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Salivary gland myoepithelioma variants

Histological, ultrastructural, and immunocytological features

Irving Dardick[1, 2], Sharon Cavell[3], Marie Boivin[3], Dianne Hoppe[3], William R. Parks[3], Janet Stinson[3], Sayoko Yamada[2], and Bruce F. Burns[3, 4]

[1] Department of Pathology, University of Toronto, Banting Institute, 100 College Street, Toronto, Ontario M5G 1L5, Canada
[2] Department of Pathology, Toronto General Hospital, Toronto, Ontario, Canada
[3] Department of Laboratory Medicine, Ottawa Civic Hospital, Ottawa, Ontario, Canada
[4] Canadian Reference Centre for Cancer Pathology, 60 Ruskin Avenue, Ottawa, Ontario, Canada

Summary. The histological and ultrastructural features of five major salivary gland tumours, which have little or no evidence of duct- or gland-type differentiation in routine sections, are described. Four of the cases have the tumour cells organized as narrow, anastomosing cords of cells separated by a myxoid and vascularized stroma; we have designated such lesions as reticular-type myoepitheliomas. The fifth case has a solid growth pattern and is largely composed of hyaline cells, that is, a plasmacytoid myoepithelioma. Ultrastructurally, one reticular myoepithelioma reveals myoepithelial cell differentiation with microfilament aggregates, while the other three examples are composed of modified myoepithelial cells displaying widened intercellular spaces, prominent synthesis of extracellular glycosaminoglycans, distinct basal lamina development, and obvious accumulations of cytoplasmic intermediate filaments. In electron micrographs, the modified myoepithelial cells of the plasmacytoid variant closely resemble the tumour cells in the reticular form. Three cases had expression of both glial fibrillary acid protein (GFAP) and vimentin, but only one of the myoepitheliomas contained muscle-specific actin. At least focally, each of the cases exhibited a considerable spectrum of cytokeratin filaments. Using double-labeled immunofluorescent microscopy of one reticular variant and the plasmacytoid myoepithelioma, there was individual tumour cell co-expression of GFAP and vimentin focally in the plasmacytoid myoepithelioma, but co-expression of cytokeratins 13, 16 and GFAP were not noted in either case.

As expected, co-expression of high- and low-molecular weight cytokeratin filaments was widespread in both myoepitheliomas. Most described myoepitheliomas have a solid growth pattern and are composed of spindle and plasmacytoid cells, but based on cytological features and growth patterns in this series, it is apparent that polygonal-shaped cells with novel architecture can occur in myoepitheliomas. The results also indicate the close relationship between pleomorphic adenoma and such variants of myoepithelioma.

Key words: Salivary gland – Myoepithelioma – Neoplasm – Electron microscopy – Immunohistochemistry – Cytoplasmic filaments

Offprint requests to: I. Dardick

Introduction

There is increasing acceptance that the counterpart of the normal myoepithelial cell of salivary gland acini and intercalated ducts has a role in producing the highly characteristic histological patterns of pleomorphic adenoma (Caselitz et al. 1986a; Dardick et al. 1983a, b; Erlandson et al. 1984; Lam 1985; Nikai et al. 1986; Palmer et al. 1985a, b). Indeed, review of ultrastructural and immunohistochemical findings in this tumour-type indicates that the organization of the tumour cells reflects the intimate association of duct luminal or acinar cells with their myoepithelial/basal cells in the normal salivary gland (Dardick et al. 1983a, b; Dardick and von Nostrand 1987). Since a myoepithelioma should be closely related to a pleomorphic adenoma, at least in terms of developmental processes and tumour cell morphology, a key question is whether intermediate filament expression in the tumour cells of myoepitheliomas reflect those evident in the neoplastic myoepithelial cell component of pleomorphic adenomas. However, there are few studies where the filament complement in myoepitheliomas are indicated expressly (Lins and

Gnepp 1986; Mori et al. 1986; Mori et al. 1987; Nikai et al. 1986; Toto et al. 1986).

Criteria for classifying myoepitheliomas of the salivary glands have never been well-defined or clearly described; they are usually simply categorized as well-circumscribed tumours composed entirely of spindle-shaped or plasmacytoid (hyaline) cells (Sciubba and Brannon 1981). There is increasing awareness that either quite well-differentiated or, more frequently, structurally modified myoepithelial cells are a major component of pleomorphic adenomas (Batsakis et al. 1983; Dardick and van Nostrand 1985, 1987; Toto et al. 1986). Because of this fact, it is reasonable to assess the cytological characteristics and growth patterns of neoplastic myoepithelium in pleomorphic adenomas and see if similar features exist in salivary gland tumours with limited or no ductal component. Using this approach, we have recently described the general features of 40 myoepitheliomas (Dardick et al. 1989b). This group of tumours have four basic growth patterns, i.e., solid, myxoid, reticular, and a mixed type, and are composed of spindle, plasmacytoid, epithelioid, and clear cells or some combination of these types of tumour cells. An additional criteria used to segregate myoepitheliomas from pleomorphic adenomas is the absence of ductal differentiation or ductal structures comprising less than 10% of the epithelial component in the former (Dardick et al. 1989b).

Using the above criteria, among the 40 myoepitheliomas, there were 10 (25 percent) with a growth pattern that has not been described in detail but has been illustrated previously (Nesland et al. 1981). In these myoepitheliomas, the tumour cells are arranged in relatively narrow, anastomosing columns separated by a relatively acellular, myxoid appearing, vascularized stroma. On the basis of the sieve- or lace-like arrangement of the modified myoepithelial cells, we have designated this variant of myoepithelioma as having a reticular or canalicular-like pattern of differentiation (Dardick et al. 1989b). In this report, we describe the details of the histology, ultrastructure, and immunohistochemistry of four myoepitheliomas with a reticular morphology and contrast the features with those of a myoepithelioma composed of plasmacytoid cells arranged in a solid growth pattern.

Materials and methods

Tissues for morphological examinations were obtained from five tumours of the parotid salivary gland at the time of surgery. The clinicopathological details of these lesions are presented in Table 1.

Immediately following superficial or deep parotidectomy and gross examination of the tumours, representative portions were appropriately fixed for light and electron microscopy. For light microscopy and immunohistochemical studies, tissue slices were fixed in modified methacarn solution, i.e., methanol 60 percent, chloroform 30 percent, and glacial acetic 10 percent (Mitchell et al. 1985), for 4 to 6 h and embedded in paraffin. Sections for review and photography were stained with haematoxylin, phloxin, and saffron.

Ultrastructural examinations were performed on minced tissue fragments from all five tumours; these were fixed overnight in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. After washing in cacodylate buffer and post-fixation in osmium tetroxide, the tissue was dehydrated in graded alcohols and propylene oxide prior to embedding in Epon-Araldite resin. Following review of semi-thin plastic sections, appropriate blocks were trimmed, and thin sections cut on a diamond knife and mounted on uncoated copper grids. After staining the sections with uranyl acetate and lead citrate, they were reviewed and photographed on a Philips EM 301 electron microscope at 60 KV.

In cases 1 to 3 and case 5, methacarn-fixed and paraffin-embedded tumour tissue was used for the immunoperoxidase and immunofluorescent studies; case 4 only had formalin-fixed tissue available and was excluded from this part of the study.

The antibodies employed, their specificities, and sources are outlined in Table 2. For immunoperoxidase studies, a peroxidase-antiperoxidase methodology was used with the polyclonal antibodies, while the Unistain detection kit (Becton Dickinson, Mississauga, Ontario) was used with the monoclonal antibodies. Details of the indirect immunoperoxidase techniques and controls employed has been described previously (Burns et al. 1988; Dardick et al. 1988).

For immunofluorescent microscopy, methacarn-fixed tissue sections were deparaffinized and washed in phosphate-buffered saline (PBS). The combination of antibodies used for double labeling always included a monoclonal and a polyclonal one so that staining for the two intermediate filaments could be done simultaneously (Achtastatter et al. 1986). Using the dilutions outlined in Table 2 and the monoclonal/polyclonal antibody combinations detailed in Table 5, each antibody mixture was applied to the sections at room temperature for 30 min. Following three brief washes with PBS, a mixture of fluorescein isothiocyanate-tagged anti-mouse IgG (diluted 1:50; Organon Teknika Corp., West Chester, PA) and rhodamine-tagged anti-rabbit IgG (diluted 1:50; Organon Teknika Corp., West Chester, PA) was applied to the primary antibody labeled tissue sections for 30 min, again at room temperature. Subsequently, the sections were washed in PBS and cover-slipped with a 50% aqueous glycerol/PBS solution containing 0.1% *p*-phenylenediamine (to retard fading of fluorescence). Following review of the sections using a Zeiss epifluorescent microscope, having both a xenon and a mercury lamp (and appropriate filters for fluorescein and rhodamine), selected areas were sequentially photographed (Ilford XP1 400 film) using each fluorescent marker.

Results

Clinicopathological data

All of the tumours occurred in the parotid gland and only one myoepithelioma, the sole malignant lesion, was in the deep lobe (Table 1). Three patients were male and two were female, and the

Table 1. Clinicopathological data of myoepithelia variants

	Case 1	Case 2	Case 3	Case 4	Case 5
Age/sex	43/F	57/M	77/M	71/M	22/F
Site (lobe)	Parotid (superficial)	Parotid (tail of superficial)	Parotid (superficial)	Parotid (superficial)	Parotid (deep)
Size (CM)	2.5 × 2.0	3.0 × 2.5	6.0 × 3.0	2.4 × 2.2	3.0 × 2.0
Symptoms	Lump for 6 weeks	Indurated area in neck, 3 months prior to surgery	Pea-sized nodule present for 30 years	Mass, angle of mandible for 9 months	Facial pain and mass
Growth pattern	Reticular	Reticular	Reticular	Reticular	Solid
Cell type(s)	Spindle, with some polygonal	Spindle	Spindle, with some polygonal	Polygonal, with some spindle	Plasmacytoid, with some polygonal
Comments	Benign. Multinodular, with some nodules having extensive mucoid stroma	Benign. Squamous cell carcinoma in base of tongue one year before lump in neck appeared	Benign. Two previous biopsies prior to excision. Died of carcinoma of pancreas one year later	Benign. Encapsulated tumour in superficial lobe	Malignant. Cellular with moderate nuclear pleomorphism. No capsular invasion but blood vessel infiltration noted

