Virchows Archiv A Pathol Anat (1989) 414:263–271

Virchows Archiv A
Pathological Anatomy and Histopathology

Heterogeneity of Wilms' tumour blastema

An immunohistological study*

F.W. Albeda[1], W.M. Molenaar[1], L. de Leij[2], and A.H. Thijs-Ipema[1]

[1] Department of Pathology,
[2] Department of Clinical Immunology, University of Groningen, Oostersingel 63, 9713 EZ Groningen, The Netherlands

Summary. Antigen expression in fourteen cases of Wilms' tumour was assessed with a panel of monoclonal antibodies. The panel included antibodies reactive with intermediate filament proteins and antibodies reactive with membrane markers originally reported to be associated with small cell lung carcinoma. The immunohistological findings in the tumours were compared to results obtained in adult and fetal kidney.

The antigen profile in the blastemal tissue component of the tumours revealed both characteristics of embryonic tissue and signs of early epithelial differentiation. In addition, the histologically apparent transition between blastema and tubules was shown to be reflected in a concurrently occurring and gradual increase in the number of expressed epithelial antigens.

Between different tumours, heterogeneity in the degree of epithelial differentiation in the blastema was found. In addition, one case showing foci of anaplasia proved to have an entirely different antigen profile when compared with the other thirteen, non-anaplastic cases. This result is discussed in relation to the different clinical behaviour of focally anaplastic tumours.

It is concluded that immunohistology can confirm and extend the histological classification of Wilms' tumour. In addition, new subtypes may be identified in this way.

Key words: Wilms' tumour – Differentiation – Monoclonal antibodies – Fetal kidney – Anaplasia

Introduction

Most cases of Wilms' tumour consist of three tissue components, blastema, epithelium and mesenchyme although biphasic and even monophasic tumours are seen. Following the introduction of radio- and chemotherapy the originally very bad prognosis has been considerably improved (Beckwith and Palmer 1978). Nowadays prognosis is dependent on a number of factors such as the age of the patient and stage of disease at presentation. As a histopathological criterion a high degree of epithelial differentiation appears to be associated with good survival (Lawler et al. 1977), whereas the occurrence of anaplasia is mostly associated with bad clinical outcome (Beckwith et al. 1978; Breslow et al. 1986; Bonadio et al. 1985).

In a previous study from our laboratory it was found that the blastemal component of Wilms' tumour may play a major role in the metastatic behaviour of the tumour, and, in addition, appears to be the component which is most sensitive to chemotherapy (Van Leeuwen et al. 1987). Therefore the exact biological characteristics of this tissue component could be a major factor in the clinical behaviour of the tumour as a whole. The (immunohistological) studies described thus far have not revealed a great deal of heterogeneity in the blastemal component in Wilms' tumour, nor within individual tumours, nor between tumours (Altmannsberger et al. 1984; Denk et al. 1985; Yeger et al. 1985, 1987; Feitz et al. 1986; Hennigar et al. 1986; Ishii et al. 1987).

To assess the antigen profile of the blastema in more detail a panel of monoclonal antibodies (mabs) was applied to fourteen Wilms' tumours. In this panel some antibodies were used which were shown to react with the different tissue components in Wilms' tumour. Mesenchyme was probed with an antibody against vimentin, muscle with an antibody against desmin, epithelium with an antibody against cytokeratin and with MOC-31, and undifferentiated tissue with MOC-1. MOC-31 and MOC-1 were originally reported to be directed against respectively epithelial- and neuroendo-

* Supported by S.K.O.G. (Foundation for pediatric oncology Groningen)

Offprint requests to: F.W. Albeda

crine-associated small cell lung carcinoma-antigens (De Leij et al. 1985, 1986, 1987a; Postmus et al. 1986). More recently has been shown that MOC-1 also reacts with early, undifferentiated epithelia present in first trimester fetal lungs (De Leij et al. 1987b). In addition, four monoclonal antibodies directed against different embryonal differentiation markers are used in the present study. These antibodies have been reported to be also reactive with mature kidney to some extent (Solter et al. 1978; Fox et al. 1981; Andrews et al. 1982, 1984; Shevinsky et al. 1982).

Materials and methods

Surgically resected Wilms' tumour specimens were received from 14 patients ranging in age from 5 to 63 months; all biopsies were taken from primary, untreated tumours. Fresh tissue was snapfrozen in freon and stored in liquid nitrogen until further use. Histologically one tumour showed a focus containing anaplastic tubules in an otherwise typical Wilms' tumour. From this patient frozen sections were taken from areas with and without anaplasia. Specimens derived from four fetal kidneys (maturation ranging from 22 to 32 weeks menstrual age), from one kidney of a four year old child and from a kidney of an adult were used as controls.

Multiple paraffin sections of each tumour were reviewed to confirm the diagnosis and to evaluate the representativeness of the frozen sections used for immunohistology, especially with regard to the relative amounts of blastema, epithelium and mesenchyme. The different components were distinguished according to the criteria of Bennington and Beckwith (1975); only clearcut tubular formations with a central lumen were considered to be epithelial. The tumours were graded according to their degree of epithelial differentiation on a scale from 0 to 3 as described by Lawler et al. (1977), with 0 corresponding to no discernible tubules and 3 corresponding to a tumour consisting almost entirely of tubules.

