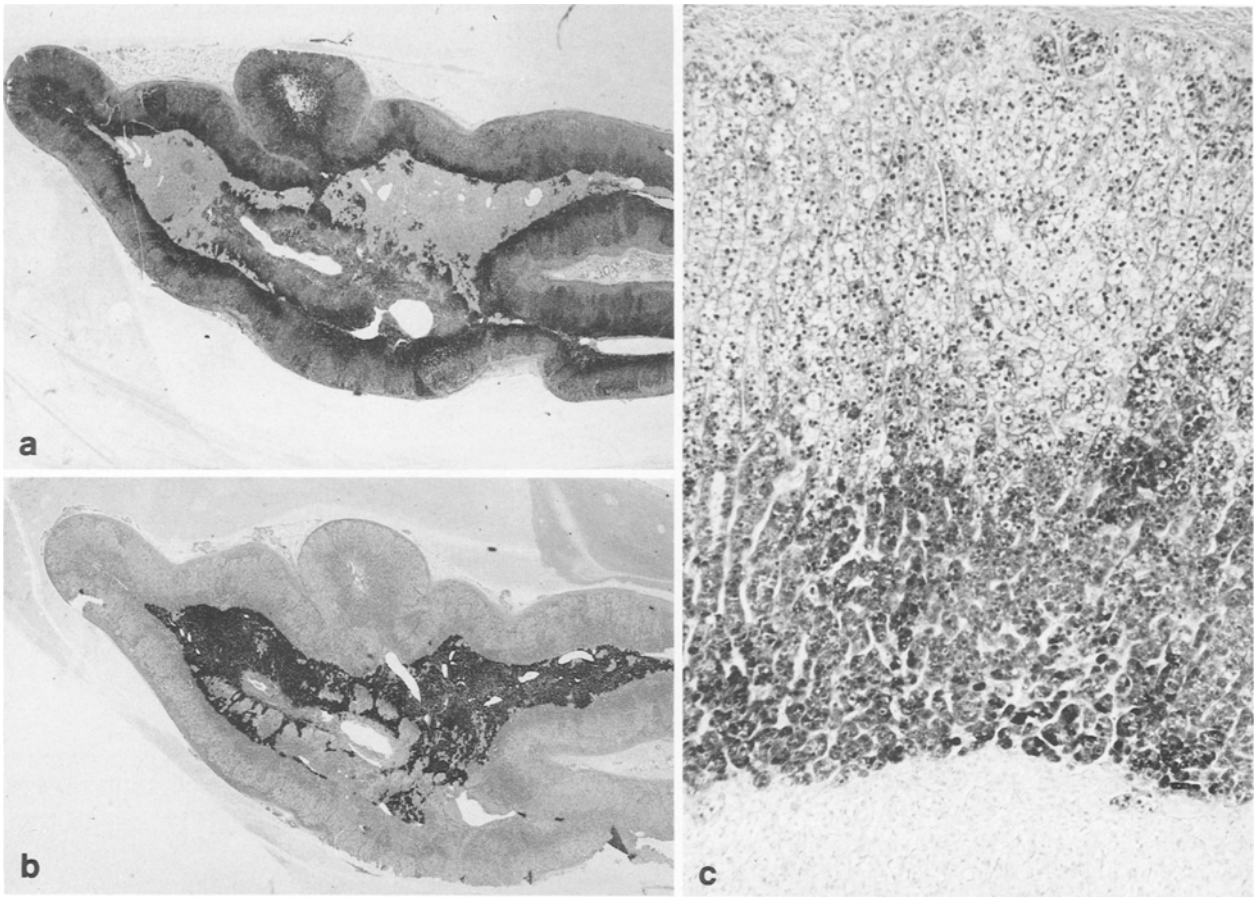

Fig. 3a–c. Immunocytochemistry of normal adrenal tissue. **a** D11 immunostaining showing exclusive positivity of the cortex with accentuation of the central portions. ×5.5. **b** Complementary aspect of chromogranin A (Chr A) immunoreactivity with intensive staining exclusively of the medulla. ×5.5. **c** Magnification from **a**: Strong nuclear D11 staining of all cortical cells with additional cytoplasmic reactivity in the central parts of the zona fasciculata and in the zona reticularis; medullary cells negative. ×65

was recorded for some tubular segments. In the pancreas, positive results were shown for duct epithelia and for acini of the exocrine parenchyma. In all of the organs examined, smooth muscle cells of arterial and venous vessel walls and fibrocytes within the organ capsules were D11-positive; D11 staining was always confined to the cytoplasm.