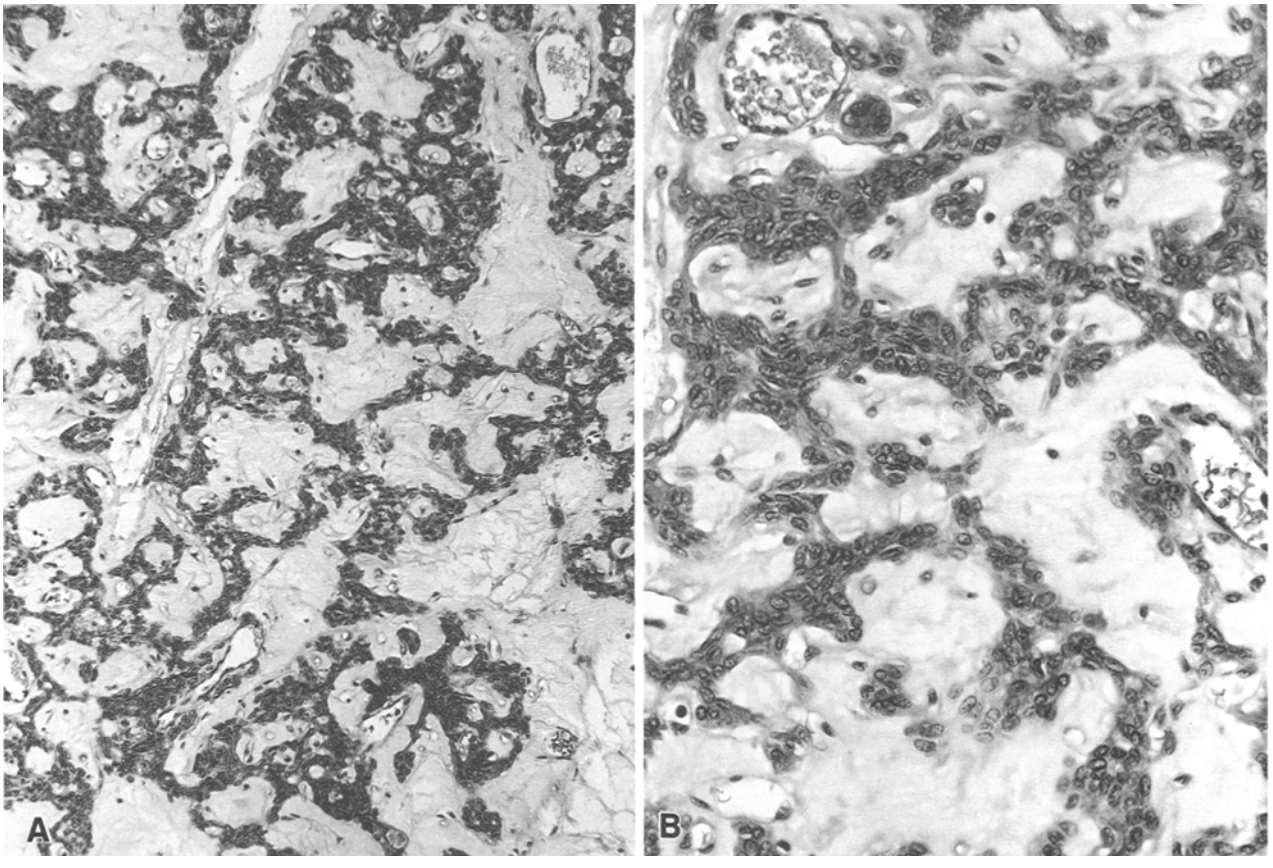

Fig. 1. Reticular myoepithelioma (case 3). **(A)** An intricate, sieve-like pattern is produced by narrow cords of tumour cells ramifying through a mucoid, relatively acellular stroma that contains capillaries and sinusoidal vessels. **(B)** The tumor is composed of plump, relatively short spindle cells with uniform appearing oval to round nuclei; the cytoplasm is not particularly prominent. Haematoxylin, phloxin, and saffron (HPS). **(A)** ×120; **(B)** ×300

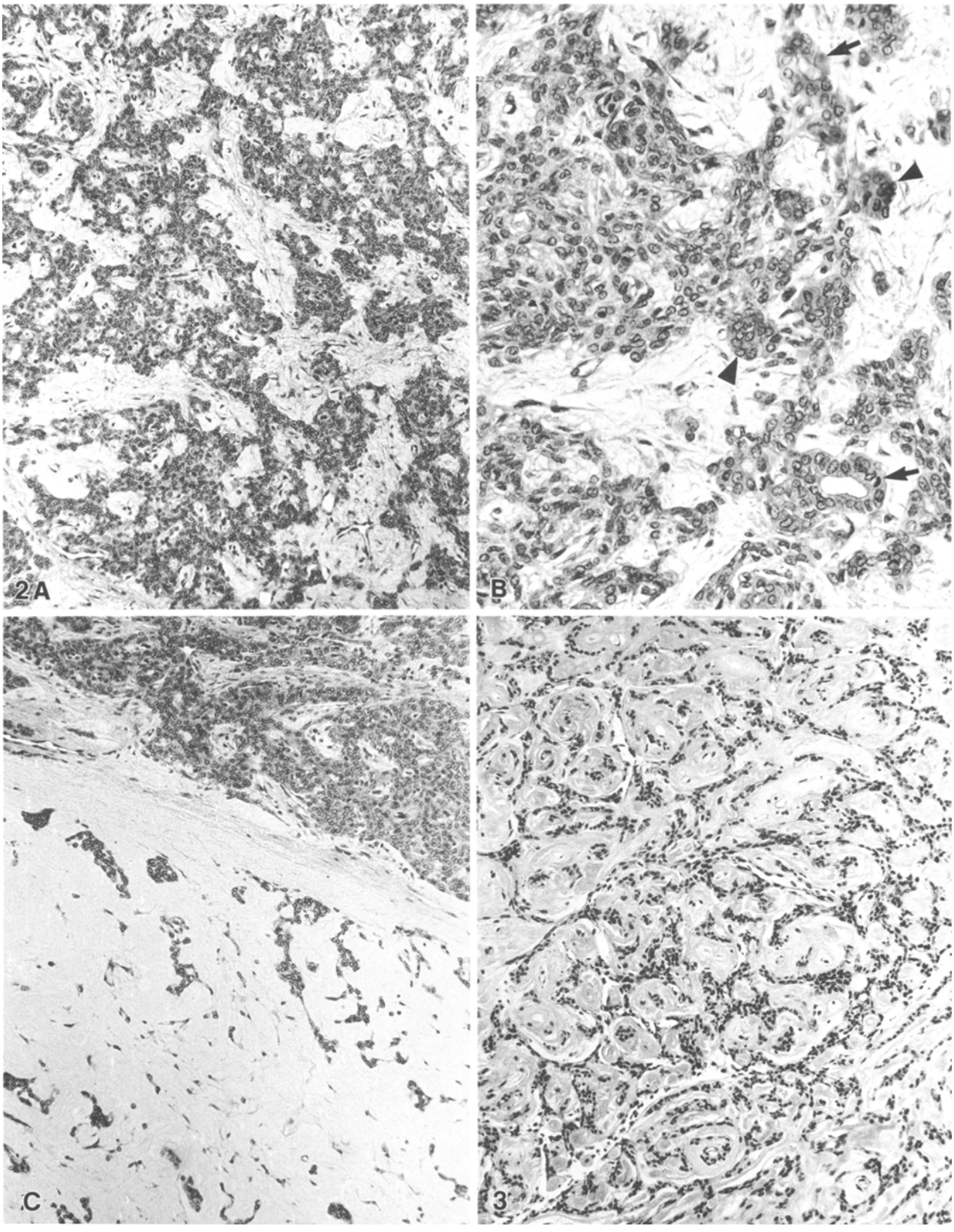

Fig. 2. Reticular myoepithelioma (case 1) (**A**) Major portions of this tumor display similar appearing cells forming complex patterns and solid groupings separated by a loose stroma. (**B**) The principal cells are polygonal to spindled in shape, but focally there are compact clusters of cells (*arrowheads*) and definite ductular formation (*arrows*). (**C**) In this tumor, there is a nodular area in which the tumour cells are considerably separated by large amounts of myxoid materials, the growth pattern seen in some pleomorphic adenomas. HPS. (**A**) and (**C**) $\times 120$; (**B**) $\times 300$

Fig. 3. Reticular myoepithelioma (case 2). In this particular parotid tumour, the anastomosing cords are separated by hyalinized stromal tissues. HPS. $\times 120$