Mabs directed against intermediate filament proteins are: Vimentin (Vim), an anti-vimentin antibody reactive with mesenchymal tissues and tumours (Ramaekers et al. 1982); Desmin (Des), an anti-desmin antibody reactive with smooth and striated muscle and their tumours (Ramaekers et al. 1983a), and RGE 53, an anti-cytokeratin antibody, reactive with a low-molecular weight cytokeratin (Ck 18) present in non-keratinizing epithelia and their tumours (Ramaekers et al. 1983b; Cooper et al. 1985).

Two antibodies, designated MOC-1 and MOC-31, were originally raised against small cell lung carcinoma (De Leij et al. 1985, 1987a). MOC-1 is reactive also with neural and neuroendocrine tumours, whereas MOC-31 reacts also with simple epithelia and their tumours (De Leij et al. 1985, 1986, 1987a; Postmus et al. 1986). MOC-1 and MOC-31 detect membrane antigens with apparent molecular weights of 120 kDa and 40 kDa respectively (De Leij et al. in press). Vim, Des, RGE 53, MOC-1 and MOC-31 were purchased from Eurodiagnostics, Apeldoorn, The Netherlands. Four mabs (SSEA-3, SSEA-3, TRA-1–60, TRA-1–81, were kind gifts of dr. P.W. Andrews, the Wistar Institute, Philadelphia, USA), were raised against embryonal cell carcinoma and appeared to be stage-specific markers of human and murine embryogenesis. These mabs are also reactive with fetal or adult kidney tissue (Solter et al. 1978; Fox et al. 1981; Andrews et al. 1982, 1984; Shevinsky et al. 1982).

Frozen sections were incubated with the various mabs and stained with a two-step immunoperoxidase method (Timens et al. 1985).

Results

Sections taken from fetal and adult kidneys were tested for reactivity with Vim, RGE 53, MOC-31, and MOC-1. The results are summarized in Table 1. A difference in immunohistological staining was observed between the childhood and various adult kidney preparations on the one hand and the fetal kidney preparations on the other. In the fetal kidney, cytokeratin 18 (probed with RGE 53) and the epithelium associated membrane antigen detected by MOC-31 reacted heterogeneously with the different cells present in the developing nephron. Before the S-shaped body stage the cells were non-reactive with both antibodies, whereas in the S-shaped body stage a reaction with MOC-31 but not with RGE 53 was apparent (for a review of the development of the nephron, see Kazimierczak 1971). After the S-body stage a gradual increase in reactivity could be noticed with MOC-31, whereas the cells became also reactive with RGE 53. This increase in expression of epithelium associated antigens apparently occurred in parallel with the acquisition of a higher degree of epithelial differentiation as could be appreciated histologically by the outgrowth of tubules. The tubules and collecting ducts reacted positively with RGE 53 and MOC-31 both in the fetal and adult kidney (see Fig. 1A). All cells in the epithelium of Bowmans capsule reacted with MOC-31 to some extent, whereas RGE 53 stained only a few. In the adult kidney no reactivity with MOC-1 could be seen. In contrast, in the fetal kidney, MOC-1 reacted with a number of different cell types. Firstly, the uninduced mesenchymal cells in the nephrogenic zone reacted positively with MOC-1.

Table 1. Immunohistology of fetal and adult kidney (fetal/mature kidney)

	MOC-1	MOC-31	RGE 53	Vim
Nephrogenic zone	+ / *	− / *	− / *	+ / +
S-shaped body	+ / *	+ / *	− / *	+ / +
Glomerulus	− / −	− / −	− / −	+ / +
Bowman's capsule	− / −	+ / w	−[2] / −[2]	− / −
Tubules	w[1] / −	+ / +	+ / +	− / +[3]
Collecting ducts	− / −	+ / +	+ / +	− / −
Interstitium	+ / −	− / −	− / −	+ / −

* = not present. w = weak staining. [1] distal tubules negative, [2] focally positive, [3] atrophic tubules only