Three adrenalectomy specimens from healthy multi-organ donors and histologically inconspicuous residual parenchyma from the periphery of 24 tumours-bearing adrenals gave the immunocytochemical results recorded in Fig. 3a and b. D11 positivity was demonstrated in the nuclei of all three cortical zones. Faint granular cytoplasmic immunostaining was demonstrated in the zona glomerulosa and in the peripheral two-thirds of the zona fasciculata. In the central third of this zone, as in the entire zona reticularis, there was intense diffuse cytoplasmic staining. Medullary tissue was completely devoid of D11 reactivity, while argyrophilia and positivity for NSE, Chr A and SYN were only seen in this region, thus producing a virtually complementary picture to D11 immunostaining.

In 15 patients, benign adrenocortical adenomas (mean weight 12 g, range 7–35 g) were classified as aldosteronomas as they were associated with clinically evident Conn's syndrome. On NSE immunostaining, 1 showed doubtful positivity. The remaining lesions proved NSE-negative and, like all of the other subsequently described cortical tumours, also gave negative results when tested for Chr A and argyrophilia. In each of the 15 cases, D11 produced the same staining pattern as described above for the normal zona glomerulosa (Fig. 4a). In 6 lesions, increased cytoplasmic D11 staining was seen focally. This never reached the intensity of the nuclear reaction.

Seven cortical tumours (mean weight 23 g, range 11–48 g) associated with Cushing's syndrome were classified as being adenomas owing to their intact capsular confinement, the lack of mitotic activity and the absence of any post-operative symptoms (mean observation period 36 months). All cases exhibited intense nuclear and cytoplasmic D11 staining (Fig. 4b, c). NSE immunocytochemistry gave negative results each time.

In 2 tumours associated with Cushing's syndrome (weights 125 g and 450 g), malignancy could not be entirely discounted because of the presence of moderate nuclear pleomorphism and of focal capsular invasion, although neither patient showed any post-operative