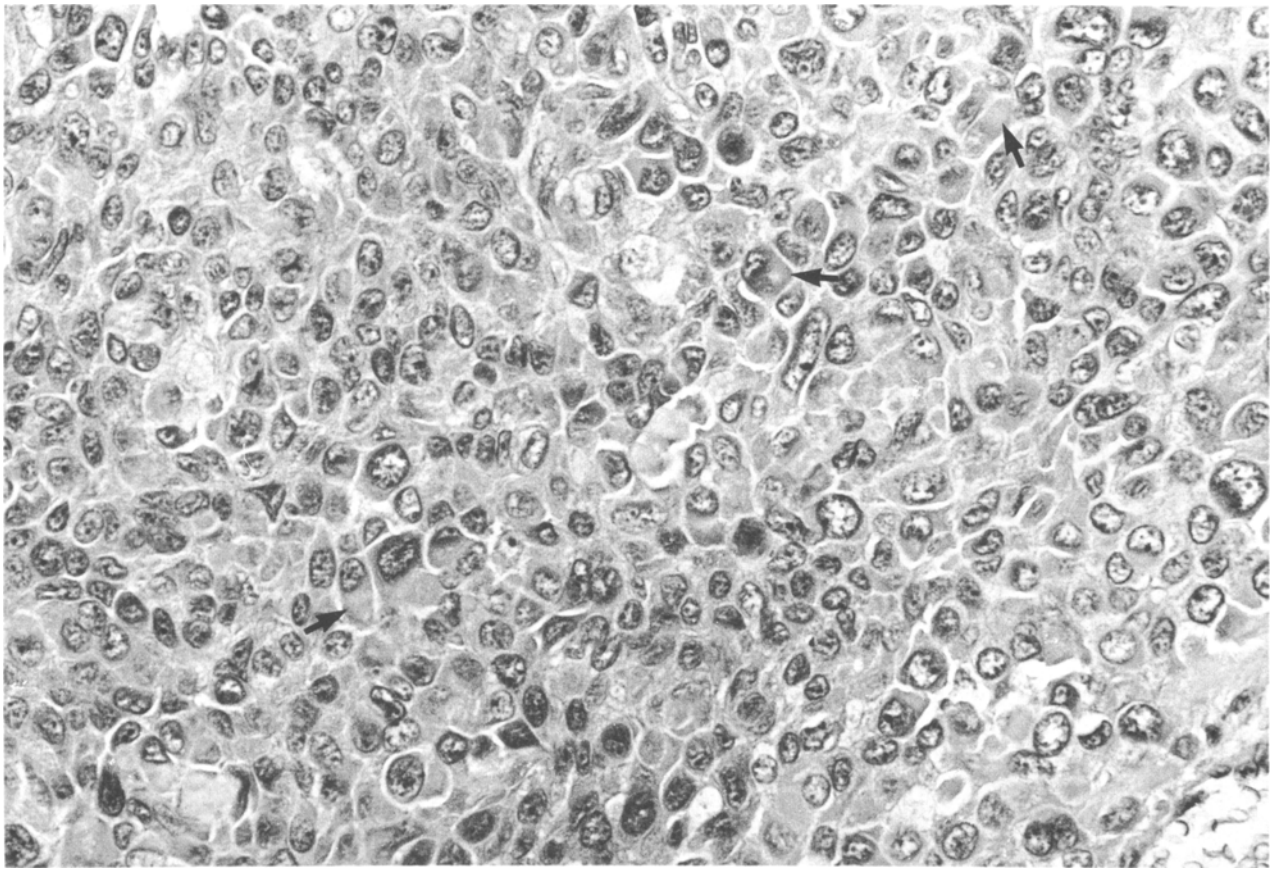

Fig. 4. Plasmacytoid myoepithelioma (case 5). In this variant of myoepithelioma with a solid growth pattern, the tumor cells are polygonal to irregular in shape and a proportion have a homogeneously staining cytoplasmic region displacing the nucleus to one side of the cell (*arrows*). HPS. ×400

mean age of presentation was 54 years. The tumours were generally in the range of 2.5 to 3.0 cm and presented as a lump detected incidentally by the patient. Three of the myoepitheliomas were composed predominantly of spindle cells with or without a component of polygonal-shaped cells, one was mainly formed by polygonal cells with a minor population of spindle cells, while one lesion was principally composed of plasmacytoid cells along with some polygonal cells (Table 1).

Histology

Four of the five parotid salivary gland tumours (cases 1 to 4) had an unusual type of growth pattern in which the tumour cells were intricately arranged. As illustrated by Figs. 1, 2a, and 3, the lesions generally had a fenestrated or lace-like histological appearance, a feature that resulted from the interplay of the tumour cells and the extracellular stroma. Relatively narrow, anastomosing columns of cells curved around vascularized, poorly cellular extracellular materials that were either myxoid in appearance (Figs. 1 and 2) or somewhat hyalinized (Fig. 3). There was a clear demarcation between the periphery of the epithelial cell cords and the stromal tissues. Case 5 differed from the others by having a solid growth pattern (Fig. 4), but there were a few focal areas in which the tumour cells were separated increasingly by mucoid extracellular materials.

In cases 1 to 4, the tumour cells tended to be oval to spindled in shape with moderate amounts of cytoplasm and round to elliptically shaped nuclei with marginated heterochromatin (Figs. 1b and 2b). At least focally in four of the tumours, some cells were polygonal in shape (Table 1), but only in case 1 were such cells organized to form a few scattered true duct- or gland-like structures (Fig. 2b). Case 5 was composed of polygonal- to oval-shaped cells showing some variation in size but a greater range of nuclear size and form (Fig. 4). Particularly, in certain regions, many of the tumour cells had a homogeneously staining and rather glassy-appearing round to oval cytoplasmic zone at one pole with the nucleus at the other pole

Table 2. Antibodies employed, their specifications and sources

Antibody	Type	Specificity	Source
Anti-GFAP	polyclonal	GFAP	Dako, Santa Barbara, CA
Anti-vimentin	polyclonal	vimentin	Euro Diagnostics BV, Holland
HHF35	monoclonal	muscle actin	Enzo Biochemicals, NY
Anti-epidermal keratin	polyclonal	CK 3, 6	Dako, Santa Barbara, CA
312C8-1	monoclonal	CK 14	Dr. S.H. Dairkee, Oakland, CA
PKK1	monoclonal	CK 7, 8, 17, 18	Lab Systems, Helsinki, Finland
8.12	monoclonal	CK 13, 16	ICN Biochemicals, Montreal, Quebec
RPN.1162	monoclonal	CK 7	Amersham Canada Ltd., Oakville, Ontario
4.62	monoclonal	CK 19	ICN Biochemicals, Montreal, Quebec
8.60	monoclonal	CK10, 11, ±1	ICN Biochemicals, Montreal, Quebec

(Fig. 4); these features fit the morphological description of hyaline (plasmacytoid) cells (Lomax-Smith and Azzopardi 1978; Sciubba and Brannon 1982).

Cases 1 to 4 were encapsulated and cytologically benign (Table 1). Although major portions of case 5 were encapsulated, there were focal regions where the tumour cells infiltration adjacent normal salivary gland and where vascular invasion was evident; because of these features, case 5 was considered histologically malignant. Case 1 was the only lesion with a separate nodular region in which the majority of the tumour cells were extensively separated by myxoid materials producing a histology similar to that seen in many pleomorphic adenomas (Fig. 1c).

Ultrastructure

The extent of expression of the main electron microscopic features of this group of myoepitheliomas are provided in Table 3. In general, the ultrastructural features of cases 1 to 4 were similar, particularly in the general appearance of the tumour cells and their organization, the prominence of cytoplasmic filaments in a proportion of the tumour cells, the excessive amounts of glycosaminoglycans extracellularly, and the expression of a discrete basal lamina. Except for the lack of widened intercellular spaces and glycosaminoglycan production, the tumour cells in case 5 had similar features to those observed in the other four cases (Table 3).

Low magnification electron micrographs in cases 1 to 4 revealed that elongated, oval, and polygonal cells formed the cords (Figs. 5 and 6). The outline of individual tumour cells was usually irregular due to a varying number of short, pointed to blunt cytoplasmic processes that abutted on adjacent tumour cells producing a series of widened intercellular spaces. Both the spaces between the tumour cells and the areas between the cellular columns contained granulofilamentous materials of the type associated with glycosaminoglycans (Fig. 5). Although usually small, desmosomes were of moderate number and often well formed (Fig. 5). In all cases, a discrete, usually narrow basal lamina was seen at the periphery of cords or clusters of tumour cells (Fig. 5), but in case 2 this was thickened in some regions (Fig. 7). Occasionally, basal lamina-lined intercellular spaces were also present.

Some cytoplasmic and nuclear features were noteworthy. Organelles, such as mitochondria, endoplasmic reticulum and the Golgi complex, were generally limited in number and the electron lucent cytoplasm often contained many randomly arranged intermediate filaments (Figs. 5–8). Only in case 2 did a small number of the tumour cells contain a narrow peripheral band of filaments associated with some densities (Fig. 7). Nuclei had two distinctive features. The heterochromatin was disaggregated into small clumps with a narrow peripheral rim below the nuclear membrane (Figs. 6 (inset) to 8). In cases 1 (Fig. 6) and 3 (Fig. 5), the heterochromatin was particularly disaggregated. Although tumour cell nuclear profiles were often variable in size and form, and had one or two indentations (Figs. 5 to 7), cases 3 (Fig. 5) and 5 (Fig. 6, inset) had a proportion of nuclear profiles with lobations as a result of deep invaginations and nuclear protrusions joined to the nucleus by segments of nuclear membranes (so-called nuclear blebs or pockets (Smith and O'Hara 1968)).

Only case 1 displayed the development of a few duct-like structures in histological sections (Fig. 2b); portions of this myoepithelioma with ducts were not available for electron microscopy. However, ultrastructurally, both case 4 and case 5 (Fig. 8, inset) revealed the differentiation of

