Virchows Arch A (1987) 410:295–307

Virchows Archiv A

Intermediate filaments and desmosomal plaque proteins in testicular seminomas and non-seminomatous germ cell tumours as revealed by immunohistochemistry *

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Summary. Seminomas and non-seminomatous testicular germ cell tumours were studied for the presence of cytokeratin and vimentin filaments and desmosomes using immunohistochemical methods. In the majority of the classical seminomas and in seminomatous areas of mixed tumours most tumour cells appeared to lack cytokeratin filaments. Some seminomas contained a focally variable proportion of cells exhibiting cytokeratin-positive structures while other cases contained only few seminoma cells with a well developed fibrillar cytokeratin network. Gel electrophoresis of cytoskeletal proteins from microdissected regions revealed cytokeratin polypeptides nos. 8 and 18 typical of simple epithelia. In one seminoma, however, all, or almost all, tumour cells contained cytokeratin filaments. This finding is in line with the assumption of transitional forms between seminoma and embryonal carcinoma. Despite the lack – or variable expression – of cytokeratin filaments most seminoma cells contained desmosomes, although often few in number and irregularly distributed at the circumference of the cells. Loosely arranged and often very sparse vimentin fibrils were found in many, but not all seminoma cells. Double label immunofluorescence microscopy suggested that the majority of desmosomes was associated with intermediate filaments of the vimentin type. In contrast, in carcinoma cells of malignant teratomas, in well differentiated epithelial cells of intermediate-type malignant teratomas and in trophoblastic cells present in trophoblastic-type malignant teratomas cytokeratin filament bundles as well as desmosomes were decorated. The arrangement and density of the cytokeratin filament skeleton and of desmosomes varied with degree of maturation of the tissue. The most regular distribution and intensive staining of cytokeratin filaments and desmoplakin was found in "mature" tissues. Vimentin was demonstrated in mesenchymal areas and stroma cells. The results show that seminomas are distinguished from most other germ cell and non-germ cell tumours by the presence of true desmosomes together with scanty vimentin filaments in most tumour cells. In addition, they indicate that seminoma cells can be heterogenous in their cytoskeletal complement and may include cells with cytokeratin expression, indicative of a multipotential character of the initially transformed cell(s).

Key words: Intermediate filaments – Desmosomes – Testicular tumours – Germ cell tumours – Immunohistochemistry

Because of their cell-type specificity which is retained during neoplastic transformation, intermediate filament (IF) proteins have been widely used as histogenetic markers in diagnostic pathology (for review see Osborn and Weber 1983; Ramaekers et al. 1983b). In most tissues a given cell type contains only one IF species. Under certain conditions and in some special tissues, however, several types of IF proteins are co-expressed. Co-expression of cytokeratin and vimentin filaments, for example, occurs in many epithelial cells in culture (Franke et al. 1979) but is rare in tissues. Nevertheless, some human epithelial cells, such as

* Supported in part by Fonds zur Förderung der wissenschaftlichen Forschung (grant No. 4708 to H.D.) and by the Deutsche Forschungsgemeinschaft (grants Mo 345/3 and Fr 308-17)

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mesothelium, ovarian granulosa cells, amnion epithelial cells, epithelia of fetal kidney at a certain stage of development, and some types of epithelial tumours, e.g. mesotheliomas, renal carcinomas, pleomorphic adenomas of salivary glands, and some carcinomas metastatic to serous cavities contain vimentin in addition to cytokeratin (e.g. Caselitz et al. 1981; Krepler et al. 1982; Holthöfer et al. 1983, 1984; Ramaekers et al. 1983a; La Rocca and Rheinwald 1984; Blobel et al. 1985; Czernobilsky et al. 1985; Denk et al. 1985b).

Cytokeratin IF are usually associated with intercellular junctions of the desmosome-type. Therefore, desmosomes and desmosomal proteins serve as additional markers of epithelial differentiation (Müller and Franke 1983; for review see Moll et al. 1986). However, recent immunohistochemical and biochemical studies revealed structures resembling desmosomes in ultrastructure and chemical composition also in association with IF of the desmin type in cardiac muscle, and of the vimentin type in arachnoidal tissue ("desmofibrocytes"), including meningiomas, in nephroblastomas and ovarian granulosa cell tumours (Franke et al. 1982; Schwechheimer et al. 1984; Denk et al. 1985b; Moll et al. 1986).

In recent investigations (Battifora et al. 1984; Ramaekers et al. 1985; Miettinen et al. 1985), the diagnostic significance of cytokeratins and vimentin was examined in testicular tumours, and lack of cytokeratin staining was reported for seminomas in contrast with embryonal carcinomas (Battifora et al. 1984). The present paper reports on immunohistochemical studies of testicular tumours using antibodies to desmosomal plaque proteins (desmoplakins; Müller and Franke 1983; Moll et al. 1986) in addition to those directed against cytokeratins and vimentin. It will be shown that desmosomes are constantly present in a number of tumour cells of seminomas and embryonal carcinomas, independent of the presence of cytokeratin IF, indicative of a non-coordinated expression of both major cytoskeletal components.

Materials and methods

Fifteen testicular tumours (Table 1) were studied. After surgical removal, several portions of the specimens were fixed in 10% phosphate-buffered (pH 7.4) formaldehyde solution and embedded in paraffin according to conventional techniques. Sections, 4 mμ thick, were subjected to routine staining (haematoxylin-eosin, PAS, Van Gieson), and the tumours were classified according to the classification proposed by the British tumour panel (Pugh 1976; Hedinger 1980; Hochstetter and Hedinger 1982) by three independent pathologists. Other pieces of the tumours were immediately frozen in isopentane cooled in liquid nitrogen and stored at −70° C for immunohistochemistry.

Table 1. Testicular seminomatous and non-seminomatous germ cell tumours studied by immunofluorescence microscopy

Histologic diagnosis	N	Age of patients (years)
CS	5	22, 23, 39, 40, 44
CS + MTI	–	
CS + MTU	2	24, 35
CS + MTT	–	
MTI	2	18, 34
MTU	3	22[a], 23, 26
MTT	3	19, 20, 41

N = number of patients; CS = classical seminoma; MTI = malignant teratoma, intermediate type; MTU = malignant teratoma, undifferentiated type; MTT = malignant teratoma, trophoblastic type