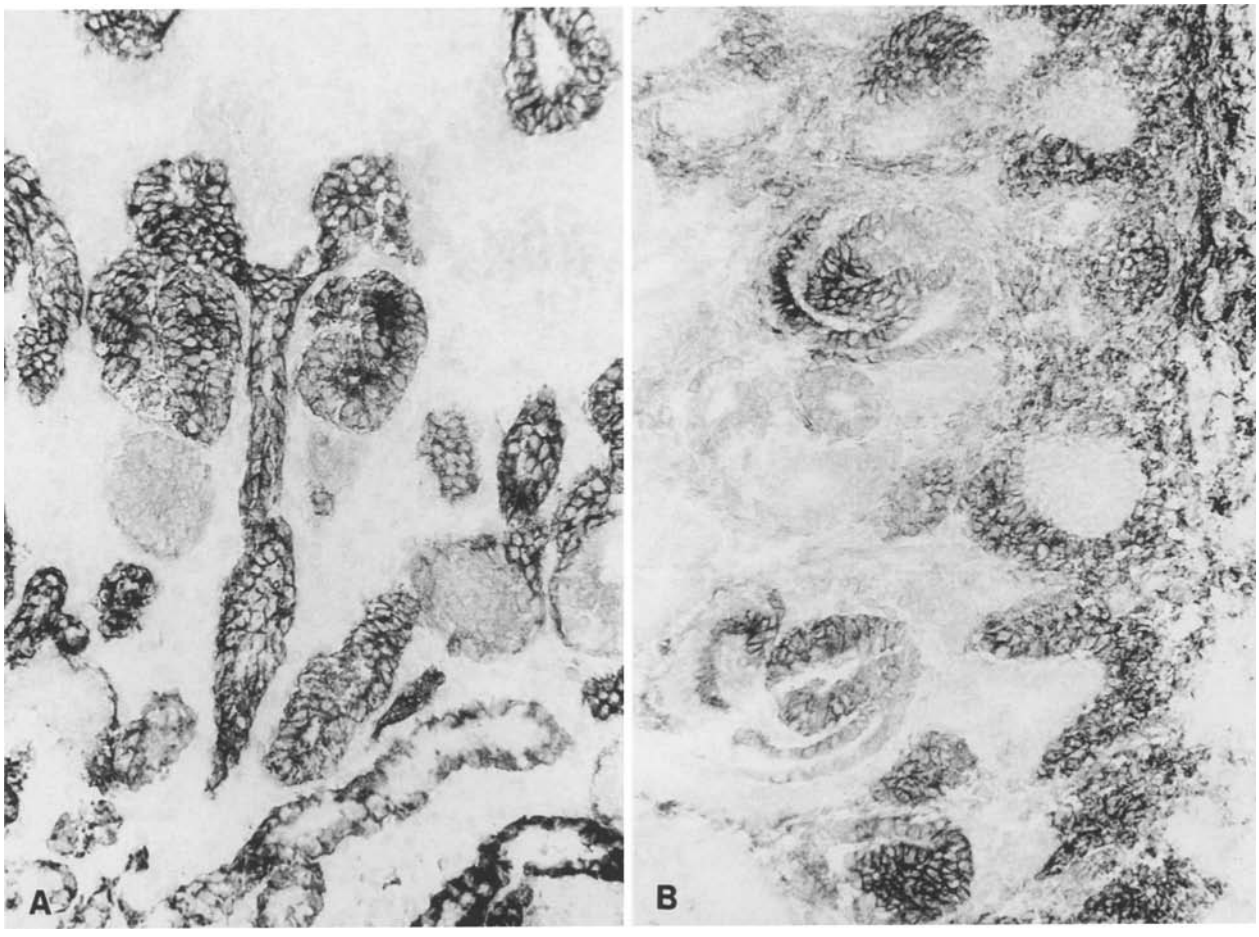


Fig. 1. A Fetal kidney, 22 weeks, MOC-31. In the central part a collecting duct can be seen, which has one ampulla on each side. Underneath each ampulla is an S-shaped body. All these structures are clearly MOC-31 positive, as are the tubules visible at the bottom. Two developing glomeruli are also visible, showing less staining with MOC-31. Magnification: 224×. **B** Fetal kidney, 22 weeks, MOC-1. Within the MOC-1 positive nephrogenic zone, two non-staining ampulla of collecting ducts are visible as punched out globules. Next to the nephrogenic zone three partly MOC-1-staining primitive glomeruli, in various stages of development, are visible. Magnification: 224×

In the developing nephron the reaction with MOC-1 appeared to diminish as the cells become more differentiated. This is illustrated in Fig. 1 B: clear reactivity with MOC-1 is present in the renal vesicle and S-shaped body, whereas staining decreases as capillary loops of the future glomerulus develop. The more developed nephron was unreactive with MOC-1, but for the proximal tubules which stained weakly. Secondly, in the fetal kidney, the loose fibrous tissue of the renal interstitium reacted positively with MOC-1. In the fetal kidney, vimentin, as probed with Vim, was present in the uninduced mesenchymal cells of the nephrogenic zone and in the S-shaped body, whereas the developing tubules were negative. Vimentin could be also detected in the cells of the fully developed glomerulus in fetus and adult: the glomerular tuft and the visceral as well as the parietal epithelium of Bowmans capsule were stained. Vimentin was detected in the glomerulus, in the renal stroma and in the epithelium of atrophic tubules in the adult kidney.

The antigenic profile of Wilms' tumour was assessed with monoclonal antibodies Des, Vim, RGE 53, SSEA-1, SSEA-3, TRA-1–60, TRA-1–81, MOC-31, and MOC-1. A reaction with Des, indicative of the presence of desmin in the cells, was observed in only one of the tumours assessed. In this case extensive rhabdomyomatous differentiation was also seen in paraffin sections. The embryonal differentiation markers detected by SSEA-1, SSEA-3, TRA-1–60 and TRA-1–81 were present only in some scattered cells, which were seen in most tumours and in all three histologically discernable tumour components. No clearly recognizable pattern could be deduced from these staining results.