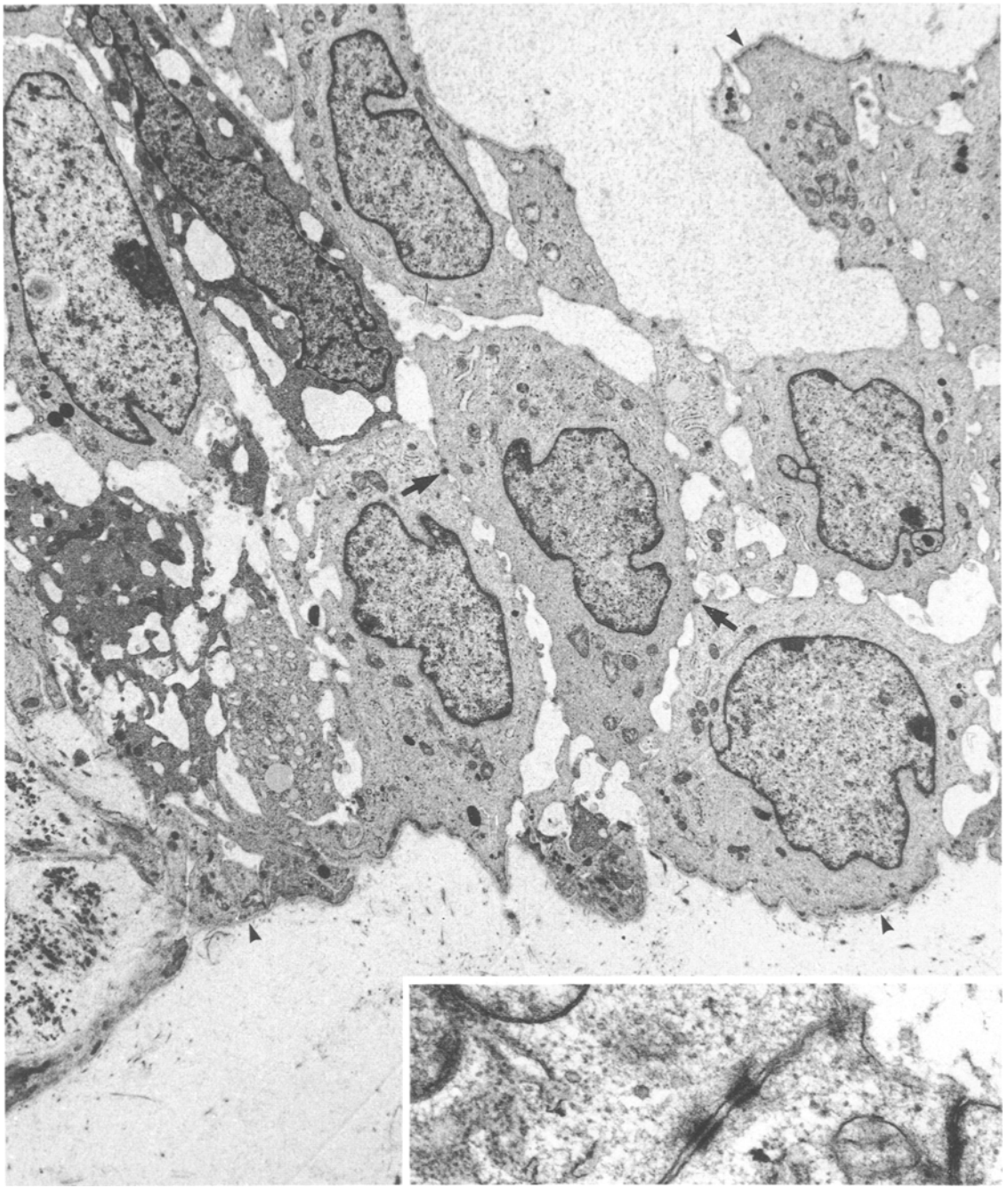

Fig. 5. Reticular myoepithelioma (case 3). Cords of polygonally to slightly irregularly shape tumour cells have widened intercellular spaces bridged by blunt cytoplasmic processes joined by small desmosomes (*arrows*). Cytoplasmic organelles are limited and a narrow basal lamina is present on some external surfaces (*arrowheads*). *Inset:* Filament-associated and well-formed desmosomes join adjacent tumour cells. Magnification, ×1400; *inset*, ×9500

small, intercellular lumens complete with tight junctions and microvilli. In these cases, the microlumens were formed by cells that had general features, including intermediate filament accumulations, identical to surrounding tumour cells (Fig. 8).

Immunoperoxidase staining

The myoepitheliomas expressed a spectrum of intermediate filaments with some cases demonstrating both vimentin and GFAP as well as a variety of cytokeratins (Table 4). Except for case 5, vimen-

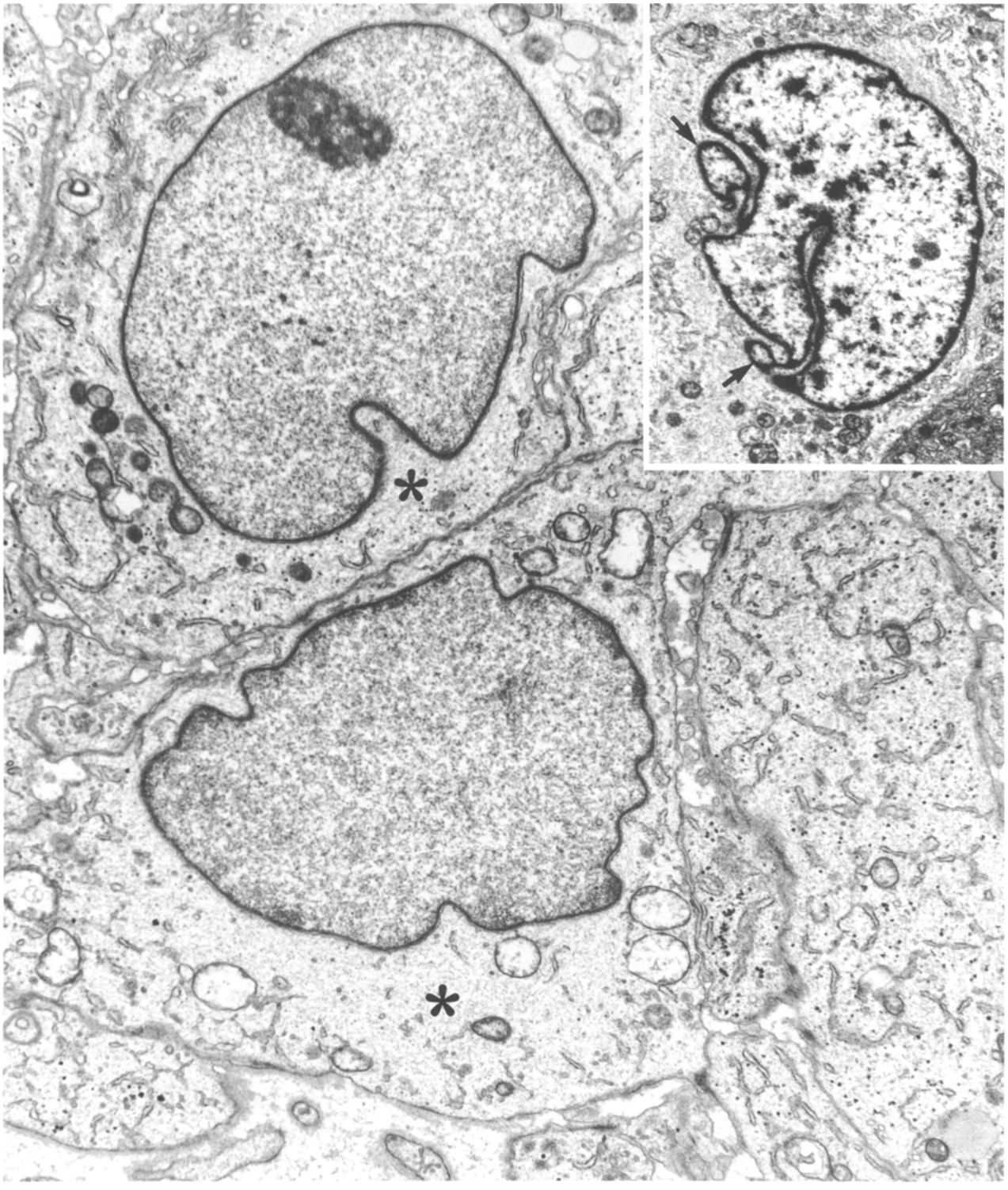

Fig. 6. Reticular myoepithelioma (case 1). Tumour cells have nuclei with markedly disaggregated condensed chromatin and limited cytoplasmic organelles, but numerous intermediate filaments (*asterisk*). *Inset:* Some nuclei (as in the plasmacytoid myoepithelioma of case 5) have deep indentations and lobations (*arrows*) joined by narrow membranous bridges of the main nuclear membrane. Magnification, ×3200; *inset*, ×1500

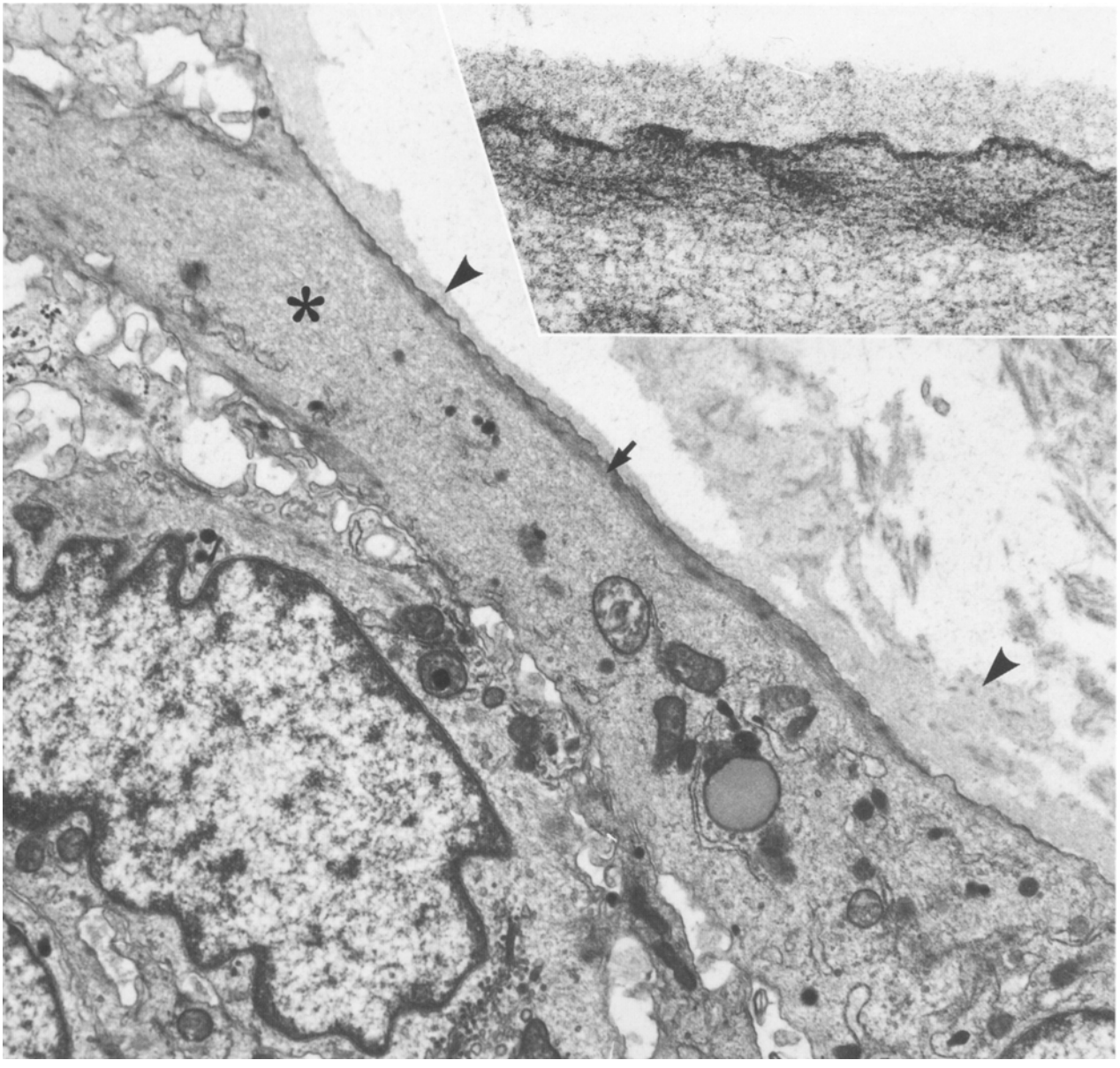

Fig. 7. Reticular myoepithelioma (case 2). Tumour cells at the periphery of the cell cords not only have accumulations of intermediate filaments (*asterisk*), but also have a narrow, peripheral band of microfilaments associated with filamentous densities (*arrow* and *inset*). A broad band of granular appearing basal lamina (*arrowheads*) is present on the external aspect of the cells and at times extends into the extracellular spaces. Magnification, ×4000; *inset*, ×21000

tin was more widely expressed than GFAP. Only case 2 demonstrated muscle-type actin and this was strongly and widely expressed in this myoepithelioma (Table 4). Of the seven anti-cytokeratins utilized, anti-epidermal keratin, PKK1, 8.12, and RPN.1162 were most consistently expressed and usually 25 percent or more of the tumour cells were moderately to strongly stained. Antibodies 312C8-1, 4.62, and 8.60 were entirely negative in most cases, but an occasional case had less than 10 percent of the tumour cells stained (Table 4). However, even those myoepitheliomas which stained positively for cytokeratins, it was infrequent for the entire tumour cell population to be stained and many focal regions were completely negative for a particular cytokeratin.