[a] see Fig. 11 of Moll et al. 1986

Four mμ thick frozen sections were prepared for immunofluorescence microscopy as described previously (Denk et al. 1985b). The following antibodies were used: (*i*) guinea pig immunoglobulins to murine hepatocytic cytokeratin component D (for designation and immunoreactivity see Franke et al. 1981; this antibody shows a broad range of immunoreactivity with epithelial cells and detects most epithelial cells, including those of simple type); (*ii*) guinea pig antibodies to total cytokeratin polypeptides from human epidermis (Denk et al. 1979); (*iii*) guinea pig antibodies reacting with human cytokeratin no. 18 and "IT-protein" but not with other cytokeratin polypeptides (Moll et al. 1982); (*iv*) monoclonal cytokeratin antibody PKK1 with broad range of cytokeratin immunoreactivity (Labsystems Inc., Helsinki, Finland); (*v*) monoclonal antibody A 53-B/A2 specific for cytokeratin no. 19 (Karsten et al. 1985); (*vi*) rabbit antibodies to vimentin prepared from human aorta (Denk et al. 1985b); (*vii*) monoclonal antibody clone VIM-9 against vimentin (obtained from Viramed, Martinsried, FRG) which reacts (immunoblotting) with vimentin and its degradation products; (*viii*) guinea-pig antibodies to desmoplakins (Moll et al. 1986); (*ix*) monoclonal antibody DP 1&2-215 against desmoplakins (Cowin et al. 1985; Moll et al. 1986); (*x*) monoclonal and polyclonal (rabbit) antibodies to human β-HCG (Dakopatts, Copenhagen, Denmark; Amersham International, Amersham, UK); (*xi*) polyclonal (rabbit) antibodies to human α-1-fetoprotein (AFP; Dako-Immunoglobulins, Copenhagen, Denmark) were used in some instances; (*xii*) binding of the first antibodies was assessed by FITC-, TRITC- or Texas-Red-coupled antibodies (IgG) to guinea pig (goat; Medac E–Y Labs., San Mateo, CA., USA, or Dakopatts, Copenhagen, Denmark), to rabbit (swine; Dakopatts, Copenhagen, Denmark) or to mouse (goat; TAGO, Burlingame, CA, USA; or Dianova, Hamburg, FRG) immunoglobulins as described previously (Denk et al. 1985b). For double immunofluorescence microscopy, both primary antibodies were applied simultaneously and binding was visualized with FITC-coupled anti-guinea pig IgG and TRITC-coupled anti-rabbit or Texas-Red-coupled anti-mouse IgG. In addition, some specimens were also subjected to immunoperoxidase staining using horse radish peroxidase-coupled rabbit antibodies to guinea pig IgG (Dakopatts, Copenhagen, Denmark) to show binding of guinea pig antibodies to cytokeratins and desmoplakins. As controls, preimmune sera, or sera not related to the antigens to be detected were used in the first layer. Slides were viewed and photographed with an epiillumination fluorescence microscope (Zeiss Photomikroskop III).

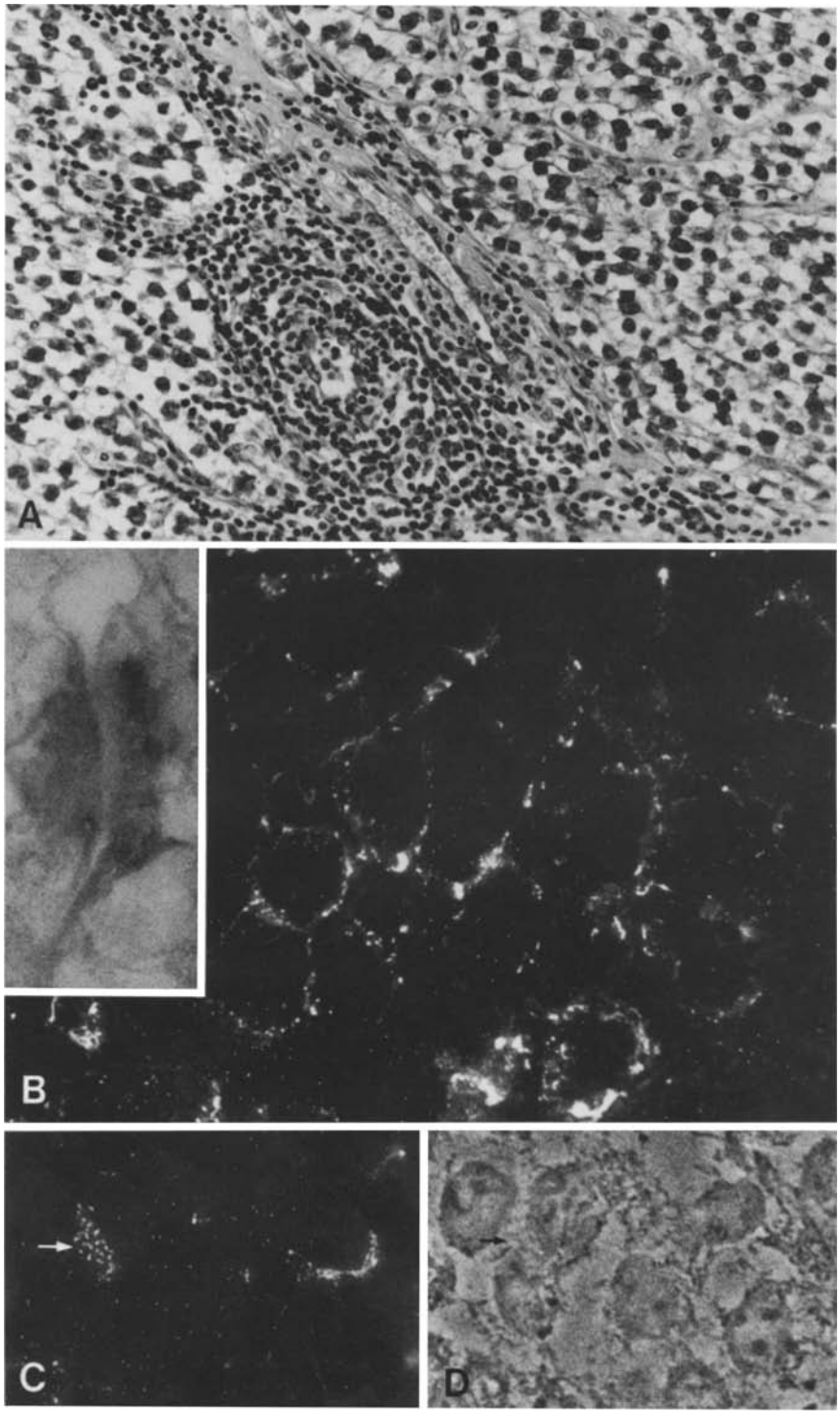

Fig. 1. **A** Classical seminoma consisting of uniform loosely arranged tumour cells with clear cytoplasm separated by connective tissue septa infiltrated by lymphocytes; **B** Demonstration of desmoplakin by specific guinea pig antibodies in immunofluorescence microscopy. Note that most tumour cells contain desmoplakin-reactive spots (desmosomes) at their periphery. Immunoelectron microscopy of a desmosome as revealed by polyclonal desmoplakin antibodies (*inset*); **C** Another case of seminoma stained by the monoclonal desmoplakin antibody DP 1 & 2-215. Note heterogenous distribution of desmosomes which are especially well recognized in the grazing section of a cell-cell boundary at the left side (arrow); **D** Phase contrast picture corresponding to **C**. (**A** HE, × 150; **B** ×800; inset in B, × 117,000; **C**, **D** ×650)

Immunoelectron microscopy. Representative specimens of two classical seminomas were studied by immunoelectron microscopy for the presence of desmosomes using antibodies to desmoplakins. For this purpose, 4 mμ thick cryostat sections were fixed in acetone for 1 min at −20° C. They were then incubated with antibodies to desmoplakins for 45 min at room temperature. Binding of primary antibodies was visualized by horse radish peroxidase-coupled rabbit anti-guinea-pig IgG (incubation time 45 min at room temperature; Dakopatts, Copenhagen, Denmark). Peroxidase was visualized by the 3,3′-diaminobenzidine (DAB) reaction. The color reaction was intensified by treatment with 1% OsO_4 for 30 min. The sections were dehydrated in a series of graded ethanol, embedded in Epon, and viewed with a Philips EM 400 electron microscope.