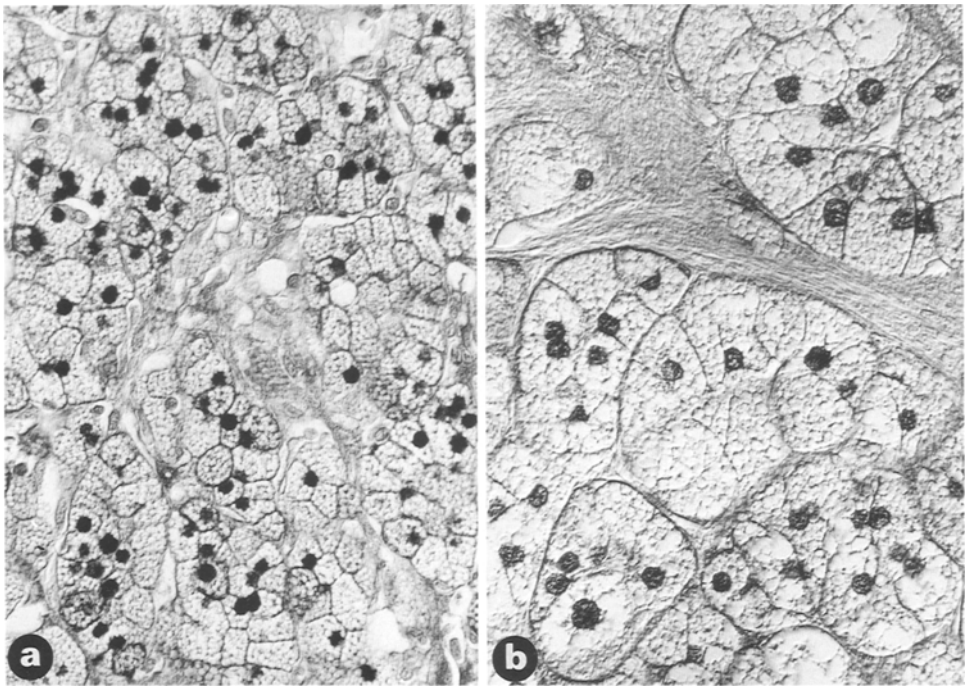

Fig. 4a, b. D11 immunocytochemistry in aldosteronomas showing intensive nuclear and only faint granular staining of the cytoplasm; vascular endothelium and stroma D11 negative. **a** × 290; **b** × 385

symptoms over a period of 6 and 13 months respectively. D11 and NSE staining provided results identical to those of the 7 adenomas described before.

In 6 patients, adrenocortical carcinomas (mean weight 900 g, range 130–2250 g) associated with Cushing's syndrome were observed. Four of these were women, in whom elevated plasma levels of testosterone was accompanied by virilisation. Three of the patients died not more than 8 months after diagnosis. The remaining patients are alive 4, 5 and 62 months later, respectively, all suffering from metastatic tumours. Upon NSE immunostaining, questionable reactivity was seen with 4 of the carcinomas. In one case, this finding initially led to the mistaken diagnosis of phaeochromocytoma when the hormonal symptoms were not recognised. In contrast, D11 gave constantly clear positive nuclear and cytoplasmic results with all 6 tumours (Fig. 4d).

In 7 cases solitary, well-defined adrenal tumours (mean weight 18 g, range 8–34 g) were found which were classified as endocrinologically silent cortical adenomas from their unremarkable histological appearances, the absence of endocrine symptoms and an uneventful post-operative course (mean observation period 32 months). All the tumours were NSE negative. On D11 immunocytochemistry strong nuclear positivity was found in each. Five lesions demonstrated intense cytoplasmic staining in addition (as described above for adrenocortical tumours associated with Cushing's syndrome), while the remaining 2 neoplasms lacked significant cytoplasmic positivity (as noted for aldosteronomas).

The biological potential of 5 larger non-functioning cortical tumours (mean weight 209 g, range 35–750 g) could not be determined by morphological means. The patients had hitherto remained free from recurrent disease over a mean post-operative observation period of 33 months. On NSE immunocytochemistry, 2 neoplasms showed doubtful staining while the others gave negative results. Conversely, all 5 tumours showed distinct nuclear staining as well as an intense diffuse cytoplasmic reactivity for D11 in 4 lesions.

Cortical carcinomas lacking endocrine symptoms were found in 3 patients, leading to death within not more than 8 months after diagnosis. In 2 cases, adrenalectomy specimens weighing 995 g and 3250 g, respectively, were investigated. In the third case, only a needle biopsy from a para-aortic lymph node metastasis was available for study. The first 2 tumours showed doubtful NSE reactivity. In contrast, all 3 carcinomas exhibited clear nuclear and cytoplasmic positivity for D11.

In the 1 case of bilateral primary adrenocortical microadenomatosis, strong cytoplasmic and nuclear D11 positivity of the hyperplastic cortical nodules was observed. The same intense D11 reactivity was demonstrated in sparsely scattered cortical cells in 2 myelolipomas and in 1 adrenal which showed tumour-like enlargement by extramedullary myelopoiesis, due to thalassaemia minor. In all 4 lesions, negative results were recorded when tested for NSE, Chr A, SYN and argyrophilia.

Sporadic adrenomedullary hyperplasia was found in 3 patients. The morphological and clinical features of these cases are described in detail elsewhere (Dralle et al. 1990). In 12 patients, benign hereditary phaeochromocytomas (PCCs) (mean weight 29 g, range 8–85 g) were found to be present in the setting of the multiple endocrine neoplasia syndrome, type IIa. In 3 cases, bilateral tumours were observed. In each of the patients, the disease was accompanied by the typical clinical symptoms of PCC (hypertensive crises, increased levels of catecholamines in plasma and urine and increased excretion rates for vanillylmandelic acid). In 24 patients (bilaterally in 1 of these), benign sporadic PCCs (mean weight 71 g,