The distribution of the various intermediate filaments in myoepitheliomas is of considerable importance. In the reticular-type tumours, both GFAP- and vimentin-positive (Fig. 9a) cells were often located at the periphery of the cords and clusters of tumour cells. In the plasmacytoid myoe-

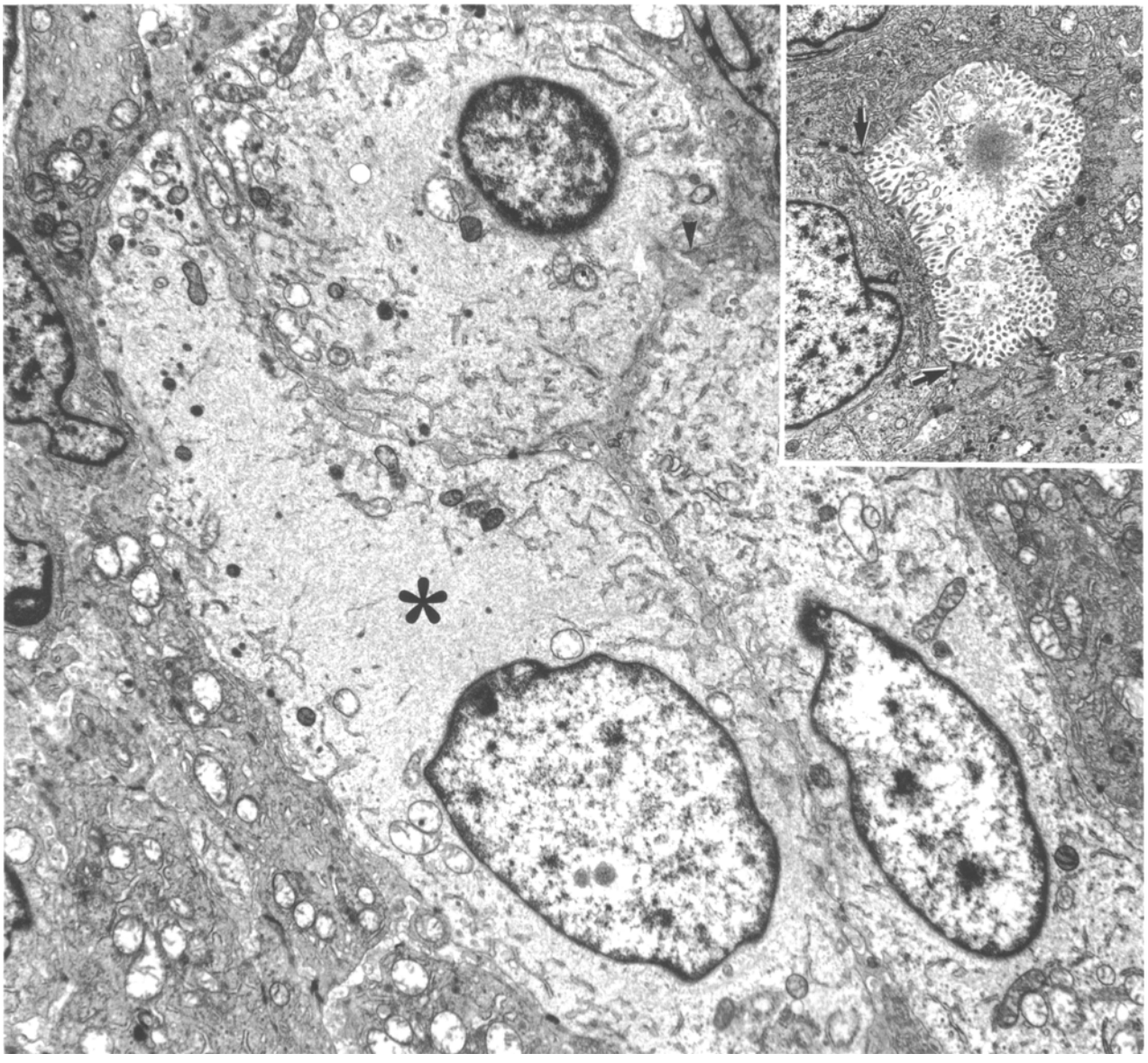

Fig. 8. Plasmacytoid myoepithelioma (case 5). Note the similarity of the cytoplasmic organelle and intermediate filament (*asterisk*) complement of these tumour cells to those from the reticular myoepitheliomas in Figs. 5 and 6. *Inset:* Focally, similar appearing tumour cells in case 5 form small intercellular lumens complete with microvilli, central secretory products, and apical tight junctions (*arrows*). Magnfications, ×8700; *inset*, ×5200

Table 3. Summary of ultrastructural characteristics in five myoepithelioma variants

Pattern/cell-type	Reticular/spindled				Solid/plasmacytoid
Feature*	Case 1	Case 2	Case 3	Case 4	Case 5
Intercellular spaces	+	+	+	±	−
Glycosaminoglycans	++	+	++	++	−
Basal lamina	+	+	+	+	+
Elastic tissue	−	−	−	+	+
Desmosomes	±	+	+	+	+
Limited organelles	++	+	++	++	+
Cytoplasmic filaments	++	+	++	++	+
Lumen formation	−	−	−	+	+
Dispersed heterochromatin	+	−	++	−	−
Nuclear blebs and lobes	±	−	+	±	+

* Degree of expression shown as: ++, extensive prominent feature; +, common feature; ±, focal or minor feature; −, absent feature

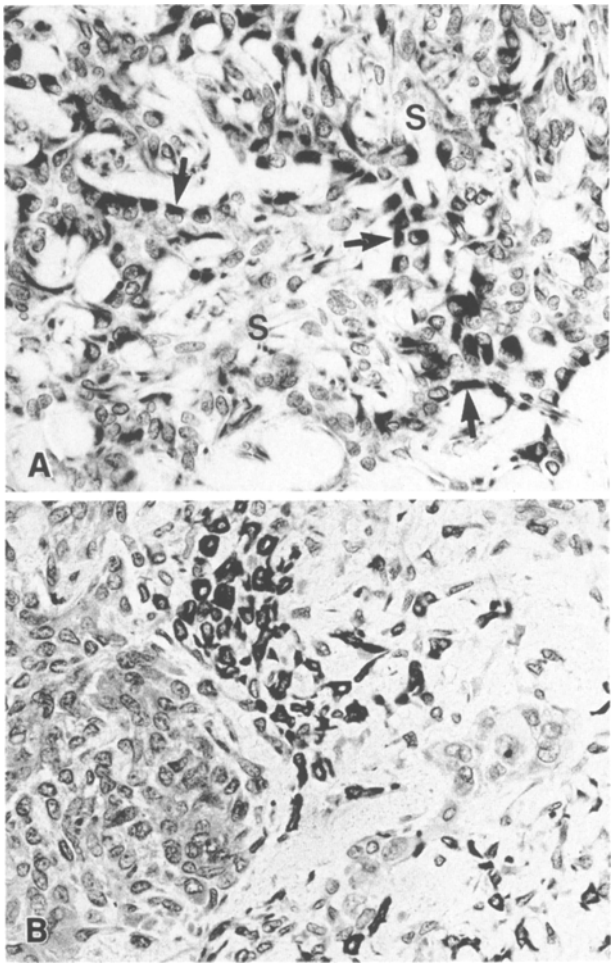

Fig. 9. (**A**). Reticular myoepithelioma (case 2). Vimentin-positive tumour cells are often located at the margins (*arrows*) of the tumour cell cords. Positive tumour cells are more strongly stained than some of the cells within the stromal tissues (S). (**B**) Plasmacytoid myoepithelioma (case 5). GFAP-positive tumour cells are mainly confined to the few foci where the cells become increasingly separated by myxoid materials. IMP with haematoxylin counterstain. (**A**) and (**B**) × 320

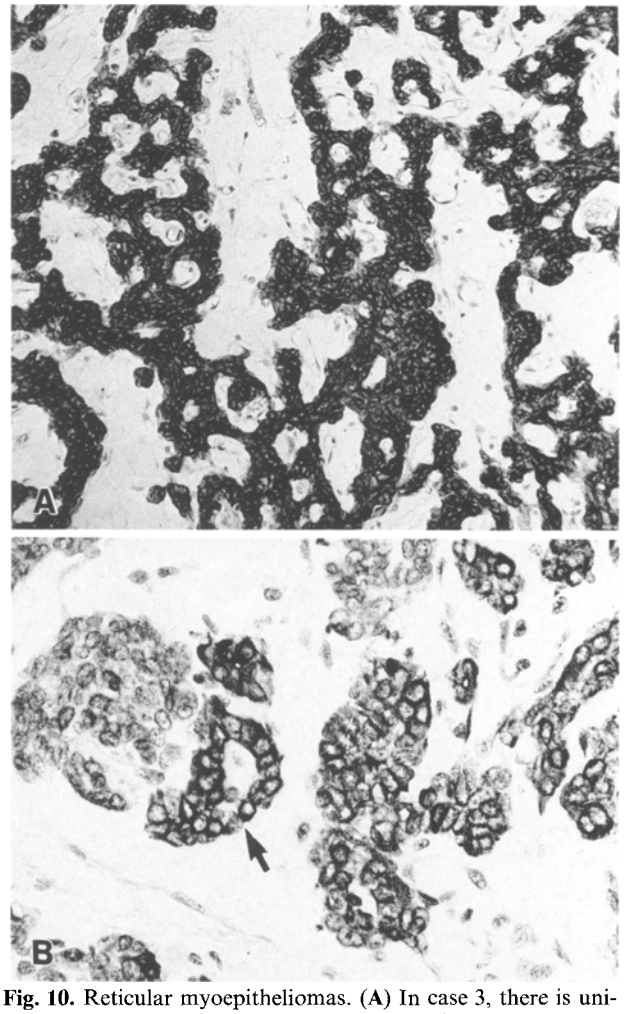

Fig. 10. Reticular myoepitheliomas. (**A**) In case 3, there is universal, strong staining with a monoclonal antibody for cytokeratins 13 and 16. (**B**) In case 1, the antibody to cytokeratins 13 and 16 stains certain compact groups of tumour cells some of which have distinct duct- or gland-type lumens (*arrows*). IMP with haematoxylin counterstain (**A**) × 130; (**B**) × 320

pithelioma, GFAP staining was most obvious in the few myxoid foci where the tumour cells were increasingly separated (Fig. 9b). In case 3 (Fig. 10a), virtually all of the tumour cells were strongly decorated by monoclonal antibody 8.12 (anticytokeratins 13 and 16), while in case 1 (Fig. 10b) this antibody stained only a proportion of the solid groups of tumour cells but did highlight the few duct-like structures in this myoepithelioma.