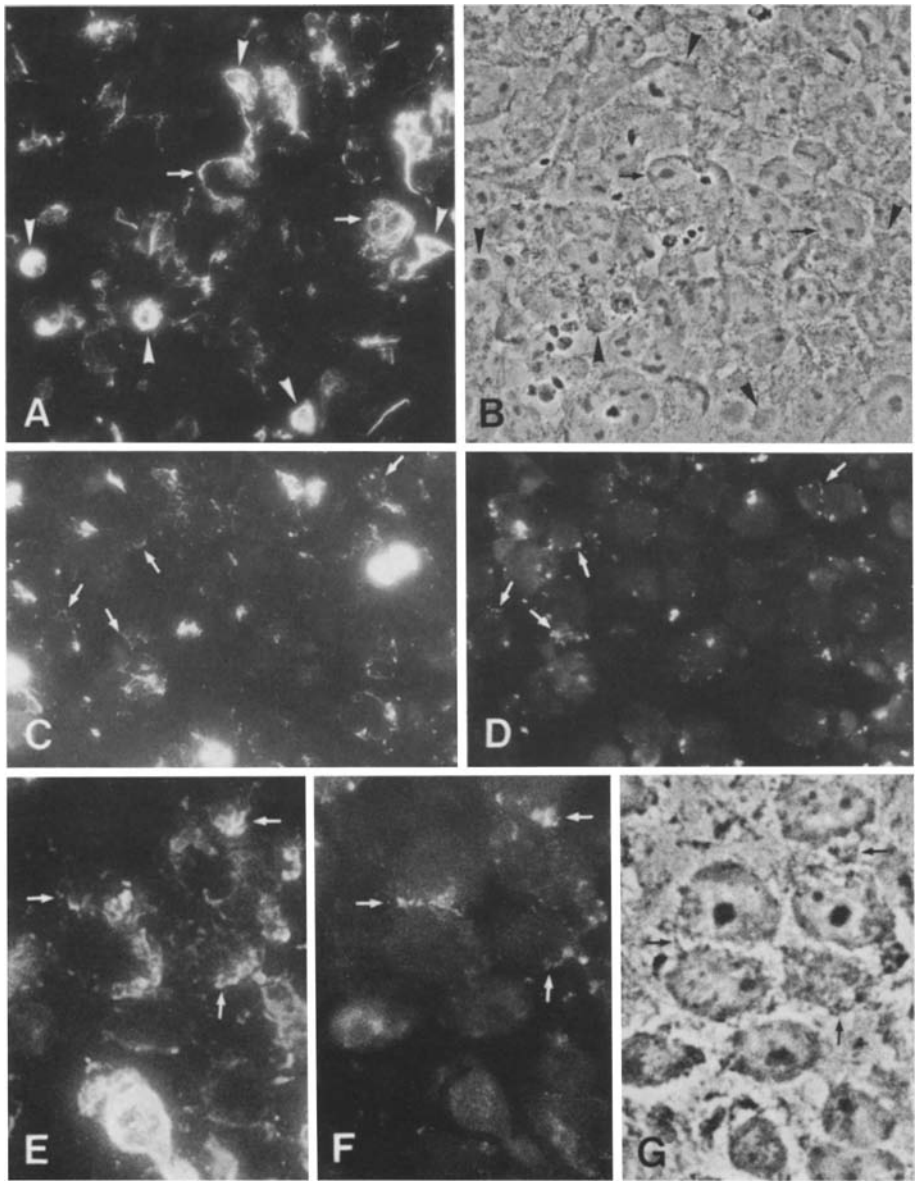

Fig. 2. A–B Most seminoma cells exhibit very scarce and delicate vimentin filaments as recognized by monoclonal vimentin antibody which are somewhat more abundant in only two seminoma cells of this field (*arrows*). Infiltrating lymphocytes are strongly vimentin-positive (*arrowheads*); **B** is the phase contrast picture corresponding to **A**; **C–D** and **E–F** show double immunofluorescence microscopy of seminoma cells using monoclonal vimentin antibodies (**C, E**) and polyclonal desmoplakin antibodies (**D, F**); **G** is the phase contrast picture corresponding to **E–F**. Note fine vimentin-positive structures (**C, E,** *arrows*) at the positions of desmoplakin-positive spots (**D, F,** *arrows*) at the periphery of seminoma cells. (**A, B** ×340; **C, D** ×380; **E–G** ×710)

Gel electrophoresis of cytoskeleton. For preparation of a cytoskeletal fraction from a seminoma (see Results) tumour areas were isolated by microdissection under stereo-microscopic control to minimize contamination of neoplastic tissue with connective tissue and necrotic material. After dissection, the tumor tissue was extracted with high-salt buffer containing Triton X-100 as described previously (Moll et al. 1982), and subjected to two-dimensional gel electrophoresis using non-equilibrium pH gradient (NEPHG) electrophoresis in the first dimension (O'Farrell et al. 1977). The separated proteins were visualized by silver staining (Oakley et al. 1980).

Results

1. Classical seminomas

Classical seminomas consisted of large, uniform tumour cells with clear or granular cytoplasm which were rather loosely arranged in sheets and lobules separated by connective tissue septa containing blood vessels and variable amounts of mesenchymal cells, namely lymphocytes and plasma cells (Fig. 1 A). Tubular or papillary structures were absent. Some areas showed considerable fibrosis. Large multinucleated giant cells resembling syncytiotrophoblastic cells in morphology and β-HCG content (Hochstetter et al. 1985) were not found in our material.

When studied by immunofluorescence microscopy using antibodies to desmoplakins, most, or at least a major proportion, of the seminoma cells of all cases studied exhibited distinct desmoplakin-reactive spots present at the cell periphery, in vari-