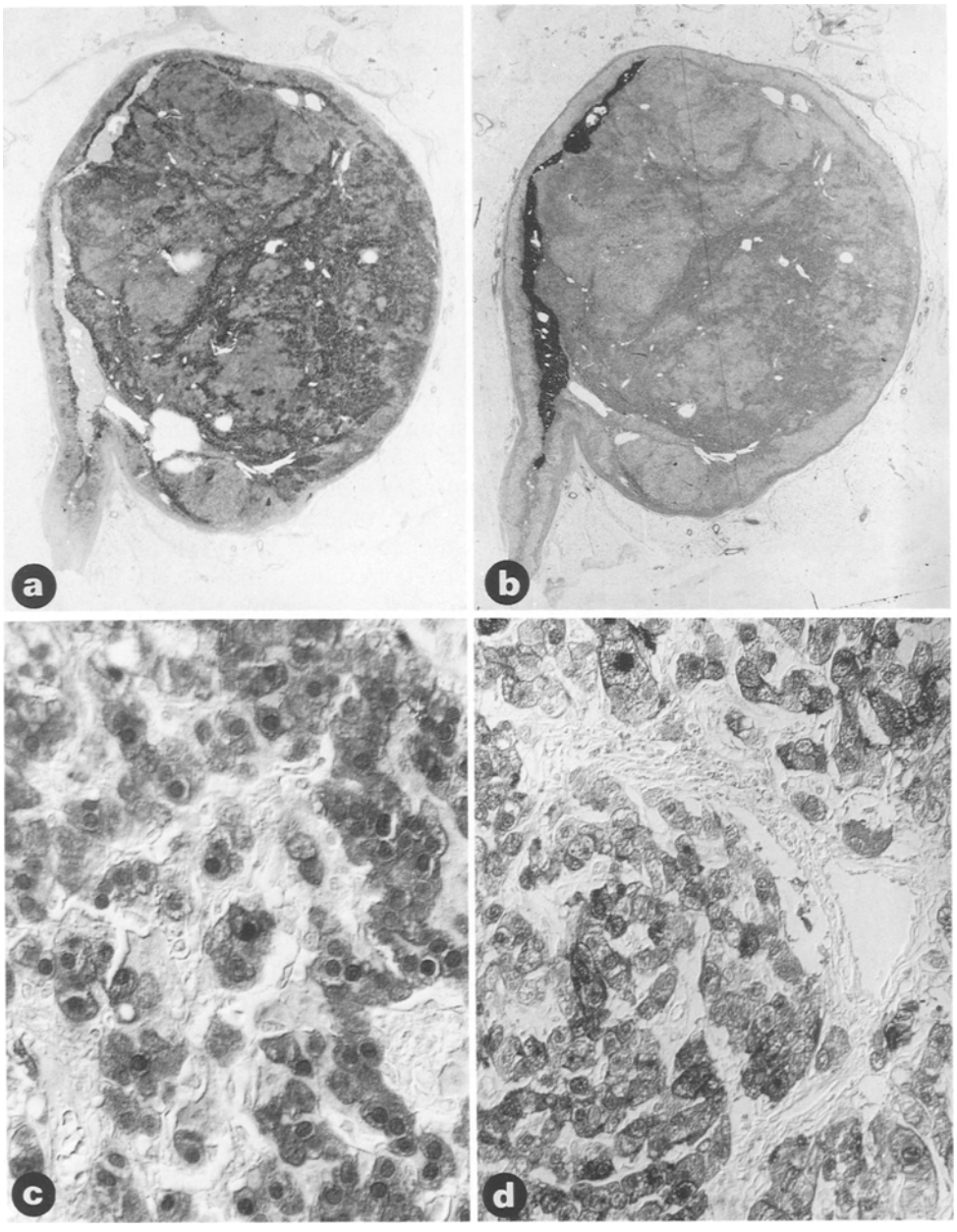

Fig. 5a–d. D11 immunocytochemistry in adrenalcortical tumours with Cushing's syndrome.
a Adenoma showing strong D11 positivity: atrophy of the darkly coloured central cortical parts in the residual parenchyma (left side of the picture). ×3.8.
b Complementary aspects of the same specimen upon immunocytochemistry for Chr A. ×3.8.
c Magnification from **a**: nuclear and cytoplasmic staining of tumour cells, the stroma being negative. ×290.
d Carcinoma showing same results as in **c**. ×150