Serial sections, immunostained for various intermediate filaments, were used to advantage to illustrate variations in distribution in the plasmacytoid myoepithelioma (case 5). Using the anti-epidermal keratin (Fig. 11a), most of the tumour cells

Table 4. Immunoperoxidase staining of myoepitheliomas

Antibody	Case 1	Case 2	Case 3	Case 4	Case 5
anti-GFAP	1+	–	1+	ND	1+
anti-vimentin	2+	3+	2+	ND	–
HHF35	–	3+	–	ND	–
anti-epidermal keratin	1+	3+	2+	ND	1+
312C8-1	–	–	–	ND	–
PKK1	1+	–	3+	ND	3+
8.12	3+	3+	3+	ND	3+
RPN.1162	2+	2+	–	ND	2+
4.62	–	ND	–	ND	–
8.60	–	–	–	ND	–

Proportion of cell population that was moderately to strongly stained is designated by the following symbols: –, none to <10%; 1+, 10 to 25%; 2+, 25 to 50%; 3+, >50%, ND, not done

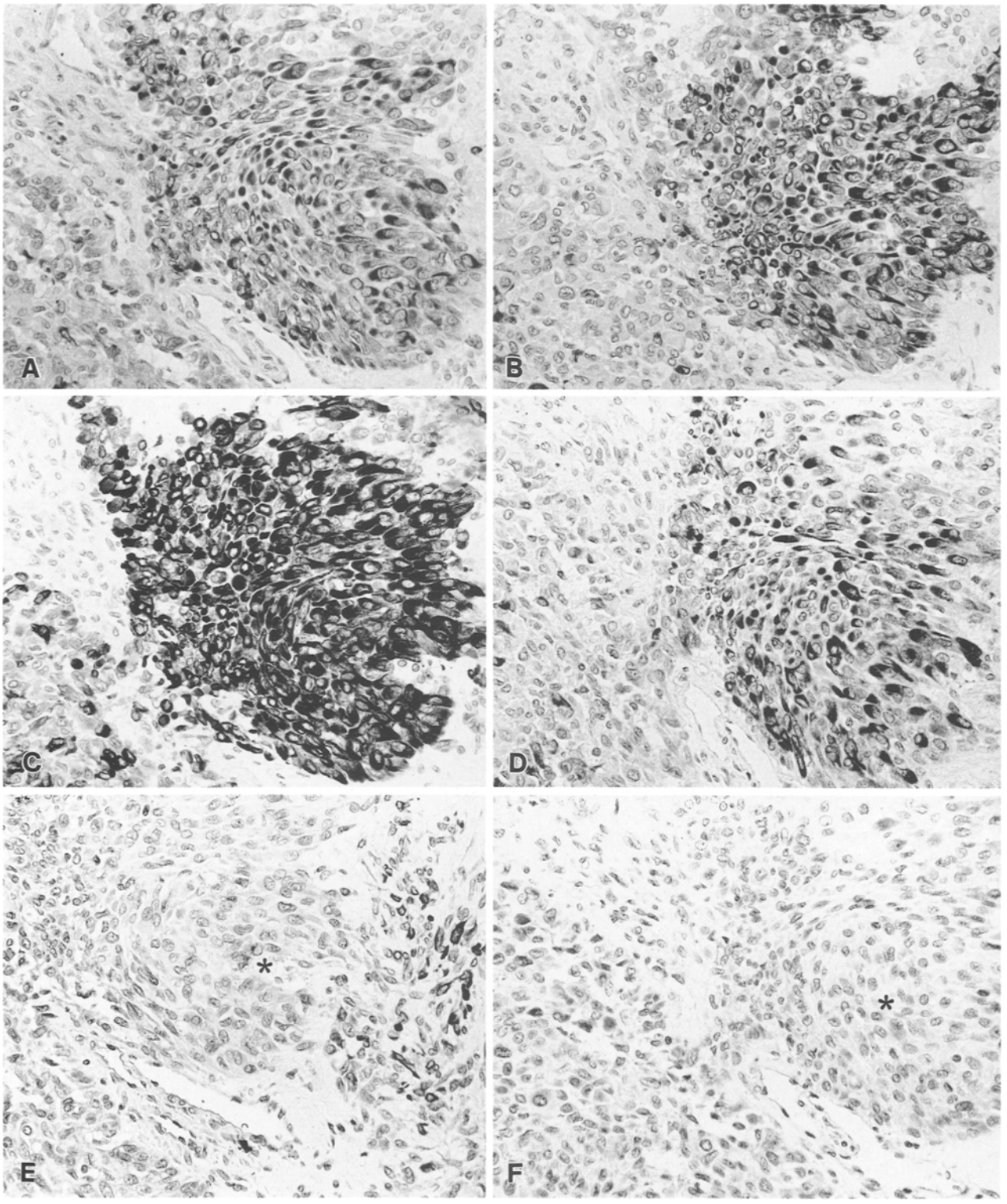

Fig. 11. Plasmacytoid myoepithelioma (case 5). Serial sections are immunostained with the following antibodies: **(A)** anti-epidermal keratin; **(B)** PKK1; **(C)** 8.12; **(D)** RPN.1162; **(E)** anti-GFAP; and **(F)** anti-vimentin. With varying intensities and proportions of the tumour cells stained, all four cytokeratin antibodies (**A** to **D**) stain a nodular region, but only a very few plasmacytoid cells are stained in the adjacent focus in the lower left corner. In contrast, the nodular region in **E** and **F** (*asterisks*) is unstained with anti-GFAP and anti-vimentin, respectively. However, anti-GFAP strongly stains tumour cells in a myxoid region to the right of the nodule in **E**, while antivimentin stains the occasional tumour cell (*arrows*) to the left of the nodule in **F**. IMP with haematoxylin counterstain. **(A)** to **(F)** ×250

Table 5. Tumour cell co-expression of intermediate filaments in double immunofluorescence labelling of myoepithelioma variants*

Antibody combination	Myoepithelioma	
	Case 1 (reticular)	Case 5 (plasmacytoid)
A 8.12 + anti-epidermal keratin	+	+
B 8.12 + GFAP	−	−
C GFAP + vimentin	−	−/+

* Symbols: +, cellular co-expression generally; −, no cellular co-expression generally; −/+, no co-expression in some cells, but definite co-expression in other cells

in one group were stained while only a minority were stained in an adjacent cluster. Antibodies PKK1 (Fig. 11b) and 8.12 (Fig. 11c) produced an identical staining pattern to the anti-epidermal keratin in both areas suggesting co-expression of at least certain of the cytokeratin filaments in some of the tumour cells. Although monoclonal antibody RPN.1162 stained only a small proportion of the tumour cells (Fig. 11d), the pattern of distribution remained similar to the other cytokeratin antibodies. All of the cytokeratin antibodies produced a ball-like or eccentric cytoplasmic area of intense staining (Figs. 11a to d) corresponding, in routinely stained sections, to the hyalinized region in the plasmacytoid tumour cells. In the zone with the most intense staining for cytokeratin filaments, anti-GFAP produced no staining, but some tumour cells at one edge of this cluster were strongly labeled (Fig. 11e). The vimentin antibody stained only a few tumour cells in either area and such staining was only weak to moderate (Fig. 11f).

Immunofluorescent staining

The results of assessing the co-expression of intermediate filaments in individual cells by a selected panel of antibodies applied to methacarn-fixed tissues of a reticular-type myoepithelioma (case 1) and the plasmacytoid myoepithelioma (case 5) are provided in Table 5.

In both tumours, there were considerable areas in which tumour cells were stained by both antibodies 8.12 (CK 13, 16) and anti-epidermal keratin