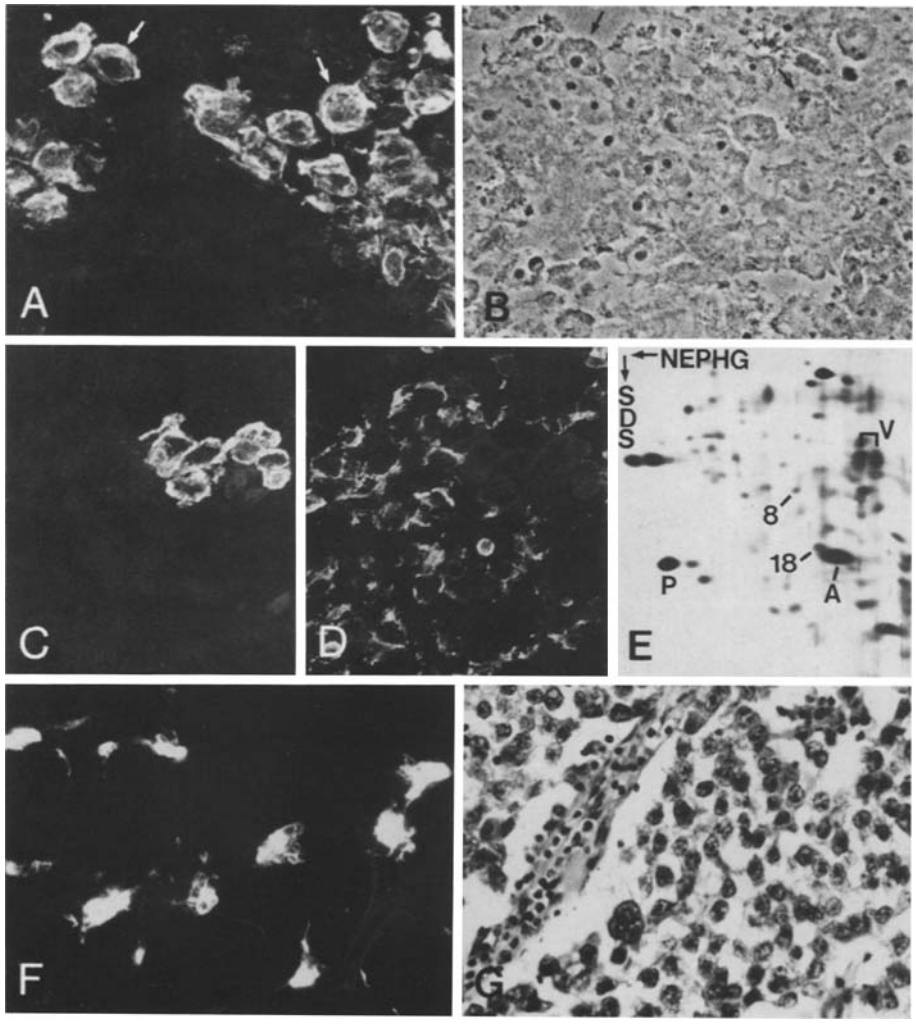

Fig. 3. A–E A case of classical seminoma: antibodies to cytokeratins decorate an extended cytokeratin fibril network in a minor proportion of tumour cells (some are denoted by *arrows*) that often are arranged in clusters (**A**, antibody PKK 1; **C** guinea pig antibodies against cytokeratin no. 18). These cells resemble the other seminoma cells by phase contrast microscopy (**B**, corresponding to **A**), but, in contrast to these, are vimentin-negative as revealed by double immunofluorescence microscopy (**C**, guinea pig antibody against cytokeratin no. 18; **D**, monoclonal vimentin antibody); **E** Two-dimensional gel electrophoresis of cytoskeletal preparations of the same seminoma case reveals major amounts of vimentin (*V*) and actin (*A*) and several unidentified polypeptides, together with the presence of small but significant amounts of cytokeratins nos. 8 and 18. These are most probably expressed in the cytokeratin-positive cells as shown in **A** and **C** (NEPHG, direction of first dimension; SDS, direction of second dimension electrophoresis in the presence of sodium dodecylsulphate; P, 3-phosphoglycerokinase from yeast added as marker polypeptide). **F–G** Another case of classical seminoma (**G**, *HE staining*) displaying irregular coiled fibrils, often aggregated to plaques that are decorated by cytokeratin antibodies (**F**, indirect immunofluorescence microscopy) in most seminoma cells. (**A**, **B** ×290; **C**, **D** ×220; **F** ×315; **G** HE, ×150)

able density and in irregular distribution (Figs. 1B–D). In some cells desmoplakin-positive streaks were visible along cell boundaries. Immunoperoxidase staining revealed results identical to those obtained by immunofluorescence microscopy (not shown). By immunoelectron microscopy, membrane plaques of desmosome-like structures were decorated by the desmoplakin antibodies (Fig. 1B, inset). Antibodies to vimentin revealed delicate, loosely arranged and often very sparse cytoplasmic fibrils in many seminoma cells, often localized at the cell periphery (Fig. 2A, B). These structures were better resolved by monoclonal than by polyclonal vimentin antibodies. Some individual seminoma cells appeared vimentin-negative. Stromal and inflammatory cells (histiocytes, lymphocytes) interspersed between seminoma cells and present in connective tissue septa displayed a strongly vimentin-positive cytoplasmic meshwork (Fig. 2A, B). Double immunofluorescence micros-

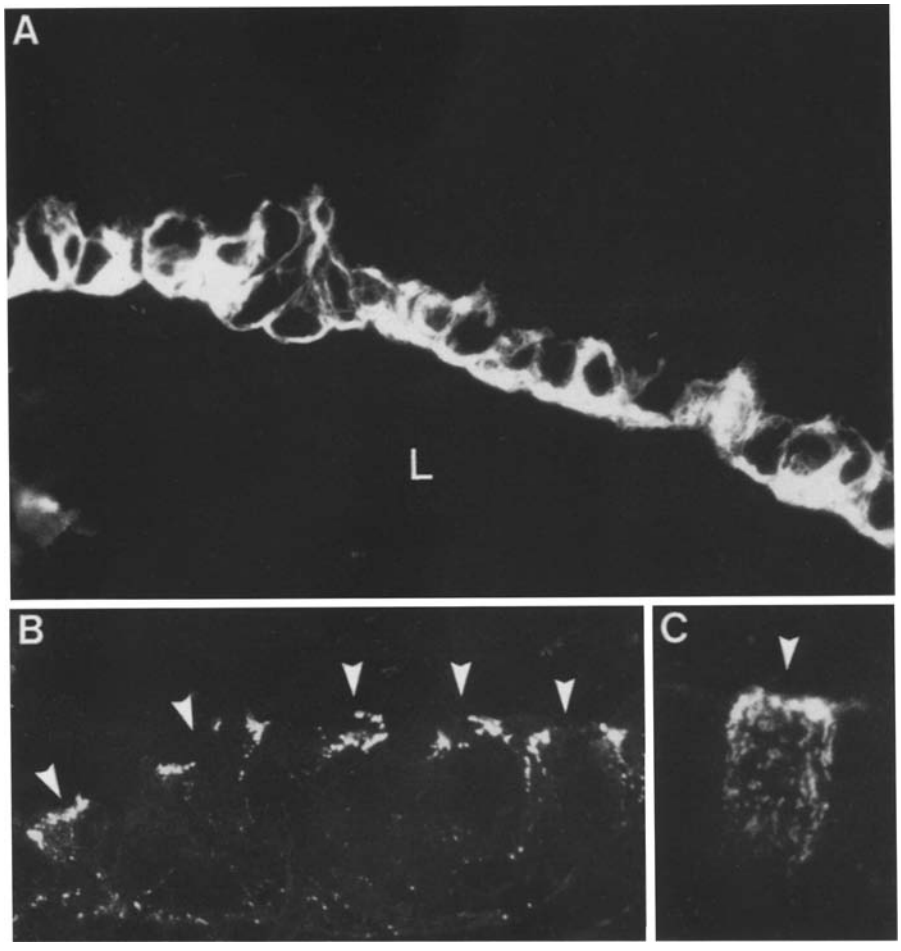

Fig. 4. A Epithelial layer of well-differentiated cystic tubule (malignant teratoma of intermediate type) contains abundant cytokeratin filament bundles (antibodies to murine hepatocytic cytokeratin component D; L, lumen of tubule); **B–C** The cells contain desmosomes in regular distribution with enrichment at the apical (luminal) pole of the cells (*arrow heads*). (**A** × 500; **B, C** × 800)

copy using desmoplakin and vimentin antibodies revealed that the delicate vimentin-positive structures of seminoma cells were usually in close spatial association with the desmoplakin-positive spots, suggesting an association of desmosomes with vimentin-type IF (Fig. 2C–G). Occasionally, however, no vimentin or other IF protein reaction could be detected at the site of desmosomes.