Table 2. Immunohistology of Wilms' tumour

A. Blastema	100%	<100-90%	90-50%	50-10%	10->0%	0%	(*n*)
MOC-1	7	4	3	0	0	0	14
MOC-31	3	1	3	4	3	0	14
RGE 53	0	0	2	1	8	3	14
Vim	1	0	2	3	7	1	14
B. Epithelium	**100%**	**<100-90%**	**90-50%**	**50-10%**	**10->0%**	**0%**	**(*n*)**
MOC-1	0	(1)*	2	3	2	3	10
MOC-31	9	1	(1)*	0	0	0	10
RGE 53	3(+1)*	5	2	0	0	0	10
Vim	0	0	0	0	3	6(+1)*	9
C. Mesenchyme	**100%**	**<100-90%**	**90-50%**	**50-10%**	**10->0%**	**0%**	**(*n*)**
MOC-1	1	0	4	6	0	3	14
MOC-31	0	0	0	0	0	14	14
RGE 53	0	0	0	0	0	14	14
Vim	14	0	0	0	0	0	14

The number of tumours showing positive staining is indicated in each column (positivity is expressed as an estimated percentage of stained cells present). The total of cases assessed is given under (*n*). Epithelial structures were not discernible in each tumour, therefore *n* is not always equal to 14 in Table 2B

* The number given in parenthesis concerns the anaplastic focus in the focal anaplastic tumour. This is not counted in the total

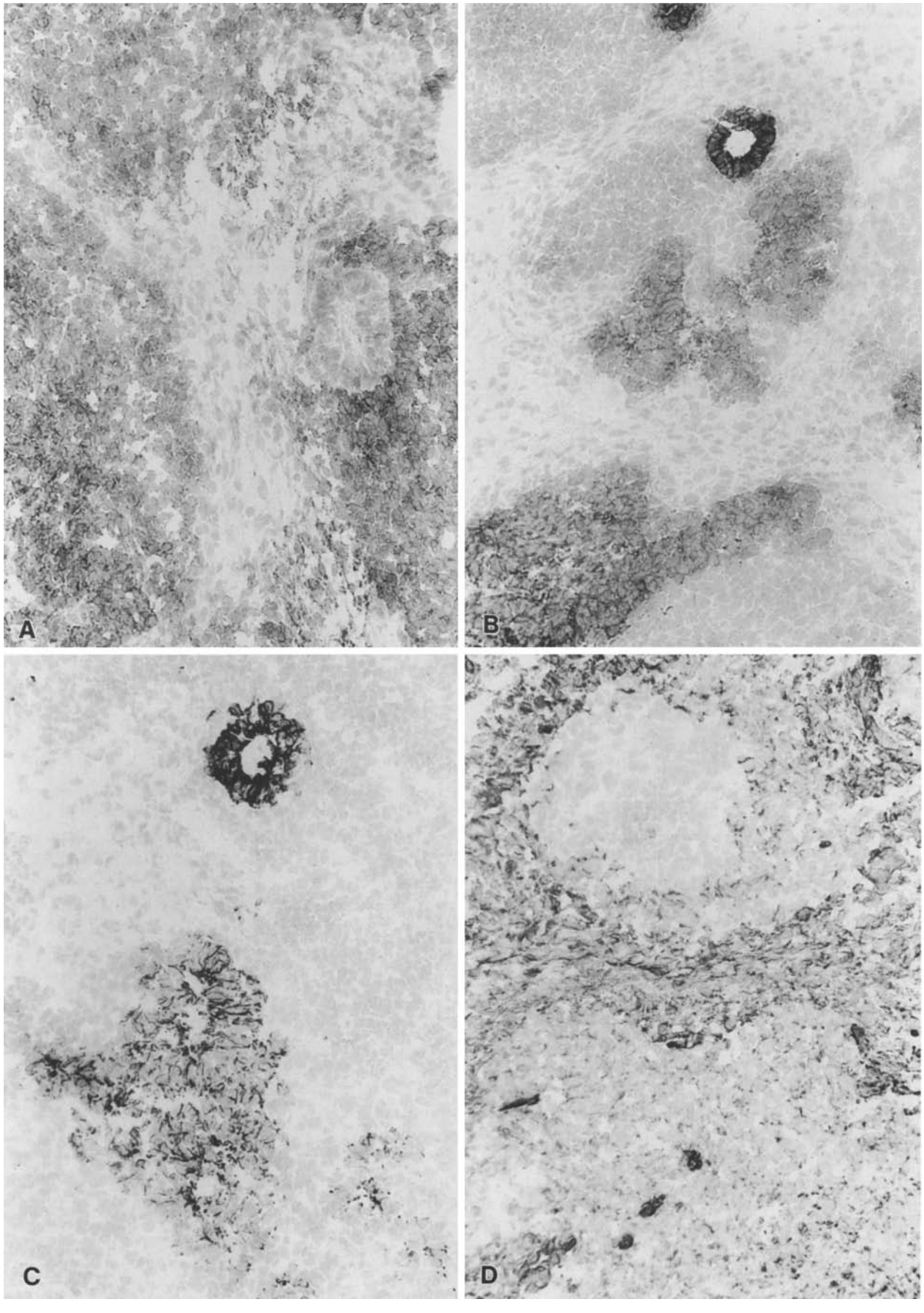
In contrast to the results given above, the antigens detected by MOC-31, MOC-1, Vim and RGE 53 showed discrete patterns. Table 2 summarizes the results obtained with these antibodies. The results in the thirteen tumours showing no signs of anaplasia are dealt with below.

