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Cytoskeletal filaments in the smooth muscle cells of uterine leiomyomata and myometrium: an ultrastructural and immunohistochemical analysis

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Summary. The filamentous components of the cytoskeleton in smooth muscle cells of leiomyomata and normal myometrium were studied by immunohistochemistry and electron microscopy. Fourteen patients hysterectomised for non-malignant disease provided leiomyomata of conventional histological type and histologically normal myometrium: four samples of fetal myometrium were studied by immunohistochemistry alone. All samples of leiomyoma and myometrium were strongly positive for α-smooth muscle actin and desmin, the latter often as paranuclear columns or granules. Vimentin was also stained in most samples but less intensely, while cytokeratin stained in about half the samples with an intensity comparable to that of vimentin. By electron microscopy, myofilaments with focal densities were abundant in both normal myometrium and leiomyomata. Intermediate filaments corresponding to the desmin and vimentin demonstrated by immunohistochemistry were also recognised in a variety of architectural arrangements. At one extreme, comparatively small numbers of filaments were loosely distributed around membranous organelles; at the other, filaments formed conspicuous aggregates, largely excluding other organelles and corresponding to the paranuclear granules seen by immunohistochemistry. A comparison of these findings with those of the literature and comments on the possible significance and origin of these aggregates are provided.

Key words: Leiomyoma – Myometrium – Cytoskeleton – Ultrastructure – Immunohistochemistry

Introduction

Leiomyomata are the commonest benign tumour of the uterus, occurring in more than 20% of women over the age of 30 years (Norris and Zaloudek 1982). Their morphology, their content of α-smooth muscle actin and desmin, as demonstrated by immunohistochemistry, and of myofilaments with focal densities as shown by electron microscopy, define them as showing smooth muscle cell differentiation. In contrast to their clearly defined intermediate filament profile as determined by immunohistochemistry (Evans et al. 1983; Abenoza and Sibley 1987; Brown et al. 1987; Norton et al. 1987; Ramaekers et al. 1988; Hyde et al. 1989) the ultrastructural organisation of intermediate filaments has received little attention. This paper (a) provides a correlated study of the cytoskeleton of uterine leiomyomata using immunohistochemistry and electron microscopy; (b) details a cytoskeletal abnormality that has so far been poorly documented; and (c) compares the findings with those on the surrounding smooth muscle cells of the myometrium.

Materials and methods

Ten uterine leiomyomata were obtained from patients undergoing hysterectomy mainly for a variety of menstrual dysfunctions including: menorrhagia; menorrhagia with dysmenorrhoea; menorrhagia with bulky uterus; utero-vaginal prolapse; and irregular cycle. Leiomyomata were of conventional histological type. Myometrium of grossly normal appearance was available from these tumour-bearing uteri, as well as from four additional hysterectomy specimens (cases 11–14; Table 1) found not to be harbouring leiomyomata. In addition, four histologically normal fetal myometrium specimens were studied. For light microscopy, tissue was fixed in 10% phosphate-buffered formalin followed by ethanol dehydration and embedding in paraffin wax. Subsequently, sections at 5 μm were stained in haematoxylin and eosin. The ABC immunoperoxidase technique was used for the detection of the following cytoskeletal proteins: cytokeratin (using CAM 5.2 from Becton-Dickinson, Erembodegem, Belgium), vimentin and desmin (both from Dako, High Wycombe, UK) and α-smooth muscle actin (Sigma, Poole, UK). Tissue for electron microscopy was available from (a) all ten conventional leiomyomata; (b) five specimens of normal myometrium from this group; (c) normal myometrium from the the four non-tumour-bearing uteri. Tissues were fixed in cacodylate-buffered 2.5% glutaraldehyde followed by osmium tetroxide, en bloc staining in uranyl acetate, dehydration in ethanol and embedding in epoxy resin. Ultra-thin sections were stained in aqueous uranyl acetate and Reynolds' lead citrate, and examined in an AEI 801 electron microscope.