range 13–140 g) were found. Follow-up study disclosed all patients to be symptom-free at the end of the observation period (mean 34 months). In 4 patients with malignant sporadic PCC, the formation of metastases was discovered during operation or became apparent 2 and 24 months later, respectively. The 4 primary tumours weighed 110–350 g (mean 245 g). Three of these patients died 3–13 months after adrenalectomy; 1 is still alive suffering from metastatic disease. Apart from 2 benign and 2 malignant non-functioning tumours, all other patients with the sporadic type of adrenomedullary neoplasia had shown typical PCC symptoms before surgery.

Chr A staining gave positive results in connection with the hyperplastic and neoplastic adrenomedullary lesions, except in the case of 2 malignant PCCs. In addition to these exceptions, NSE immunohistochemistry and silver staining were negative in 1, or where applicable, 2 benign PCCs. All of the 47 lesions were positive for SYN, while D11 stainig invariable produced negative results.

Two patients showed adrenal metastases from renal cell carcinomas which, in both cases, represented the primary manifestation of an undiscovered kidney tumour. Both tumours, weighing 32 g and 34 g, respectively, were initially thought to be functionally inactive cortical adenomas on account of their clear-cell appearance and the absence of any capsular infiltration. In one case, a contralateral nephrectomy was carried out 5 months later because of a highly differentiated renal cell carcinoma (G1). The other patient died 12 months after adrenalectomy. The post-mortem revealed a hitherto clinically undiagnosed (ipsilateral) renal cell carcinoma (also

G1) with lung and osseous metastases. Immunocytochemistry with D11 and for NSE, Chr A and SYN in both neoplasms gave negative results.

In 2 patients, adrenal metastases, weighing 90 g and 195 g, respectively, offered the first indication of a large cell lung carcinoma. One tumour was initially mistaken for a non-functioning PCC as a result of a questionable (false-positive) NSE immunostaining. The patient died 2 months after adrenalectomy. Post-mortem confirmed the presence of a metastatic lung carcinoma. Immunohistological reclassification revealed doubtful NSE reactivity in the adrenal metastasis and in the primary tumour (post-mortem histology) with no staining for Chr A and SYN or D11. In the second case, the adrenal neoplasm was typed initially as non-functioning adrenocortical tumour of indeterminate biological potential. Twelve months later, biopsies carried out on enlarged cervical lymph nodes and on a lung tumour which had, in the meantime, been located radiologically, showed the same histological appearance. Immunocytochemically, D11 and antibodies against NSE, Chr A and SYN gave negative results in all three specimens.

Two parasympathetic and 3 extra-adrenal sympathetic paragangliomas all gave negative results with D11 and positive results when tested for NSE, Chr A and SYN. Of 25 primary renal cell carcinomas (10 graded as G1 and G2 respectively and 5 graded as G3), no tumour showed D11 immunoreactivity. Out of 60 primary thyroid carcinomas (15 examples of each papillary, follicular, medullary and anaplastic tumours), only in 1 papillary neoplasm was there a focal diffuse-cytoplasmic D11 positivity, accompanied by the absence of any nuclear staining.

Discussion

Our findings show that the monoclonal antibody D11 is capable of immunocytochemical identification of adrenocortical cells, irrespective of their function or biological potential. All 27 specimens of normal cortex and all 49 adrenocortical tumours or tumour-like lesions were D11 positive, whereas all 50 specimens of normal, hyperplastic of neoplastic adrenomedullary tissue and 4 secondary adrenal tumours, 5 paragangliomas, 25 renal and 59 of 60 thyroid carcinomas were D11 negative. The significance of D11 for tumour diagnosis lies initially, therefore, in its ability to distinguish clearly cortical from medullary or secondary adrenal tumours. Moreover, our results shows that D11 also provides evidence of the histogenesis in individual adrenocortical tumours.