The blastemal tissue component in Wilms' tumour always reacted with MOC-1, although some negative cells were present in six of the thirteen tumours. In these cases MOC-1 negative cells were present almost exclusively in those parts of the blastema which appeared to have a more compact tissue structure and in which cell rosettes could be seen (Fig. 2A). These areas were partly localized around tubules and could be characterized as having an epitheloid aspect.

MOC-31 reacted with all or almost all cells present in the blastema of four out of thirteen tumours, with the strongest reaction in the more compact blastemal parts (Fig. 2B). In the blastema of the remaining nine tumours a variable reaction with MOC-31 was observed, with the number of positive cells ranging between 5 and 80%. In one of these cases MOC-31 reacted with blastemal tissue only in peritubular foci. In the other cases strong staining was seen in the compact tissue areas of the blastema, whereas the remaining blastemal tissue reacted less, weakly or not at all. All tumours stained with MOC-31 in the blastema to some extent. Depending on the extent of this staining, the reactivity of MOC-31 and MOC-1 overlapped.

The staining pattern of RGE 53 in the blastema

Fig. 2. (**A**) Wilms' tumour, MOC-1. Blastemal nodules are almost entirely positively stained, although the staining intensity varies. In the mesenchymal bands located between the nodules some positively stained cells can also be seen. One non-stained tubule can be identified in the nodule located at the right. Magnification: 224×. (**B**) Wilms' tumour, MOC-31. Strongly stained tubule and a moderately stained part of the blastema is shown. In the remaining of the blastemal nodule weak staining can be appreciated as finely dispersed stripes and dots. Magnification: 224×. (**C**) Wilms' tumour, RGE 53. One strongly stained tubule is seen next to positively stained blastema. Magnification: 224×. (**D**) Wilms' tumour, Vim. A positively stained band of mesenchyme is located at the center, a non-stained blastemal nodule at the top and a weakly stained nodule at the bottom of the picture. A few positively stained small vessels are visible in the lower half. Magnification: 224×


A
B
C
D

resembled that of MOC-31, although staining was generally less (Fig. 2C). Three tumours showed no staining with RGE 53 in the blastema, whereas in ten tumours a (partial) reaction was noticed. From these last cases, seven showed a reaction limited only to the most compact parts of the blastema, (1→10% of the blastemal cells). In contrast to the findings with MOC-31, only a little overlap in staining of RGE 53 and MOC-1 was observed.

A comparison of the relative reactivity of RGE 53 or MOC-31 in the blastema with the relative amount of epithelium present, graded according to Lawler et al. (1977), revealed no correlation in the assessed tumour cases.

The presence of vimentin (assessed with Vim) in the blastema of various tumours varied considerably, ranging from no staining (one case) to staining of all (one case) cells. Vim negative cells were preferentially found in the peritubular parts of the blastema (Fig. 2D).

In the various tumours both segregation and a certain degree of coexpression of the various markers could be noticed. For instance, depending on the degree of Vim staining, an overlap between Vim and MOC-1 reactivity was found. In addition, in six tumours part of the blastema stained both with Vim and with MOC-31, whereas overlap between Vim and RGE 53 was noted in two tumours. One of these last two cases was characterized by the presence of monomorphous blastema, without formation of tubules or compact cell groups. In this case 40% of blastemal cells stained with Vim, 80% with RGE 53, whereas both MOC-31 and MOC-1 reacted with all blastemal cells.

Tubules were identified in ten cases. The cells present in these tubules were partly reactive with MOC-1 in seven cases, whereas in three cases also a focal reaction with Vim was noted (Fig. 2A–2C). In contrast, all tubular cells were stained with MOC-31, whereas the majority was reactive with RGE 53. A few completely RGE 53-negative tubules were seen.

All mesenchymal cells reacted with Vim, whereas no reaction was noted with RGE 53 and MOC-31 (Fig. 2A–2D). In eleven cases an additional staining with MOC-1 was seen in the mesenchyme with staining varying between 20% and 100%. In three cases no reactivity of MOC-1 in the mesenchyme was found.

The one case showing focal anaplasia in the epithelial component had an antigenic profile quite different from the findings with the other cases. Inside the blastemal nodules, a highly heterogeneous staining pattern was found. Some nodules did not react with any of the assessed mabs. In other nodules some staining with MOC-1 was seen. MOC-1 positive cells occurred in these nodules, partly as scattered cells and partly as small cell-groups in otherwise negative blastemal tissue. MOC-1 positive cells accounted for about 10% of blastemal cells, Vim stained about 15%, whereas about 40% was reactive with MOC-31 and RGE 53. Therefore, in contrast to the findings given above, only a minority of MOC-31 and RGE 53 positive cells in the blastema also appeared to react with MOC-1.