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Table 1. Immunohistochemical findings

Case number	Desmin	Alpha-smooth muscle actin	Vimentin	Cytokeratin
Leiomyomata and normal adult myometrium				
1	3+	2+	0	0
2	3+	3+	1+/2+	0
3	3+	2+	0	1+
4	3+	3+	2+	0
5	3+	3+	2+	0
6	3+	3+	2+	1+/2+
7	3+	3+	2+	0
8	3+	2+	1+	0
9	3+	3+	1+	1+
10	3+	3+	2+	0
Normal adult myometrium				
11	3+	2+	1+	1+/2+
12	3+	3+	1+	1+
13	3+	3+	1+	1+/2+
14	3+	3+	1+/2+	1+/2+
Normal fetal myometrium				
15	2+	2+	1+	0
16	3+	3+	0	0
17	3+	2+	1+	0
18	2+	2+	0	0

Results

Light microscopy

Leiomyomata showed an architecture consisting of fascicles of spindle-shaped cells with nuclei having rounded ends and a substantial, eosinophilic cytoplasm. Collagenous stroma between the fascicles was abundant. Myometrium had a similar cellular composition but had a more ordered architecture and a smaller fibrous tissue component.

Immunohistochemistry

All specimens of leiomyomata and myometrium (adult and fetal) were strongly positive for both α-smooth muscle actin (Fig. 1A) and desmin (Fig. 1B; Table 1). Leiomyoma and myometrium from a given case were immunohistochemically indistinguishable. Positive vimentin staining was found in all but two cases and was less intense, while CAM 5.2 antibody stained about half of the specimens with a similar degree of intensity as vimentin. The α-smooth muscle actin antibody stained the cell periphery whereas the desmin, vimentin and cytokeratin were found centrally, often in the form of a cone or column based on a nuclear pole. In some cases of leiomyomata and adult and fetal myometrium, desmin and vimentin were seen as large, rounded or oval, paranuclear inclusions.

Electron microscopy

Leiomyomatous and myometrial smooth muscle cells showed features typical of smooth muscle differentiation