In general, cytokeratin antibodies with a broad range of polypeptide reactivity failed to react with the majority of seminoma cells (and with tumour cells in seminomatous areas of mixed tumours). However, differences between different cases were observed: one case was completely negative with all cytokeratin antibodies (not shown). In a further case, a minor proportion of seminoma cells exhibited cytokeratin-positive plaque-like structures and short fibrils in some tumour regions. In two more cases, a minor proportion of the tumour cells displayed an extended cytoplasmic network of cytokeratin-positive fibrils (Fig. 3A–C) but were negative for vimentin (Fig. 3D). These cells occured in an isolated position usually in proximity to the connective tissue septa, or, more frequently, in clusters. They appeared morphologically as typical seminoma cells and could not be distinguished from the surrounding tumour cells by phase contrast microscopy (Fig. 3A, B) nor by examination of parallel H & E stained sections. Gel electrophoresis of a cytoskeletal preparation from microdissected tumour tissue derived from one of these specimens revealed cytokeratin polypeptides nos. 8 and 18 as the major, if not exclusive, constituent polypeptides of the cytokeratin filaments of these cells (Fig. 3E) and this was confirmed by the lack of reactivity of this tumour with the antibody A53-B/A2 against cytokeratin no. 19 (not shown). Finally, in one case of seminoma (Fig. 3G) most cells contained circumscribed arrays of irregular, coiled fibrils and irregular plaques that reacted with broad-range cytokeratin antibodies (Fig. 3F). Antibodies to AFP and β-HCG did not reveal a positive immunoreaction in this specimen and in another only focally positive for cytokeratins.

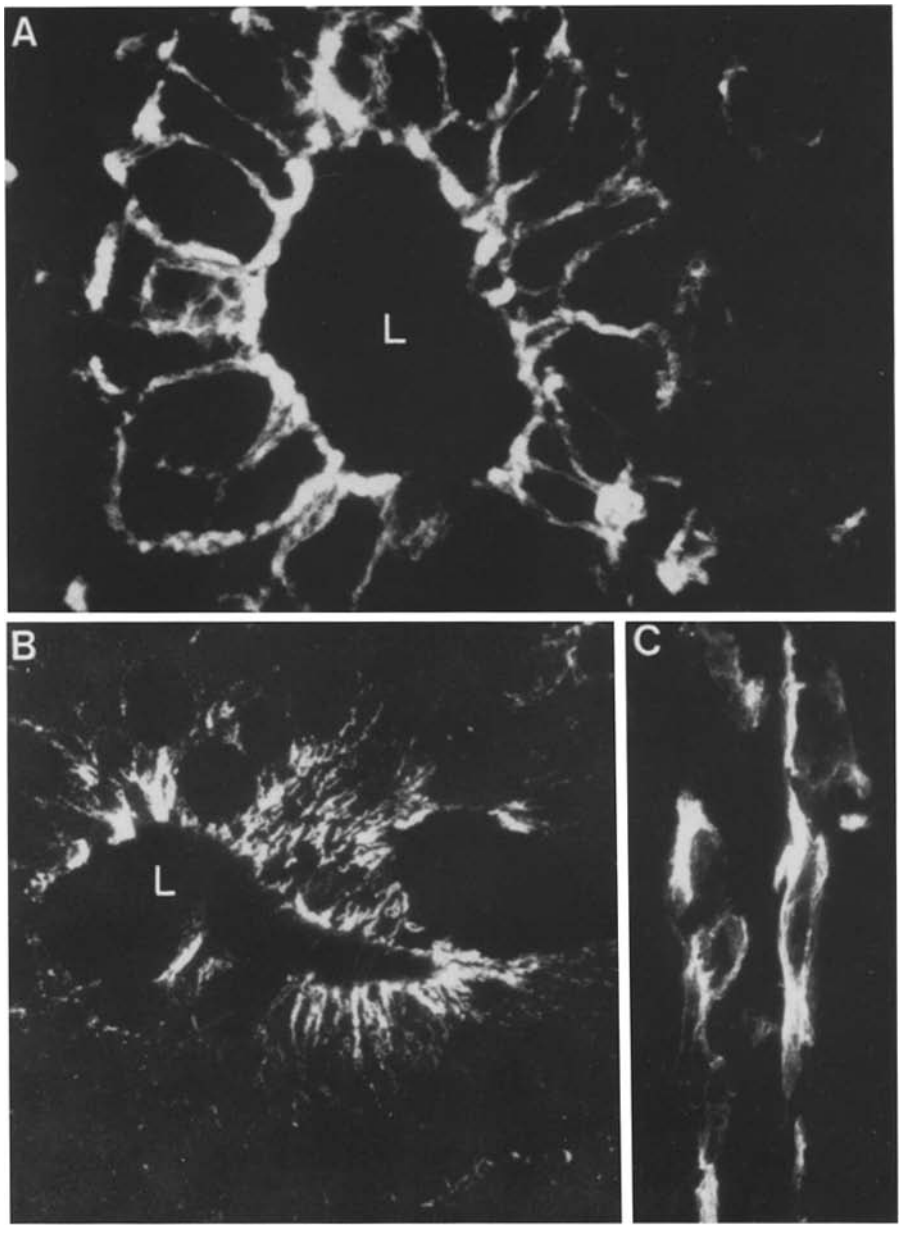

Fig. 5. A Well differentiated tubule (*L, lumen*) present in malignant teratoma of intermediate type contains cytokeratin bundles (antibodies to cytokeratin D) concentrated at the cell periphery; **B** Tubular cells (*L, lumen*) show streak-like desmoplakin-reactive sites. Spindle cells reactive with cytokeratin antibodies are shown in **C**. (**A** ×800; **B** ×800; **C** ×800)

Antibodies specific for epidermal type cytokeratins did not decorate any cells present in seminomas (not shown).

2. Malignant teratomas

Malignant teratomas studied included tumours of intermediate (teratocarcinoma), undifferentiated (embryonal carcinoma), and trophoblastic (choriocarcinoma) types.

Malignant teratoma of intermediate type. In addition to mature structures with organoid patterns resembling respiratory and gastrointestinal tissue and including mesenchymal elements such as cartilage and smooth muscle, the malignant teratomas of intermediate type displayed tissues with varying degrees of differentiation. Malignant tumour cells mostly resembled epithelial cells at the light microscopic level. The cells were large and pleomorphic with poorly contoured borders. They were arranged in solid, reticular, tubular or papillary patterns. Mitotic figures were numerous.