In the non-anaplastic part of the tumour, only a few tubules could be seen. These reacted positively with MOC-1, MOC-31 and also focally with RGE 53. The anaplastic epithelium as a whole was strongly reactive with both MOC-1, MOC-31 and RGE 53. Mesenchyme did not stain with MOC-1.

Discussion

The objective of the present study was to provide a further immunohistological characterization of Wilms' tumour blastema using a panel of mabs. The immunohistological patterns found in the blastema indicated a considerable degree of heterogeneity in this tumour component, both within one tumour and between different tumours. Heterogeneity of the blastema of Wilms' tumour is a phenomenon that has received little attention so far (Altmannsberger et al. 1984; Denk et al. 1985; Yeger et al. 1985, 1987; Feitz et al. 1986; Hennigar et al. 1986; Ishii et al. 1987).

For the non-anaplastic tumours the following general staining patterns was evident. MOC-1 appears to be a marker of undifferentiated blastemal cells especially. Decreased expression of MOC-1 was noted in those parts of the blastema showing a more condensated pattern, especially around tubules, whereas the tubules themselves were virtually unreactive. A similar staining tendency was observed with Vim, whereas the reverse appears to apply to MOC-31 and especially RGE 53. These epithelial differentiation markers showed the strongest staining in the tubules, with decreased reactivity in peritubular blastema and some (MOC-31) or no (RGE 53) staining in the undifferentiated blastema. The results strongly suggest the occurrence of a gradient of increasing epithelial differentiation starting from the blastema and extending to the tubules. Similarities in antigen expression between peritubular blastema and tubules have been described previously, among others by Denk et al. (1985) using mabs against desmoplakin, which is also a marker for epithelial differentiation (Yeger et al. 1985, 1987; Hennigar et al.

1986; Ishii et al. 1987). The finding in the present study that both vimentin (assessed with Vim) and cytokeratin (assessed with RGE 53) are expressed in the blastema confirms the results from others and may reflect the primitive or pluripotential character of blastema (Altmannsberger et al. 1984; Denk et al. 1985; Yeger et al. 1985; Feitz et al. 1986).

Comparison of the staining patterns of various assessed mabs with the matching normal histology in fetal and adult kidneys suggests that MOC-1 is the most 'primitive' marker. According to this scheme MOC-31, being present already at very early stages of differentiation, appears to be a general marker of epithelial differentiation in the kidney, whereas RGE 53, which is known to be reactive with cytokeratin 18, characterizes more established epithelial differentiation. The indication of MOC-1 as a marker for primitive cells in the kidney appears remarkable since this mab was originally reported to be a specific marker for neuroendocrine differentiation (De Leij et al. 1985, 1986, 1987a; Postmus et al. 1986). The interpretation of MOC-1 as being a marker of undifferentiated cells in fetal kidneys (and Wilms' tumour) is supported by similar findings in the fetal lung, where MOC-1 staining was also observed in the epithelium of the primitive lung only in the first trimester of pregnancy, whereas lung epithelia in the second trimester and more mature stages were unreactive (De Leij et al. 1987b).

The tubular structures present in Wilms' tumour are thought to be related to the tubules in normal (fetal) kidney (Hennigar et al. 1986; Ishii et al. 1987; Yeger et al. 1987). In agreement with this MOC-1 showed only limited reactivity both with the primitive tubules of the S-shaped body in the fetal kidney and with tubules present in Wilms' tumour. Similarily, Vim (indicative for the presence of vimentin) reacted both with the S-shaped body and with some tubules present in Wilms' tumour. Although mature kidney epithelium is generally unreactive with Vim, it may express vimentin under certain circumstances, as has been described for damaged and regenerating tubules (Gröne et al. 1988). In the present study a similar observation was made for the presence of vimentin in the atrophic tubules of adult kidney.

Both MOC-31, detecting a membrane antigen with apparent molecular weight of 40 kDa (De Leij et al. in press) and RGE 53, detecting cytokeratin 18, have been reported to be markers for epithelial differentiation. In the present study it was found that, at least in normal adult and fetal kidney tissue, MOC-31 is a more general marker for epithelial differentiation than RGE 53. For example the S-shaped body in fetal kidneys reacted with MOC-31, whereas RGE 53 was unreactive. In agreement with this we have reported previously that in a large number of epithelium derived tumours staining with MOC-31 is generally much more extensive than staining with RGE 53 (De Leij et al. 1986, 1987a). More importantly, MOC-31 is unreactive with non-epithelial tissues expressing cytokeratin such as normal or malignant mesothelia (De Leij et al. 1987a), epithelioid sarcomas and synovial sarcomas (unpublished observations). It can be concluded therefore that the reactivity of MOC-31 in Wilms' tumour blastema indicates 'real' epithelial differentiation. In this respect it is interesting to note that heterogeneity in reactivity with MOC-31 in the blastema can be demonstrated between different Wilms' tumour cases. Reactivity with MOC-31 in the blastema was homogeneously positive in only three of thirteen cases, whereas in the other tumours a variable degree of staining could be observed. However all tumour cases assessed showed some staining with MOC-31 in the blastema. Since, firstly, MOC-31 detects a differentiation marker which is already present on developing epithelia before morphological apparent differentiation and, secondly, since undifferentiated blastema appears to play a key role in the process of metastasis as well as the sensitivity of the tumour to chemotherapy (Van Leeuwen et al. 1987) the prognosis of Wilms' tumour may be related to the degree of epithelial differentiation in the blastema as assessed by MOC-31. To resolve this question a more extended clinical study has to be done. The number of cases in the present study is too small to obtain any reliable evaluation.