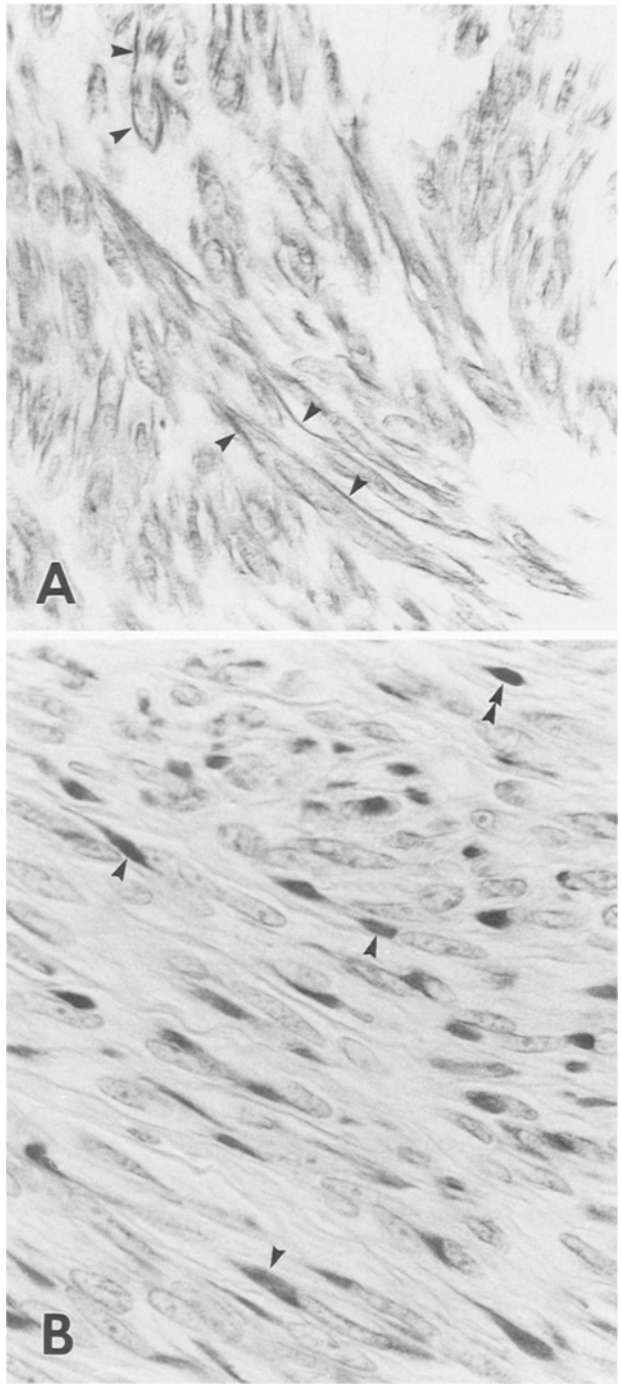

Fig. 1A, B. Leiomyoma – case 2 (30-year-old with menorrhagia). Immunohistochemical demonstration of α-smooth muscle actin (**A**) and desmin (**B**). Note the peripheral linear staining pattern in **A** and the ovoid (*double arrowhead*) or more elongate paranuclear staining pattern in **B**. × 800

(Fig. 2A). Myofilaments with focal densities were generally abundant (Fig. 2A), though in a few cells they were more modestly developed at the expense of other cellular constituents. Organelles such as rough endoplasmic reticulum (rER) cisternae, mitochondria, an occasional lysosomal body and Golgi apparatus tended to be concentrated around the nuclear poles or in other paranuclear locations. Prominent subplasmalemmal densities, focally