Epithelial cells present in well-differentiated areas displayed a regular cytoplasmic filament skeleton upon reaction with broad-range cytokeratin antibodies (Fig. 4A, Fig. 5A). The cytokeratin fibril bundles traversed the cytoplasm but were somewhat concentrated at the cellular periphery

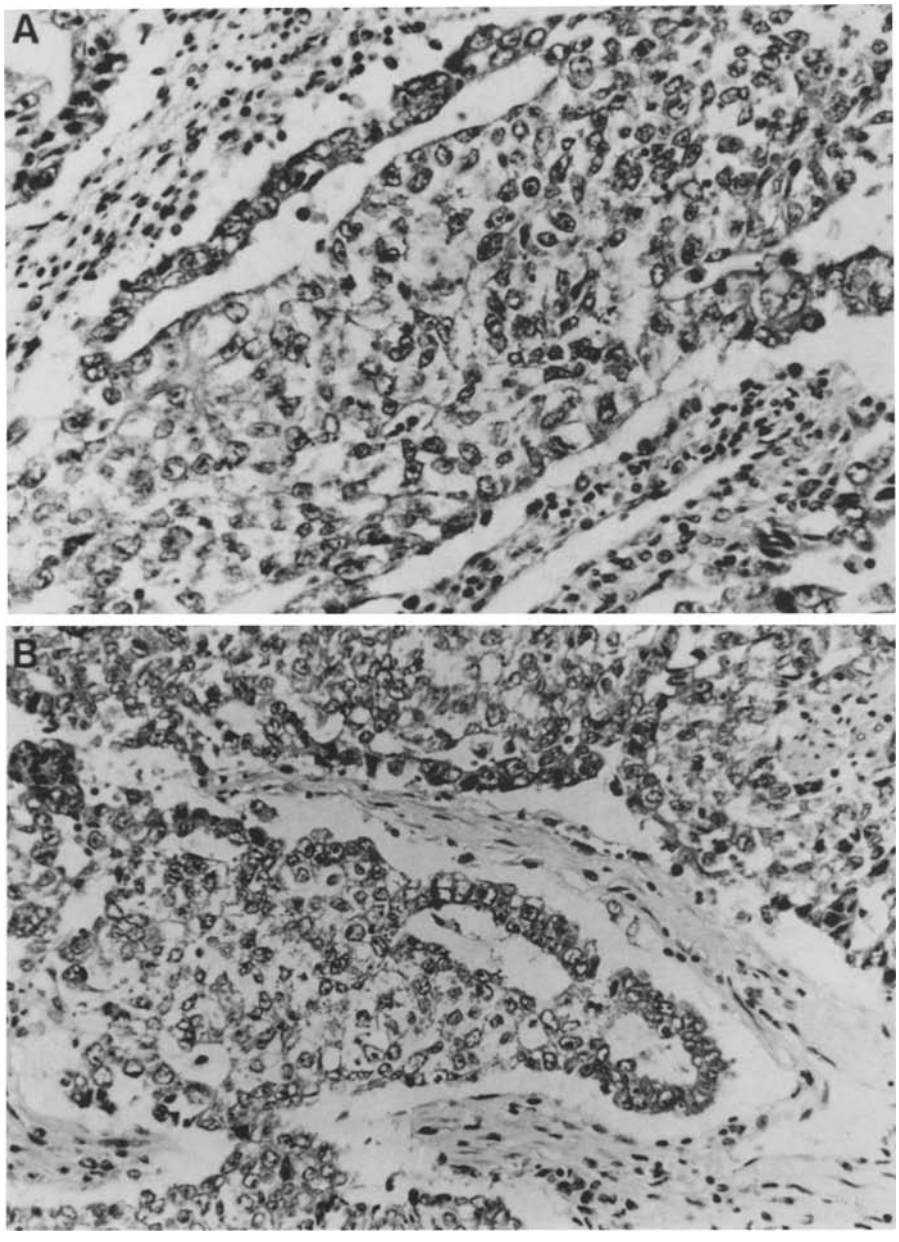

Fig. 6. A–B Malignant teratoma of undifferentiated type (*HE staining*). Tumour cells show pleomorphic appearance and are arranged in solid and reticular patterns, but also in cell layers (*periphery*). (**A** ×300; **B** ×300)

with a polygonal staining pattern (Fig. 5A). In carcinoma areas, the distribution of cytokeratin filament bundles was more regular and dense in tumour regions where cells were arranged in layers or showed tubular differentiation (see also Fig. 6). In regions with reticular arrangement of tumour cells cytokeratin filament bundles were usually sparse, shorter, thicker, and irregularly distributed (see also Figs. 7 and 8A–C). Some cells with elongated shape (spindle cells) also reacted with cytokeratin antibodies (Fig. 5C). Occasional tumour cells showed an irregular "dotted" appearance of cytokeratin staining. In some, but not all cases, a few epithelial cells reacted with antibodies to epidermal cytokeratins. The staining patterns of desmoplakin antibodies also displayed various degrees of heterogeneity. In mature structures, desmoplakin spots were frequent, regularly distributed, and apical enrichment was conspicuous in tubules (Fig. 4B, C). In less well differentiated tumour areas, desmosomes were less orderly arranged and fewer in number. Occasionally, desmoplakin-reactive sites appeared to be whisker-like elongations instead of spotlike (Fig. 5B), probably reflecting regions with closely spread desmosomes or even fused desmosomes (for example, see Cowin et al.

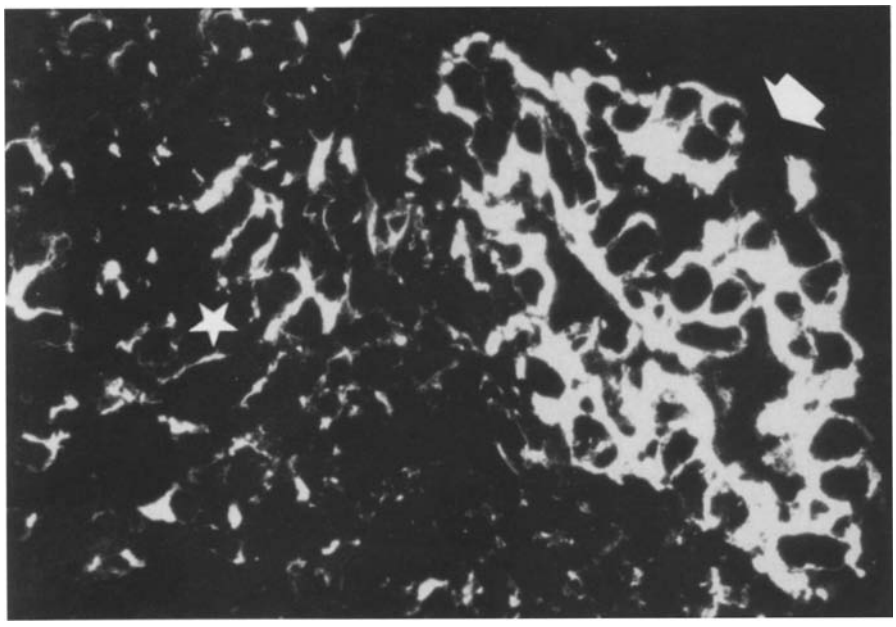

Fig. 7. Malignant teratoma of intermediate type: whereas cytokeratin filament bundles (antibodies to cytokeratin D) are dense in cells of areas with abortive tubular differentiation (*arrow*), they are irregularly arranged, shorter, thicker, and fewer per cell in cells of reticular areas (*asterisk*). (× 500)

1985). In reticular or solid regions only few desmoplakin spots per cell were found which varied considerably in size, and were distributed around the entire surface of the cells (see also Fig. 8D, D). Vimentin antibodies reacted with stromal cells and teratoma areas with mesenchymal differentiation in a filamentous pattern but did not stain cells with epithelial morphology.