In contrast to the immunohistologically apparent gradual transition from undifferentiated blastema to tubules, such a transition between blastema and mesenchyme was not found. Nevertheless some of the mesenchymal cells also appeared to react with MOC-1. In this respect, the tumour mesenchyme resembles the interstitium of the fetal kidney.

It is apparent from the results that it was not possible to detect early signs of muscle differentiation in morphological undifferentiated mesenchyme (or blastema) with Des mab.

The immunohistological findings in the one case showing focal anaplasia, are interesting, since the staining patterns are remarkably different from those seen in the other tumours. The fact that the anaplastic tubules in this case were reactive with MOC-1 could be taken as an indication of a more undifferentiated or embryonal make-up of these

tubules despite the histologically apparent differentiation. This might be interpreted as a sign of greater disregulation in this tumour when compared with non-anaplastic Wilms' tumours. In addition, the immunohistological findings in the non-anaplastic component of this tumour also demonstrated a deviating pattern when compared with the non-anaplastic tumours. These results indicate that the morphologically 'normal' parts of the tumour have in fact an 'anaplastic' phenotype. If these findings can be confirmed in more cases of focally anaplastic Wilms' tumour, immunohistology using a panel of mabs appears to be indicated in order to alert the pathologist to the probability of a (focal) anaplastic tumour. These considerations are based on the findings in only one case. Nevertheless, the fact that the prognosis of this kind of tumour is similar to the prognosis those with diffuse anaplasia (Bonadio et al. 1985), also points towards a more basic difference. The immunohistological observations in this case are further supported by results obtained with DNA-flow cytometry. Hyperdiploidy was found in both the non-anaplastic and in the separately measured anaplastic parts of the tumour (unpublished observation). Since hyperdiploidy is associated with anaplasia (Douglass et al. 1986), it appears therefore that this focal anaplastic tumour displays features of anaplasia in its non-anaplastic parts at the chromosomal level.

Acknowledgement. We wish to thank mr. H. Wierenga for his assistance with photography.

References

Altmannsberger M, Osborn M, Schäfer H, Schauer A, Weber K (1984) Distinction of nephroblastomas from other childhood tumors using antibodies to intermediate filaments. Virchows Arch [B] 45:113–124

Andrews PW, Goodfellow PN, Shevinsky LH, Bronson DL, Knowles BB (1982) Cell surface antigens of a clonal human embryonal carcinoma cell line: morphological and antigenic differentiation in culture. Int J Cancer 29:523–531

Andrews PW, Banting G, Damjanov I, Arnaud D, Avner P (1984) Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells. Hybridoma 3:347–361

Beckwith JB, Palmer NF (1978) Histopathology and prognosis of Wilms' tumor. Results from the First National Wilms' Tumor Study. Cancer 41:1937–1948

Bennington JL, Beckwith JB (1975) Tumors of the kidney, renal pelvis and ureter. Atlas of tumor pathology, second series, fascicle 12. Armed Forces Institute of Pathology, Washington DC, USA

Bonadio JF, Storer B, Norkool P, Farewell VT, Beckwith B, D'Angio GJ (1985) Anaplastic Wilms' tumor: Clinical and pathological studies. J Clin Oncol 3:513–520

Breslow NE, Churchill G, Nesmith B, Thomas PRM, Beckwith JB, Biemann Othersen H, D'Angio GJ (1986) Clinicopathologic features and prognosis for Wilms' tumor patients with metastases at diagnosis. Cancer 58:2501–2511

Cooper D, Schermer A, Sun T-T (1985) Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: Strategies, applications and limitations. Lab Invest 52:243–256

De Leij L, Poppema S, Klein Nulend J, ter Haar A, Schwander E, Ebbens F, Postmus PE, The TH (1985) Neuroendocrine differentiation antigen on human lung carcinoma and Kulchitski cells. Cancer Res 45:2192–2200

De Leij L, Postmus PE, Poppema S, Elema JD, The TH (1986) The use of monoclonal antibodies for the pathological diagnosis of lung cancer. In: Hansen HH (ed) Lung cancer: Basic and clinical aspects. Martinus Nijhoff Publishers, Boston, pp 31–48