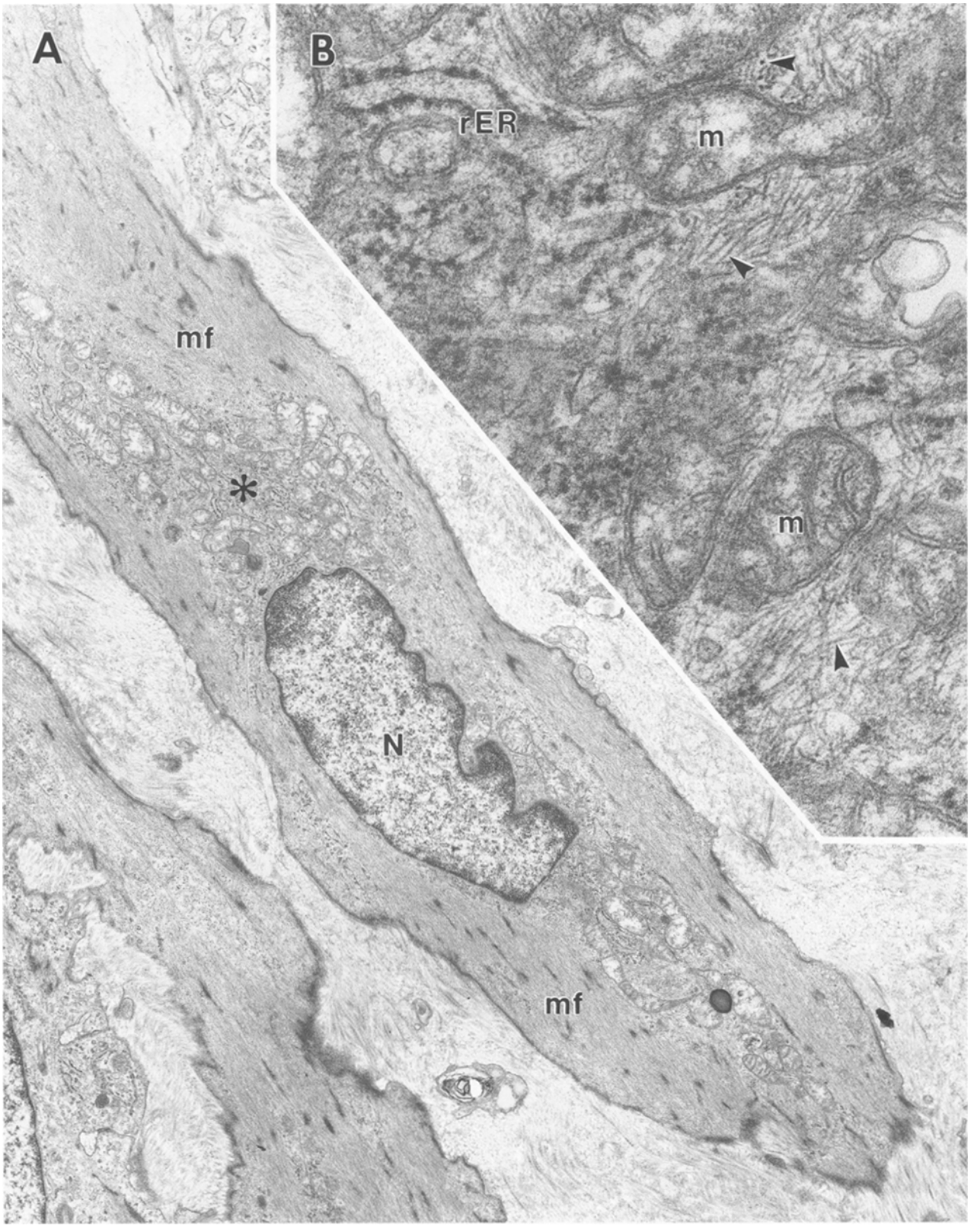

Fig. 2A, B. Leiomyoma – case 5 (49-year-old with irregular cycle). **A** Tumour cell showing typical smooth muscle cell features of central nucleus (*N*), paranuclear aggregate of organelles (*) and peripheral sheath of myofilaments with focal densities (*mf*). ×9000. **B** Detail of asterisked area in Fig. 2A showing intermediate filaments in long and cross-section (*arrowheads*) loosely distributed around membranous organelles (*rER*, rough endoplasmic reticulum; *m*, mitochondria). ×71000