Malignant teratomas of undifferentiated type. Malignant teratomas of undifferentiated type reflected the carcinomatous areas in cytokeratin, desmoplakin and vimentin staining (Figs. 6–8) described for intermediate type teratomas. Co-expression of cytokeratin and vimentin in tumour cells was not observed.

Malignant teratomas of trophoblastic type. In these tumours, syncytiotrophoblastic and cytotrophoblastic cells displayed a rather regular and pronounced cytokeratin skeleton in addition to desmosomes present around the entire circumference (Fig. 9A, B). Carcinoma cells resembled in their immunoreactivity those of the other types of teratomas. Vimentin antibodies stained mesenchymal cells. Trophoblastic cells were vimentin-negative (Fig. 9C).

3. Germ cell tumour with more than one histological pattern

In testicular tumours consisting of seminomatous and non-seminomatous neoplastic components the fluorescent patterns resembled the individual components described above. The seminomatous areas displayed only a few cytokeratin positive cells interspersed between cells lacking cytokeratin staining as described above.

Discussion

Electron microscopic reports on the presence of desmosomes in tumour cells of classical and anaplastic seminomas have been controversial. Mostofi and Price (1973), Pierce (1966), and Rosai and Levine (1976) did not detect desmosomes in seminomas arising at diverse sites, whereas Levine (1973), Holstein and Körner (1974), Janssen and Johnston (1978), Henderson and Papadimitriou (1982), and Battifora et al. (1984) found desmosomes in seminoma cells, although in low numbers and less well developed than in embryonal carcinomas. The detection of desmosomes in pathological material by electron microscopy is, however, problematic because of the possibility of sampling errors, and the fact that the morphological appearance of desmosomes is rather variable. Junctional structures similar to, but not identical with, desmosomes may occur in diverse cell types (for discussion see the reviews by Erlandson (1981) and Ghadially (1980, 1982). Using immunohistochemistry with antibodies to major desmosomal plaque proteins (desmoplakins) which overcomes these difficulties (for review see Moll et al. 1986) we have been able to demonstrate the presence of true desmosomes as a constant feature of seminomas.

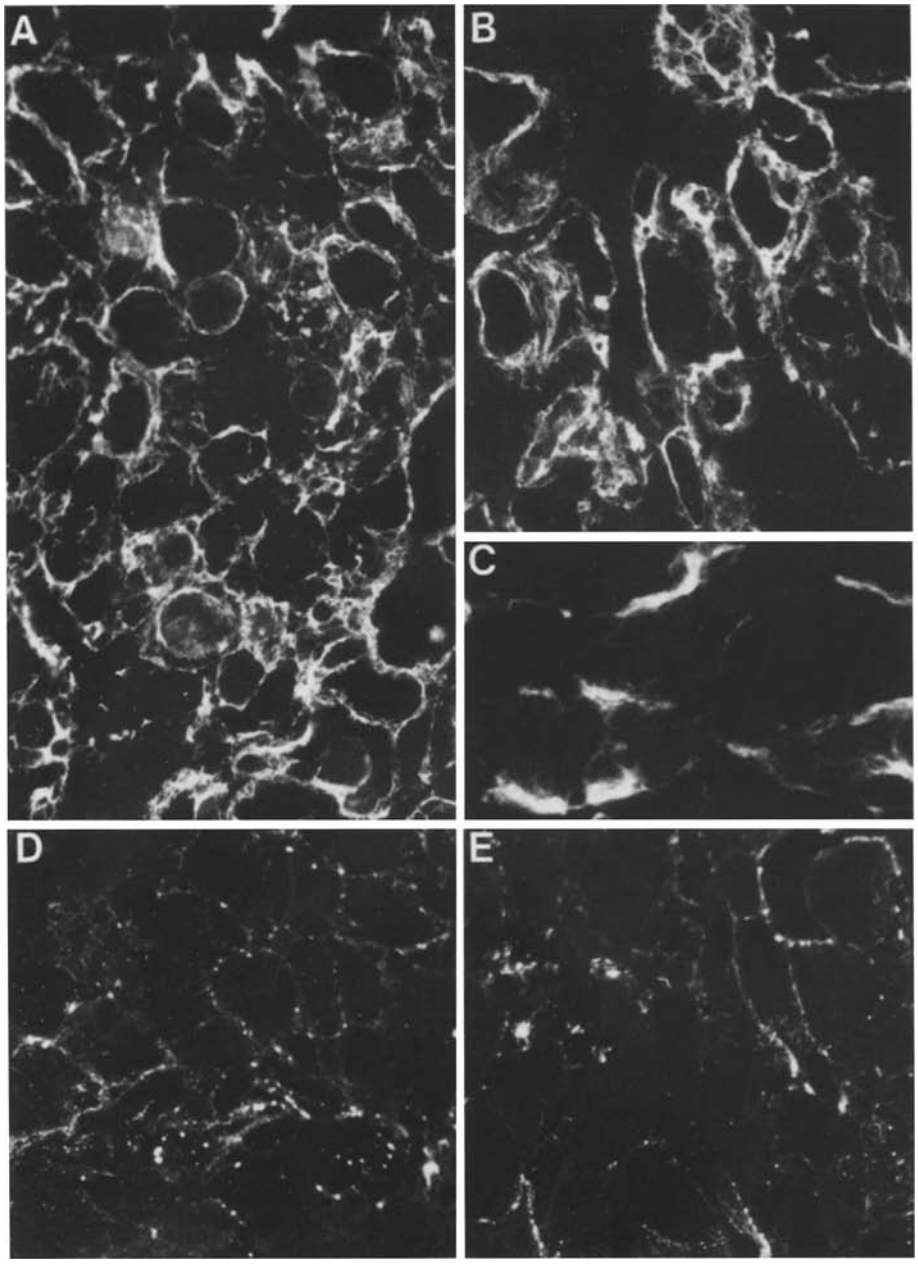

Fig. 8. Malignant teratoma of undifferentiated type: tumour cells arranged in reticular patterns contain irregular cytokeratin filament bundles concentrated at the cell periphery (antibodies to cytokeratin D; **A, B**). In some areas, the filament bundles are thick and sparse (**C**). Tumour cells contain desmosomes at their periphery as revealed by desmoplakin antibodies (**D, E**). (**A** ×500; **B** ×500; **C** ×800; **D, E** ×800)

While in most other normal and malignant cells containing desmosomes these junctions are associated with cytokeratin filaments, thereby defining these cells as epithelial, the desmosomes of seminomas usually appear in close spatial relationship to delicate vimentin filament arrays. If the attachment of vimentin filaments to desmosomal plaques in seminoma cells could be confirmed by future immunoelectron microscopic examinations, seminomas would then represent a further tumour type with an exceptional desmosomes-IF relationship, thus resembling ovarian granulosa cell tumours (Moll et al. 1986) and meningiomas (Schwechheimer et al. 1984). Seminomas differ, however, from meningiomas and granulosa cell tumours in their more scanty and irregular expression of both desmosomes and vimentin filaments. Our observation of the scarcity of vimentin filaments in seminoma cells is in general agreement with previous studies (Miettinen et al. 1985; Ramaekers et al. 1985).