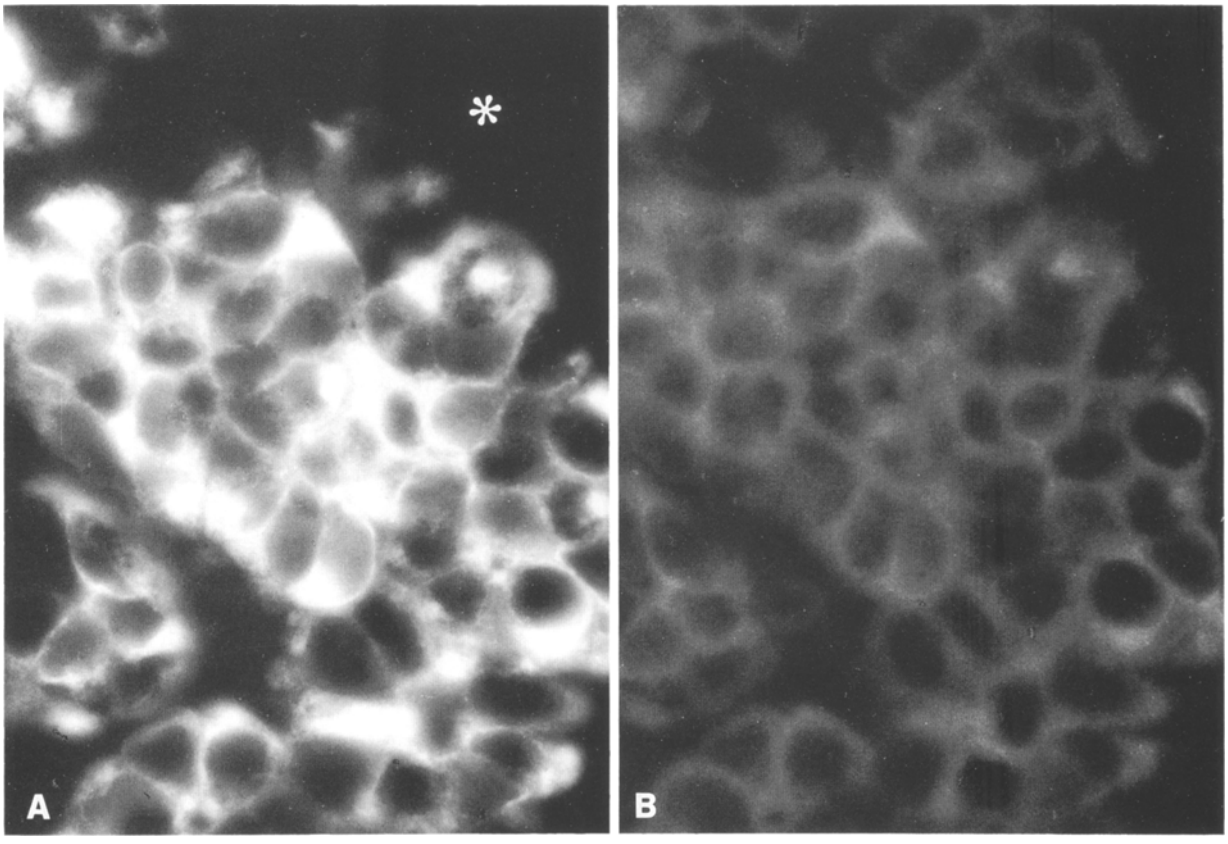

Fig. 12. Reticular myoepithelioma (case 1). Double-labeling with monoclonal antibody 8.12 (**A**) and a polyclonal anti-epidermal keratin antibody (**B**) reveals that most tumour cells are reactive with both, but some cells in this field in **A** are not decorated by antibody 8.12 (*asterisk*). Immunofluorescence with fluorescein isothiocyanate (**A**) and rhodamine (**B**). (**A**) and (**B**) × 315

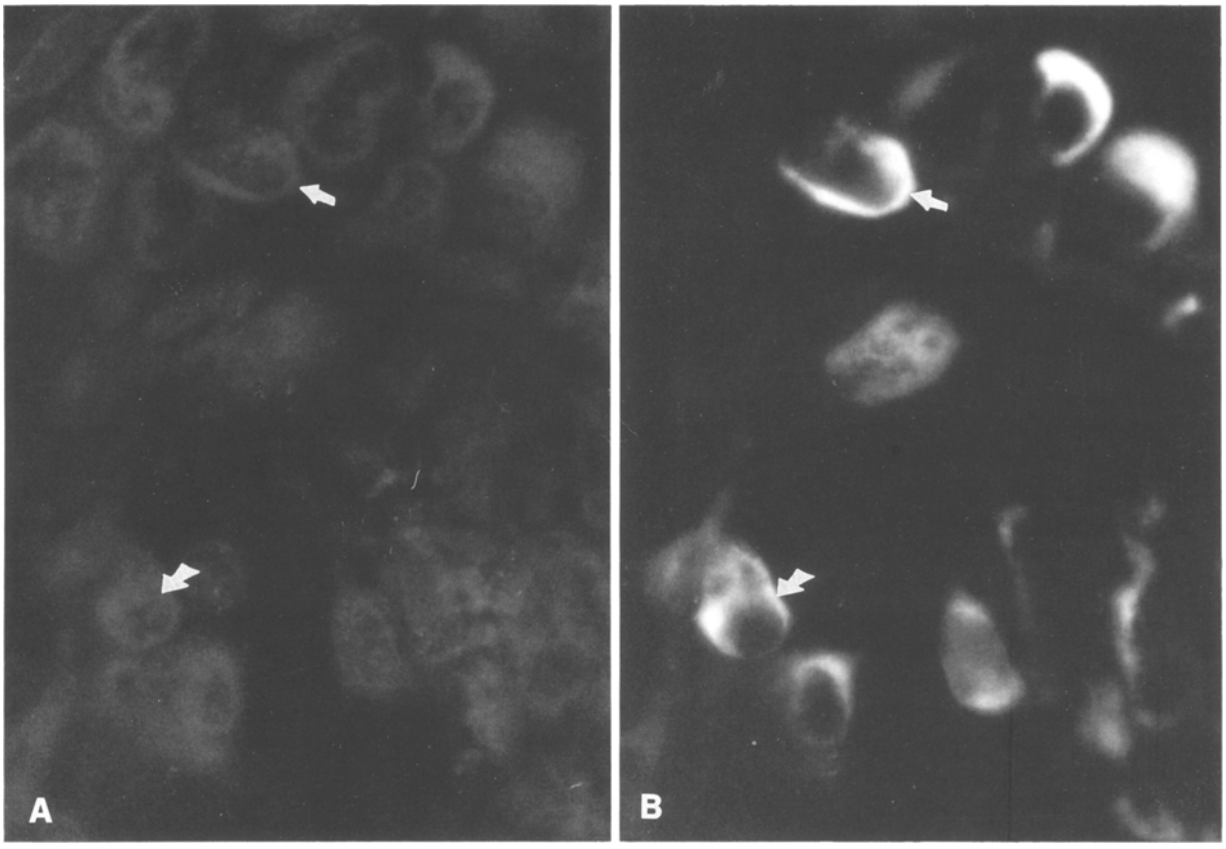

Fig. 13. Plasmacytoid myoepithelioma (case 5). In this particular area, double labeling reveals no staining with antibody 8.12 (**A**), but their slight autofluorescence shows that most of the tumour cells in this region are strongly decorated by anti-GFAP (**B**). Immunofluorescence with fluorescein isothiocyanate (**A**) and rhodamine (**B**). (**A**) and (**B**) × 315

(CK 3, 6) (Fig. 12). However, there were foci in which anti-epidermal keratin was positive but no staining was evident with 8.12 (Fig. 12), and, as is evident from the immunoperoxidase technique (Table 4), staining was more widespread with 8.12 than with the anti-epidermal keratin.

Although both cases 1 and 5 demonstrated staining with 8.12 and the GFAP antibody (Table 4), neither tumour revealed coexpression for CK 13, 16 and GFAP within individual tumour cells (Fig. 13 and Table 5). Using the combination of antibodies to vimentin and GFAP, immunofluorescent staining patterns were more complex (Table 5). In case 1, despite minimal staining for GFAP and moderate staining for vimentin (Table 4), simultaneous co-expression of these two intermediate filaments was not detected (Table 5). Case 5 demonstrated some 10 to 25 percent of the tumour cells to be positive for GFAP, but only a few small foci of vimentin-positive cells (Table 4). As a result, most areas in the sections from this myoepithelioma did not reveal individual cell co-expression for GFAP and vimentin (Table 5). However, in the few foci that showed definite immunofluorescent staining for vimentin, it was evident that some of these tumour cells did co-express GFAP while others did not (Fig. 14 and Table 5).

Discussion

Traditionally, myoepitheliomas of salivary gland have been considered to be mainly composed of spindle cells. However, using pleomorphic adenomas and other salivary gland tumours as a model, it is evident that the neoplastic component consisting of structurally modified myoepithelial cells can take the form of stellate, spindle, round or polygonal shaped cells, and that hyaline (plasmacytoid), chondroid and clear cells may also be present. It was on this basis that myoepitheliomas were subdivided into spindle-cell, hyaline-cell and mixed spindle- and hyaline-cell types (Sciubba and Brannon 1982). Usually, such tumours are associated with a nodular but solid growth pattern, but a number of myoepitheliomas reported in the literature have shown a focal to extensive myxoid,

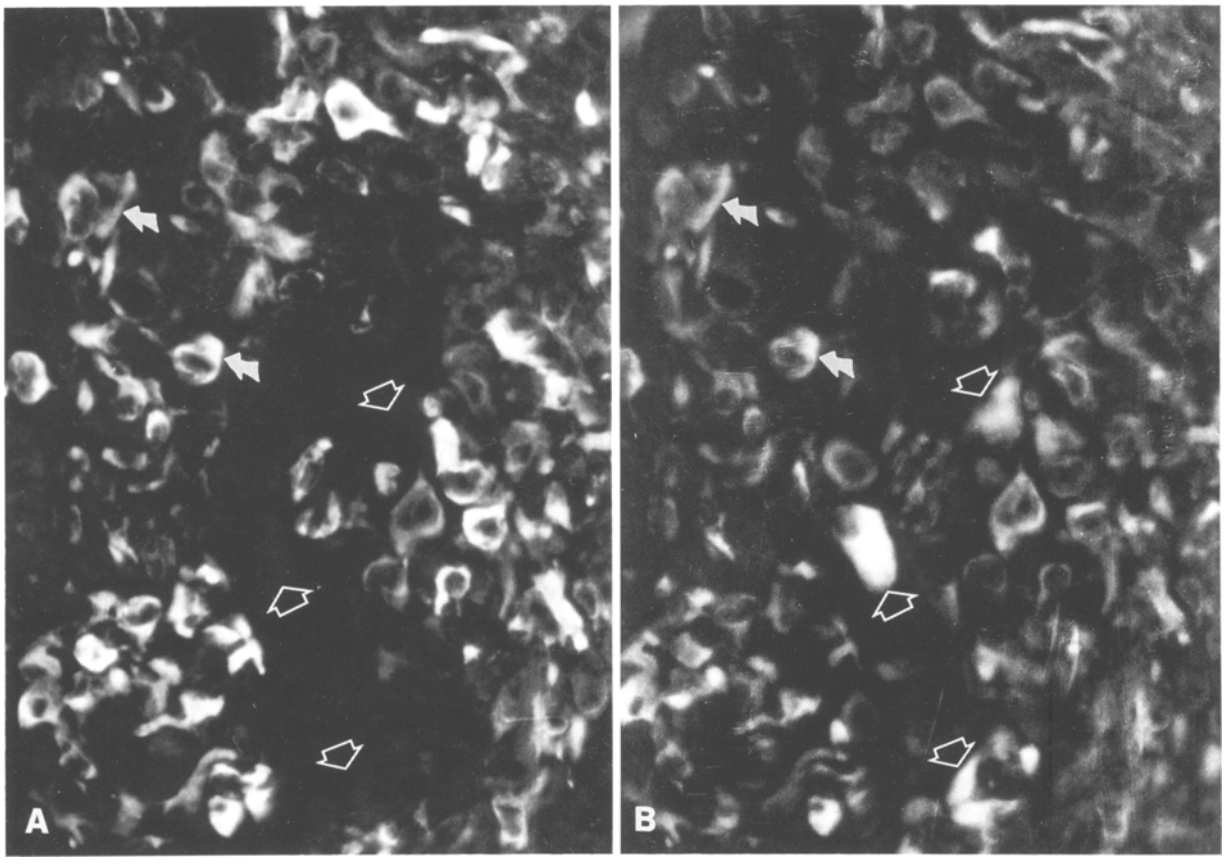

Fig. 14. Plasmacytoid myoepithelioma (case 5). (**A**) Anti-GFAP staining. (**B**) Anti-vimentin staining. Many of the tumour cells co-express GFAP and vimentin (*solid arrows*), while some cells express vimentin but not GFAP (*open arrows*). Immunofluorescence with fluorescein isothiocyanate (**A**) and rhodamine (**B**). (**A**) and (**B**) ×200

and even a chondroid component (Dardick et al. 1989b). With certain of the cell-types and the myxoid stroma characteristic of pleomorphic adenoma evident in some myoepitheliomas, perhaps other cellular organizations can be identified in pleomorphic adenoma that are reflected in additional variants of myoepithelioma.