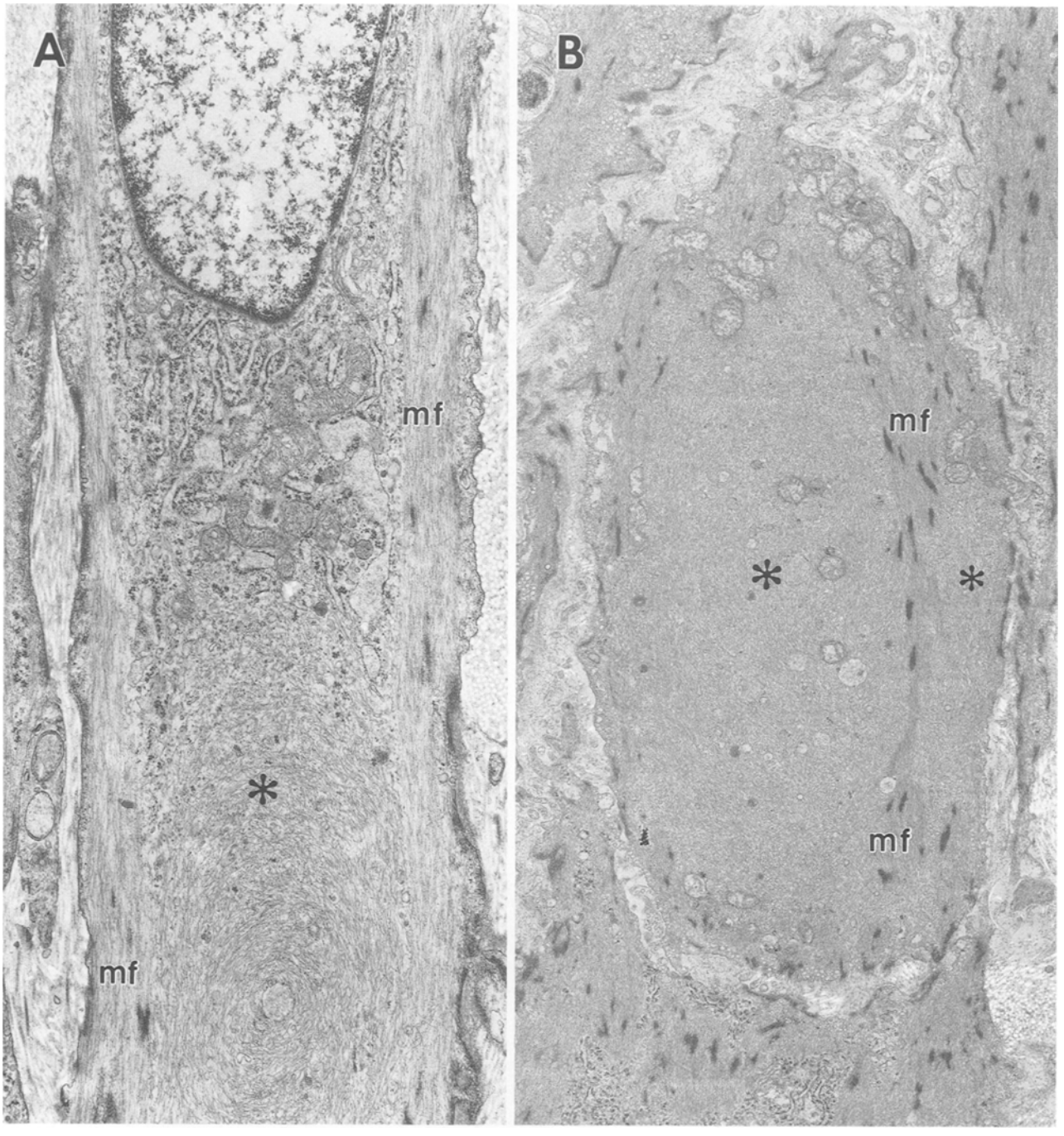

Fig. 3A, B. Leiomyoma – cases 3 (34-year-old with menorrhagia and bulky uterus) (A) and 6 (55-year-old with utero-vaginal prolapse) (**B**). **A** Intermediate filament aggregate (*) distal to paranuclear assembly of membranous organelles, both surrounded by peripheral myofilaments (*mf*). ×14000. **B** Large, oval intermediate filament aggregate (*asterisks*) occupying a large proportion of a leiomyoma tumour cell, surrounded and partially dissected by myofilaments (*mf*) with focal densities. ×9000

conspicuous surface membrane caveolae and a well-defined but discontinuous lamina, were observed.

Intermediate filaments were recognised as 10-nm-thick structures, noticeably coarser and more discrete than myofilaments, and corresponding approximately to the thickness of the plasmalemma. Often, they had a wavy or gently curving profile. They were found interspersed between the cellular constituents outlined above, in almost all cells of all ten leiomyomata and all nine samples of myometrium. The numbers of filaments varied within any given sample. Small numbers were frequently found between organelles such as mitochondria, rER cisternae, and the peripheral sheath of myofilaments (Fig. 2B). Larger numbers were organised into fairly sharply delineated aggregates that were usually rounded, oval or rather elongate in outline (Figs. 3A, B, 4A). Typically they were 4–10 μm long and often about twice as long as wide; the two largest examples