In all normal and neoplastic cortical cells we found nuclear D11 positivity hitherto unencountered in any other organ. An additional intense cytoplasmic D11 reactivity appeared only in the central zones of the cortex, which histochemical and experimental findings showed to be the site of glucocorticoid, androgen and oestrogen synthesis (Dhom 1981; Page et al. 1986). The aldosterone-producing peripheral cortex, however, showed only a faint granular cytoplasmic staining at most. Both of these patterns of D11 reactivity proved to be the same in all functioning cortical tumours in which the sites of origin could be deduced by means of the clinical symptoms – either Cushing's or Conn's syndrome. The constantly obvious correlation between the D11 pattern and the endocrine cell capacity found in functioning adrenocortical tumours is noteworthy, since electron microscopically there is no continuous homology in the ultrastructure of normal and neoplastic cells of the various cortical zones (Probst 1965; MacKay 1969; Sommers and Terzakis 1970; Mitschke et al. 1973). Even the recently described detection of P-450 cytochromes in adrenocortical adenomas (Sasano et al. 1988a, b) does not allow an exact topographical classification, since this enzyme system is present in many cells from all three cortical zones (Geuze et al. 1987).

Since D11 staining also gave a uniformly positive result in non-functioning adrenocortical tumours, this method does not allow conclusions to be drawn about the functional capacity of individual neoplasms. The detection of the two distinct D11 patterns described above in different cortical layers and in both functioning neoplasms and tumours not presenting with endocrine abnormalities suggests that in these particular zones of the adrenal cortex, structural proteins exist which are not associated with specific hormonal synthetic capacity. These proteins also appear to be expressed after neoplastic transformation and make histogenetic typing possible.

The proteins responsible for the D11 staining of adrenocortical cells have not been identified. From our biochemical experiments, D11 recognises several 59 kDa antigens which possess the same antigenetic determinants and are present in various organs. In vitro findings show that these proteins are capable of binding apolipoprotein E (Apo E), which is present in trigylceride-rich chylomicron remnants in high concentrations (Beisiegel et al. 1988). One of the 59 kDa proteins was identified by cDNA-sequencing as being protein disulphide-isomerase (PDI) (Ihrke and Stoffel, unpublished results). The cytoplasmic D11 positivity of cultivated HepG2 cells after membrane permeabilisation agrees with the results reported for the immunolocalisation of PDI by Kaetzel et al. (1987). Further analogies exist in the D11 positivity of fibrocytes in various organ capsules, since, according to the findings of Myllylä et al. (1983), PDI activity and rates of procollagen synthesis are positively correlated. No plausible explanation can be found, however, for the presence of PDI and/or of an Apo E-binding protein in the adrenal cortex. The organ-specific nuclear and the cytoplasmic D11 immunoreactivities of adrenocortical cells are thus presumably based on two different 59 kDa proteins which correspond physiologically neither to the PDI nor to an Apo E-binding protein and need to be more accurately determined in further tests.

The diagnosis relevance of the antibody D11 (as confirmed through the certain exclusion of PCC) is independent of the biochemical characterisation of the D11 immunoreactive adrenocortical proteins. Our observation of cortical neoplasms with phenotypic similarity to medullary tumours (and vice versa) emphasises the well-known likelihood of confusion between the two tumour

forms, as has been described authoritively in monographs (Page et al. 1986) as well as in individual case reports (Ramsay et al. 1987). Furthermore, such confusion cannot always be avoided by applying commercial antibodies. According to our own and others experience (Heitz 1987), the diagnostic usefulness of NSE immunostaining is hampered by an occasionally inconclusive result. Thus in 9 lesions which were clearly related to the cortex (and which were D11 positive), we observed an NSE reaction which could not clearly be defined as negative. Conversely, amongst the PCCs in this series, in one case a questionable and in another case a negative NSE reaction was observed. As shown by ourselves and by others (Hacker et al. 1988; Kimura et al. 1988) PCCs exhibiting either doubtful or no NSE reactivity can, in the majority of cases, be defined with certainty by Chr A or SYN immunocytochemistry or by silver staining. The absence of argyrophilia and Chr A reactivity in respectively, 5 and 2 of the PCCs in our material and in several cases described in other series (Hamid et al. 1987; Hacker et al. 1988) indicate the limitations of using these methods. It should be added that in about 5% of the adrenomedullary tumours – 9% (4/44), in our study – there is no clinical indication of PCC symptoms (Scott et al. 1982; Shapiro et al. 1984; Plouin et al. 1987; Samaan and Hickey 1987a; Krause et al. 1988). Thus, in such cases, the correct diagnosis can only be determined by the pathologist.