De Leij L, Broers J, Ramaekers F, Berendsen H, Wagenaar SjSc (1987a) Monoclonal antibodies in clinical and experimental pathology of lung cancer. In: Ruiter DJ, Fleuren GJ, Warnaar SO (eds) Application of monoclonal antibodies in tumor pathology. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp 191–210

De Leij L, ter Haar JG, Schwander E, Berendsen HH, The TH (1987b) Small cell lung cancer and embryonic lung epithelium share monoclonal antibody defined neuroendocrine-related antigens. Chest Suppl 91S:9S–11S

De Leij L, Berendsen H, Spakman H, Ter Haar J, The TH (1988) Neuroendocrine and epithelial antigens in SCLC. Proceedings Workshop on SCLC Lung Cancer (in press)

Denk H, Weybora W, Ratschek M, Sohar R, Franke WW (1985) Distribution of vimentin, cytokeratins and desmosomal-plaque proteins in human nephroblastoma as revealed by specific antibodies: co-existence of cell groups of different degrees of epithelial differentiation. Differentiation 29:88–97

Douglass EC, Look AT, Webber B, Parham D, Wilimas JA, Green AA, Roberson PK (1986) Hyperdiploidy and chromosomal rearrangements define the anaplastic variant of Wilms' tumor. J Clin Oncol 4:975–981

Feitz WFJ, Debruyne FMJ, Vooys GP, Herman CJ, Ramaekers FCS (1986) Intermediate filament proteins as tissue specific markers in normal and malignant urological tissues. J Urol 136:922–931

Fox N, Damjanov I, Martinez-Hernandez A, Knowles BB, Solter D (1981) Immunohistochemical localization of early embryonic antigen (SSEA-1) in postimplantation mouse embryos and fetal and adult tissues. Dev Biol 83:391–398

Gröne HJ, Weber K, Gröne E, Helmchen U, Osborn M (1988) Coexpression of keratin and vimentin in damaged and regenerating tubular epithelium of the kidney. Am J Pathol 129:1–8

Hennigar RA, Spicer SA, Sens DA, Biemann Othersen H, Garvin J (1986) Histochemical evidence for tubule segmentation in a case of Wilms' tumor. Am J Clin Pathol 85:724–731

Ishii E, Fujimoto J, Tanaka S, Hata J (1987) Immunohistochemical analysis on normal nephrogenesis and Wilms' tumour using monoclonal antibodies reactive with lymphohaemopoietic antigens. Virchows Arch [A] 411:315–322

Kazimierczak J (1971) Development of the renal corpuscle and the juxta-glomerular apparatus. Acta Pathol Microbiol Scand A, Suppl 218

Lawler W, Marsden HB, Palmer MK (1977) Histopathological study of the first medical research council nephroblastoma trial. Cancer 40:1519–1525

Postmus PE, Hirschler-Schulte TJW, de Leij L, Poppema S, Elema JD, Edens ET, Mesander G, Sluiter HJ, The TH

(1986) Diagnostic application of a monoclonal antibody against small cell lung cancer. Cancer 57:60–63

Ramaekers F, Puts J, Kant A, Moesker O, Jap P, Vooys P (1982) Differential diagnosis of human carcinomas, sarcomas and their metastases using antibodies to intermediate-sized filaments. Eur J Cancer Clin Oncol 18:1251–1257

Ramaekers FCS, Puts JJG, Moesker O, Kant A, Huysmans A, Haag D, Jap PHK, Herman CJ, Vooys GP (1983a) Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumours: An overview. Histochem J 15:691–731

Ramaekers F, Huysmans A, Moesker O, Kant A, Jap P, Herman C, Vooys P (1983b) Monoclonal antibody to keratin filaments, specific for glandular epithelia and their tumors. Lab Invest 49:353–361

Shevinsky LH, Knowles BB, Damjanov I, Solter D (1982) Monoclonal antibody to murine embryos defines a stage-specific embryonic antigen expressed on mouse embryos and human teratocarcinoma cells. Cell 30:697–705

Solter D, Knowles BB (1978) Monoclonal antibody defining a stage-specific mouse embryonic antibody (SSEA-1). Proc Natl Acad Sci USA 75:5565–5569

Timens W, Poppema S (1985) Lymphocyte compartments in human spleen. An immunohistologic study in normal spleens and non-involved spleens in Hodgkin's disease. Am J Pathol 120:443–454

Van Leeuwen EH, Postma A, Oosterhuis JW, Meiring A, Cornelisse CJ, Koudstaal J, Molenaar WM (1987) An analysis of histology and DNA-ploidy and primary Wilms' tumors and their metastases and a study of the morphological effects of therapy. Virchows Arch [A] 410:487–494

Yeger H, Baumal R, Bailey D, Pawlin G, Phillips MJ (1985) Histochemical and immunohistochemical characterization of surgically resected and heterotransplanted Wilms' tumor. Cancer Res 45:2350–2357

Yeger H, Baumal R, Harason P, Phillips MJ (1987) Lectin histochemistry of Wilms' tumor. Comparison with normal adult and fetal kidney. Am J Clin Pathol 88:278–285

Received August 8, 1988 / Accepted October 10, 1988