In some tumour regions, we have found desmosomes in cells that seemed to lack IF. In such cases, some vimentin filaments might be attached at the

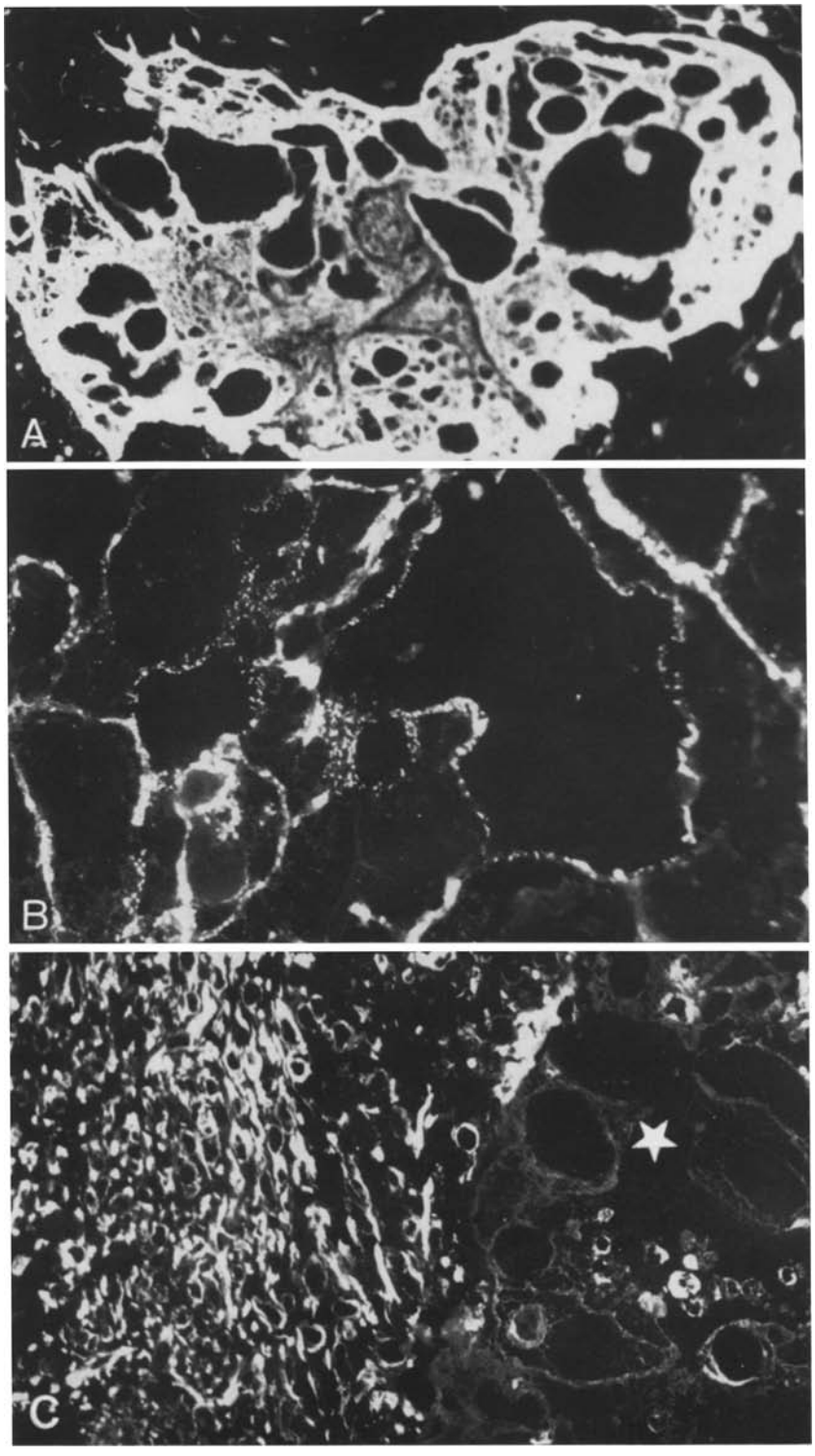

Fig. 9. A Syncytiotrophoblastic elements present in malignant teratoma of trophoblastic type exhibit a conspicuous cytokeratin skeleton (antibodies to cytokeratin *D*); **B** Desmoplakin antibodies reveal desmosomes at the cell periphery; **C** Trophoblastic elements (*asterisk*) lack vimentin filament staining whereas surrounding mesenchymal cells are decorated by vimentin antibodies (**A** ×500; **B** ×800; **C** ×315)

desmosomes but too low in amount to be detected by the vimentin antibodies. Alternatively, it may indicate a non-coordinated expression of both cytoskeletal components. From experiments using mouse epidermal cells in vitro, Jones and Goldman (1985) have concluded that a close interaction between IF bundles and desmoplakins is the initiation step in the formation of desmosomal structures (Jones and Goldman 1985). However, in vivo desmosomal development can be independent of

the presence of any detectable IF (Denk et al. 1985a) as is also suggested by our present results.

Our results show that, in contrast to malignant teratomas, in classical seminomas most tumour cells lack cytokeratin, thus confirming reports by other authors (Battifora et al. 1984; Ramaekers et al. 1985; Miettinen et al. 1985). However, a minor proportion of seminoma cells in some cases, and most seminoma cells in one case did contain short cytokeratin fibrils similar to those of reticular areas of undifferentiated malignant teratoma (embryonal carcinoma). A similar observation has been reported by Ramaekers et al. (1985) who found a major proportion of cytokeratin-positive cells in one of their seminomas. The almost constant presence of desmosomes, together with the variable presence of often rudimentary cytokeratin-positive structures, might be interpreted as an incomplete and in its extent variable expression of an epithelial differentiation program in these tumours.

The variability in cytokeratin expression suggests that even morphologically homogenous-looking classical seminomas are heterogeneous (for heterogeneity of seminoma cells see also Holstein and Körner 1974; Schulze and Holstein 1977). The similarity in the expression of cytoskeletal components between some seminomas and most embryonal carcinomas is intriguing and might point to a more unifying concept of testicular germ cell tumours. It is noteworthy in this context that on the basis of occasional morphological and immunohistochemical (e.g. expression of AFP) similarities between seminoma, embryonal carcinoma and even yolk sac tumour a close relationship between these different tumour types as well as the existence of transitional forms have been discussed (Hochstetter and Hedinger 1982; Walt et al. 1986). It would be interesting to know whether the biological behaviour of the seminomas with high level of cytokeratin expression resembles that of embryonal carcinomas and thus differ from other seminomas.

The arrangement and distribution of desmosomes and cytokeratin filaments in undifferentiated carcinomatous structures of malignant teratomas resembled those of blastema cells present in nephroblastomas (Denk et al. 1985b). In less differentiated areas, tumour cells contained few and irregular cytokeratin bundles, which appeared to be more regular with maturation and, in particular, tubule formation. The number and density of desmoplakin-reactive spots, resembling desmosomes also increased accordingly. It is important to note that all these non-seminomatous germ cell tumours are characterized by the common desmosome-cytokeratin filament complex, in contrast to the desmosome-vimentin filament relationship observed in typical seminoma cells. The question remains whether the latter complex is also expressed in anaplastic seminomas, which often pose special problems of differential diagnosis.

Acknowledgement. The photographical work of Mr. I Georgiev and the secretarial assistance of Ms. E. Ogriseg are gratefully acknowledged.

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Accepted September 24, 1986