More important than the exclusion of PCC would appear to be the possibility of differentiating between primary and secondary adrenal neoplasms. In this series 3 secondary lesions were mistaken initially for adrenal tumours, 2 of which were thought to be benign. The D11 negativity of 2 clear-cell renal tumours metastatic to the adrenal was confirmed by identical results in 25 primary renal carcinomas. This result is of great value; it has barely been possible to distinguish between adrenal infiltrations of such renal secondaries and primary adrenocortical neoplasms because of their phenotypic similarity in the absence of a specific means of identification. The single case of one D11 positive papillary neoplasm occurring amongst 60 primary thyroid carcinomas does not cast doubt on this evidence, but underlies a demand for further systematic analyses of the expression patterns of D11-positive antigens in other normal and neoplastic tissues. In so far as such studies sustain our assumption of a specificity of the nuclear D11 reactivity type for adrenocortical tumours, it follows that this antibody must be introduced into the panel of immunocytochemical probes used to type occult neoplasms. The reason for this is that up to 60% of adrenocortical carcinomas do not produce endocrine manifestations; 50% of the cases have developed metastasis at the time of clinical diagnosis (Samaan and Hickey 1987b); this may represent the first signs of the illness (Page et al. 1986).

References

Beisiegel U, Kita T, Anderson RGW, Schneider WJ, Brown MS, Goldstein JL (1981) Immunologic cross-reactivity of the low density lipoprotein receptor from bovine adrenal cortex, human fibroblasts, canine liver and adrenal gland, and rat liver. J Biol Chem 1256:4071–4078

Beisiegel U, Weber W, Havinga JR, Ihrke G, Hui DY, Wernette-Hammond ME, Turck CW, Innerarity TL, Mahley RW (1988) Apolipoprotein E-binding proteins isolated from dog and human liver. Arteriosclerosis 8:288–297

Dhom G (1981) Die Nebennierenrinde. In: Doerr W, Seifert G, Uehlinger E (eds) Spezielle pathologische Anatomie, vol 14/ii. Pathologie der endokrinen Organe II. Springer, Berlin Heidelberg New York, pp 729–970

Dietel M, Arps H, Gerding D, Trapp M, Niendorf A (1987) Establishment of primary cell cultures: experiences with 155 cell strains. Klin Wochenschr 65:507–512

Dralle H, Schröder S, Gratz KF, Grote R, Padberg B, Hesch RD (1990) Sporadic unilateral adrenomedullary hyperplasia with hypertension cured by adrenalectomy. World J Surg (in press)

Geuze HJ, Slot JW, Yanagibashi K, McCracken JA, Schwartz AL, Hall PF (1987) Immunogold cytochemistry of cytochromes P-450 in porcine adrenal cortex. Two enzymes (side-chain cleavage and 11 beta-hydroxylase) are co-localized in the same mitochondria. Histochemistry 86:551–557

Grimelius L, Wilander E (1984) Silver impregnation and other non-immunocytochemical staining methods. In: Polak JM, Bloom SR (eds) Endocrine tumours: the pathobiology of regulatory peptide-containing tumours. Churchill-Livingstone, Edinburgh, pp 95–115

Hacker GW, Bishop AE, Terenghi G, Varndell IM, Aghahowa J, Pollard K, Thurner J, Polak JM (1988) Multiple peptide production and presence of general neuroendocrine markers detected in 12 cases of human phaeochromocytoma and in mammalian adrenal glands. Virchows Arch [A] 412:399–411

Hamid Q, Varndell IM, Ibrahim NB, Mingazzini P, Polak JM (1987) Extraadrenal paragangliomas. An immunocytochemical and ultrastructural report. Cancer 60:1776–1781