Pleomorphic adenomas are typified by a variety of architectural and organizational patterns even within any one tumour. In some, in which there is a retention of cellular regions (containing both ductal structures and their associated modified myoepithelial cells) among the myxochondroid portions with their isolated tumour cells, there may be areas in which the neoplastic myoepithelium has a reticular arrangement, intact margins where tumour cells abut the stromal tissues, and little or no ductal development. In occasional pleomorphic adenomas, this growth pattern may predominate. As an extension of this process, the tumours in this series are exclusively or virtually entirely formed of modified myoepithelial cells with this particular growth pattern and a mucoid-appearing vascularized stroma and are thus reticular-type myoepitheliomas. That some lesions of this type have more typical myxoid foci of the type seen in pleomorphic adenomas (Nesland et al. 1981 and case 1 in the present series) and a few ducts supports the relationship with pleomorphic adenoma and the myoepitheliomatous nature of the tumour cells. Since the differentiation of tumour cells in pleomorphic adenoma results in duct luminal epithelial and myoepithelial cells to varying degrees, it does not seem unreasonable that myoepitheliomas might contain a few foci of distinct ducts or electron microscopic evidence for the formation of microlumens. In fact, we have recently reported an example of polymorphous low-grade adenocarcinoma that was largely formed by well-differentiated myoepithelial cells, and in which in a few foci, there was also a gradual developmental transition of the cells to form small duct-type lumens (Dardick and van Nostrand 1988). Based on the report by Nesland et al. (1981), myoepitheliomas with a reticular pattern can also be composed of hyaline (plasmacytoid) cells.

Other features suggest that these salivary gland neoplasms with a reticular growth pattern are a form of myoepithelioma. If the plasmacytoid tumour cells of pleomorphic adenomas and myoepitheliomas are considered modified myoepithelial cells, and bear no microscopic similarity to normal myoepithelium, the similarity of the ultrastructural features of plasmacytoid cells in case 5 to those of the tumour cells in the reticular-type of myoepitheliomas (cases 1 to 4) indicates that the latter are also a form of modified myoepithelium. Furthermore, the finding of a peripheral band of filaments associated with focal densities in some tumour cells in case 2 is a feature that has also been seen in some pleomorphic adenomas (Erlandson et al. 1984) and at least two myoepitheliomas (Chaudry et al. 1982; MacKay et al. 1988). Like plasmacytoid myoepitheliomas, reticular myoepitheliomas display prominent intermediate filaments. Indeed, the ultrastructural findings in the myoepithelial cell component of pleomorphic adenomas (Dardick et al. 1983a, b), malignant mixed tumours (Dardick et al. 1989a), adenoid cystic carcinomas (Dardick and van Nostrand 1987; Orenstein et al. 1985), and even some basal cell adenomas (Dardick et al. 1984) are also evident in myoepitheliomas.

It is interesting that there is a morphological spectrum of basal epithelial and modified myoepithelial cells associated with intra- and interlobular salivary gland ducts (Dardick et al. 1987). Since such cells share certain key characteristics with the myoepithelium of acini and intercalated ducts (Dardick et al. 1987; Dardick et al. 1988), perhaps the tumour cells in myoepitheliomas may be the neoplastic counterpart of duct basal cells in some cases and more typical myoepithelial cells in others (or any mixture of these cell-types), along with development of a few luminal-type cells in either case. Immunohistochemically, such basal cells have cytokeratin filament complements (Dardick et al. 1988) that more closely resemble those found in the non-ductal component (i.e., modified myoepithelial cells) of pleomorhic adenoma (Burns et al. 1988) and in this series, then that found in myoepithelial cells of acini and intercalated ducts (Burns et al. 1988). In particular, normal myoepithelial cells do not immunolabel with anti-epidermal keratin, PKKl, 8.60, or 8.12 antisera, but these antibodies do decorate basal cells of normal salivary ducts. In the myoepitheliomas of this report, with this series of antibodies, only antibody 8.60 failed to stain the tumour cells. The extent of differentiation possible in these myoepitheliomas may be reflected in the one case positively stained with antibody HHF35 (for muscle specific actin), an antibody that has also identified a subset of basal cells in striated ducts of normal salivary gland (Dardick et al. 1988). Thus, the lack of development of the complete features of normal myoepithelium in most salivary gland tumours may simply reflect features of the heterogeneous population of basal cells found in normal major salivary gland ducts.

Pleomorphic adenomas demonstrate considerable variability in expression of cytokeratin filaments both within individual cases and between examples (Burns et al. 1988; Leoncini et al. 1988; Mori et al. 1986, 1987). Of the various cytokeratin filaments, those that are preferentially expressed in the myoepithelial and/or basal cells of normal salivary gland and breast are CK 4 to 6, 13, 14 and 16 (Born et al. 1987; Burns et al. 1987; Caselitz et al. 1986a, b; Dairkee et al. 1985, 1986; Dardick et al. 1987; Dardick et al. 1988; Geiger et al. 1987; Leoncini et al. 1988; Nagle et al. 1986). With this background, it is noteworthy that these myoepitheliomas, whether reticular or plasmacytoid, while expressing a variety of cytokeratins, at least in some tumour cells, do not express CK 14 (antibody 312C8-1). Similarly, although duct luminal epithelial and myoepithelial/basal cells all stain for CK 19 in the normal salivary gland (Born et al. 1987; Burns et al. 1988; Geiger et al. 1987), this cytokeratin filament was not expressed in these myoepitheliomas. However, with the gradual modification of the neoplastic and modified myoepithelial cells of the myxo-chondroid regions in pleomorphic adenomas, a similar alteration of filament expression occurs (Burns et al. 1988). Indeed, there is a range of morphological and cytoplasmic filament expression of the non-duct epithelial cells in pleomorphic adenoma and myoepithelioma, with only occasional tumour cells of this type expressing recognizable myoepithelial cell characteristics (Chisholm et al. 1972; Dardick et al. 1987; Erlandson et al. 1984; Palmer et al. 1985b); only the infrequent case is composed largely of well-differentiated myoepithelial cells (Chaudhry et al. 1982; MacKay et al. 1988). The infrequency of complete differentiation even in myoepitheliomas is evidenced by the fact that only case 2 stained immunohistochemically for muscle-specific actin (Table 4) and had cytoplasmic microfilament arrays ultrastructurally, but did not express CK 14. In parallel with the tumour cells situated peripheral to ductal structures or in the solid regions of pleomorphic adenoma (Burns et al. 1988), tumour cells in the myoepitheliomas of the current series express vimentin and GFAP either individual or simulataneously.

Both the reticular-type and plasmacytoid cell myoepitheliomas have a rich cytoplasmic filament network ultrastructurally that corresponds to the prominent cytoplasmic zonal staining for a variety of intermediate filaments immunohistochemically and to the focal glassy appearing regions of the plasmacytoid cells in routinely stained sections. Since dual expression of cytokeratins and vimentin (Caselitz et al. 1982; Erlandson et al. 1984; Krepler et al. 1982; Rozell et al. 1985), as well as GFAP and cytokeratins (Achtstatter et al. 1986), have been demonstrated in some tumour cells of pleomorphic adenoma, these myoepitheliomas provide an opportunity to see if these and other combinations of intermediate filaments exist in the tumour cells of this lesion. Not unexpectedly, a considerable number of different cytokeratin filaments can be found in the same tumour cell. Despite the fact that antibody 8.12 (anti-CK 13 and 16) is strongly and extensively expressed in all the myoepitheliomas (Table 4), no co-expression of these cytokeratins and GFAP was detected (Table 5). The combination of GFAP and vimentin in individual cells was only focally evident in the plasmacytoid myoepithelioma (case 5) and not at all in the reticular myoepithelioma (case 1). Yet both of these intermediate filaments were detected immunohistochemically in case 1. This indicates that in most tumour cells in myoepithelioma, GFAP and vimentin are expressed independently. Although cytokeratins can be expressed with either vimentin or GFAP, and possibly all three can be present in tumour cells of pleomorphic adenoma (Achtstatter et al. 1986), this does not appear to be the case in the majority of the tumour cells in myoepitheliomas.

In summary, we have described and compared the histological, immunocytochemical, and ultrastructural features of two variants of myoepithelioma, one of which has an unique, reticular form of growth pattern, while the other has a solid pattern composed of plasmacytoid (hyaline) cells. These tumour-types share many light and electron microscopic characteristics and the tumour cells have similar cytoplasmic filament complements to those observed in pleomorphic adenomas indicating the close morphogenetic relationship of these two salivary gland neoplasms. In fact, the tumours described in this report have likely been classified in the past as a form of pleomorphic adenoma. Undoubtedly, they bear a close relationship to pleomorphic adenoma but differ in the lack of a prominent ductular component and the sharp demarcation of the periphery of the cellular cords from the myxo-vascular stroma. Because of their unusual morphology and infrequent occurrence, the reticular variant of myoepithelioma should be identified to ensure recognition of the full spectrum of myoepitheliomas and to avoid their misclassification as some form of malignant tumour.

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