Heitz PU (1987) Neuroendocrine tumor markers. In: Seifert G (ed) Morphological tumor markers. Springer, Berlin Heidelberg New York, pp 279–306

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Hui DY, Brecht WJ, Hall EA, Friedman G, Innerarity TL, Mahley RW (1986) Isolation and characterization of the apolipoprotein E receptor from canine and human liver. J Biol Chem 261:4256–4267

Kaetzel CS, Rao CK, Lamm ME (1987) Protein disulphide-isomerase from human placental and rat liver. Purification and immunological characterization with monoclonal antibodies. Biochem J 241:39–47

Kimura N, Sasano N, Yamada R, Satoh J (1988) Immunohistochemical study of chromogranin in 100 cases of pheochromocytoma, carotid body tumour, medullary thryoid carcinoma and carcinoid tumour. Virchows Arch [A] 413:33–38

Krause M, Reinhardt D, Kruse K (1988) Phaeochromocytoma without symptoms: desensitization of the α- and β-adrenoceptors. Eur J Pediatr 147:121–122

Mackay AM (1969) Atlas of human adrenal cortex ultrastructure. In: Symington T (ed) Functional pathology of the human adrenal gland, part IV. Williams and Wilkins, Baltimore, pp 346–489

Mitschke H, Saeger W, Breustedt JJ (1973) Zur Ultrastruktur der Nebennierenrindentumoren beim Cushing-Syndrom. Virchows Arch [A] 360:253–264

Myllylä R, Koivu J, Pihlajaniemi T, Kivirikko KI (1983) Protein disulphide-isomerase activity in various cells synthesizing collagen. Eur J Biochem 134:7–11

Neville DM (1971) Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. J Biol Chem 246:6328–6334

Page DL, DeLellis RA, Hough AJ (1986) Tumors of the adrenal. Atlas of tumor pathology, 2nd series, fascicle 23. Armed Forces Institute of Pathology, Washington, DC

Plouin PF, Chatellier G, Delahousse M, Rougeot MA, Duclos JM, Pagny JY, Corvol P, Ménard J (1987) Recherche, diagnostic et localisation du phéochromocytome. 77 case dans une population de 21 420 hypertendus. Presse Med 16:2211–2215

Probst A (1965) Elektronenmikroskopie der Nebennierenrinde bei primärem Aldosteronismus. Beitr Pathol Anat 131:1–21

Ramsay JA, Asa SL, Nostrand AWP van, Hassaram ST, Harven EP de (1987) Lipid degeneration in pheochromocytomas mimicking adrenal cortical tumors. Am J Surg Pathol 11:480–486

Samaan NA, Hickey RC (1987a) Pheochromocytoma. Semin Oncol 14:297–305

Samaan NA, Hickey RC (1978b) Adrenal cortical carcinoma. Semin Oncol 14:292–296

Sasano H, Okamoto M, Sasano N (1988a) Immunohistochemical study of cytochrome P-450 11 β-hydroxylase in human adrenal cortex with mineralo- and glucocorticoid excess. Virchows Arch [A] 413:313–318

Sasano H, White PC, New MI, Sasano N (1988b) Immunohistochemical localization of cytochrome P-450 C21 in human adrenal cortex and its relation to endocrine function. Hum Pathol 19:181–185

Schröder S, Niendorf A, Dietel M, Beisiegel U (1989) Immunocytochemical differential diagnosis of adrenocortical neoplasm through new monoclonal antibody D11 (A 1193). Proc Am Assoc Cancer Res 30:300

Scott HW, Reynolds V, Green N, Page D, Oates JA, Robertson D (1982) Clinical experience with malignant pheochromocytomas. Surg Gynecol Obstet 154:801–818

Shapiro B, Sisson JC, Lloyd R, Nakajo M, Satterlee W, Beierwaltes WH (1984) Malignant pheochromocytoma: clinical, biochemical and scinigraphic characterization. Clin Endocrinol 20:189–203

Sommers SC, Terzakis JA (1970) Ultrastructural study of aldosterone-secreting cells of the adrenal cortex. Am J Clin Pathol 54:303–308