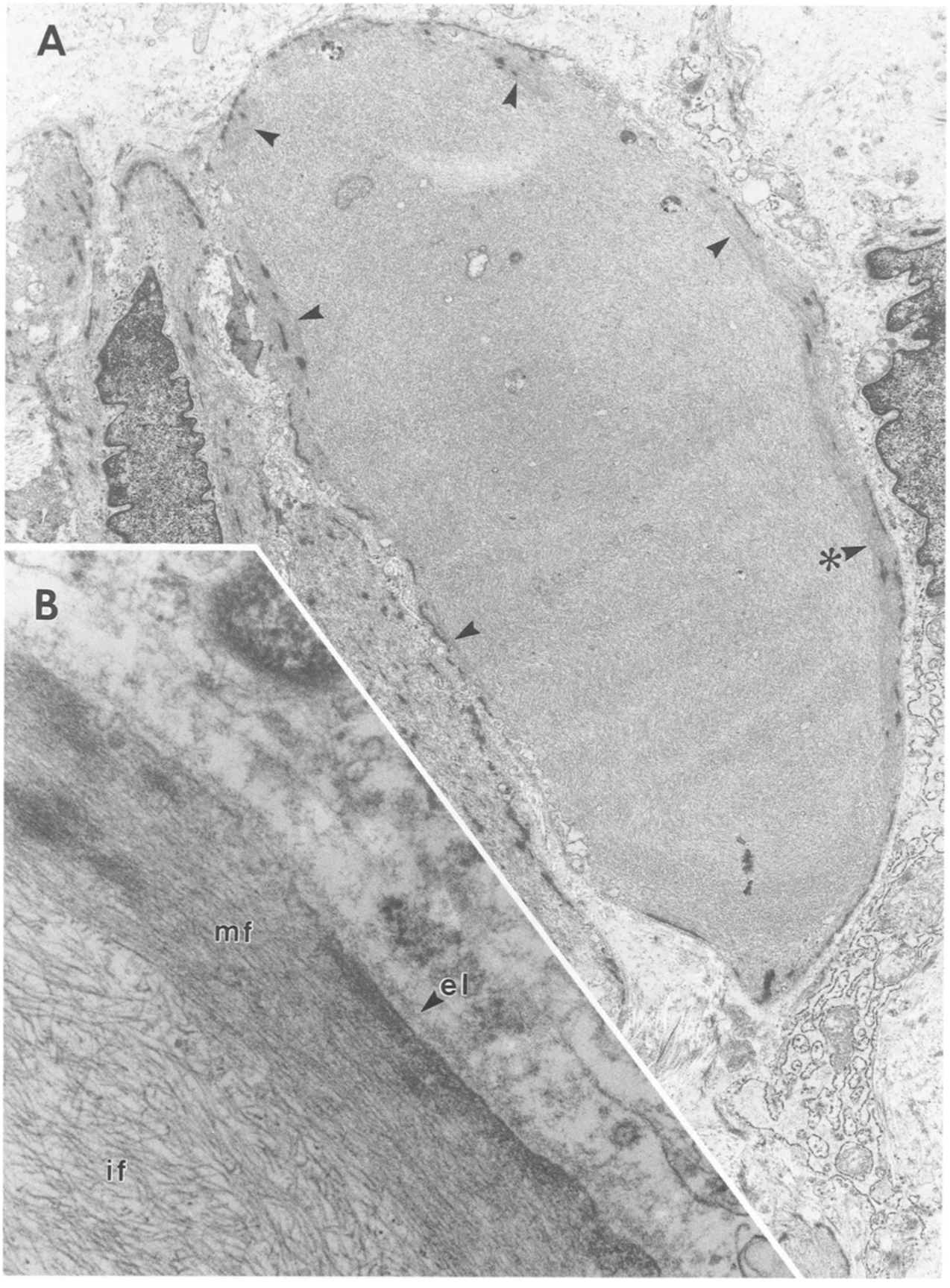

Fig. 4A, B. Leiomyoma – case 6. **A** Giant intermediate filament aggregate occupying almost the entirety of a tumour cell with only a narrow and incomplete peripheral rim of myofilaments (*arrowheads*) with focal densities. ×9000. **B** Detail of the asterisked area of Fig. 4A showing the distinction between the finer, more uniformly orientated myofilaments (*mf*) and the inner mass of anisotropic intermediate filaments (*if*). *el*, external lamina. ×71000

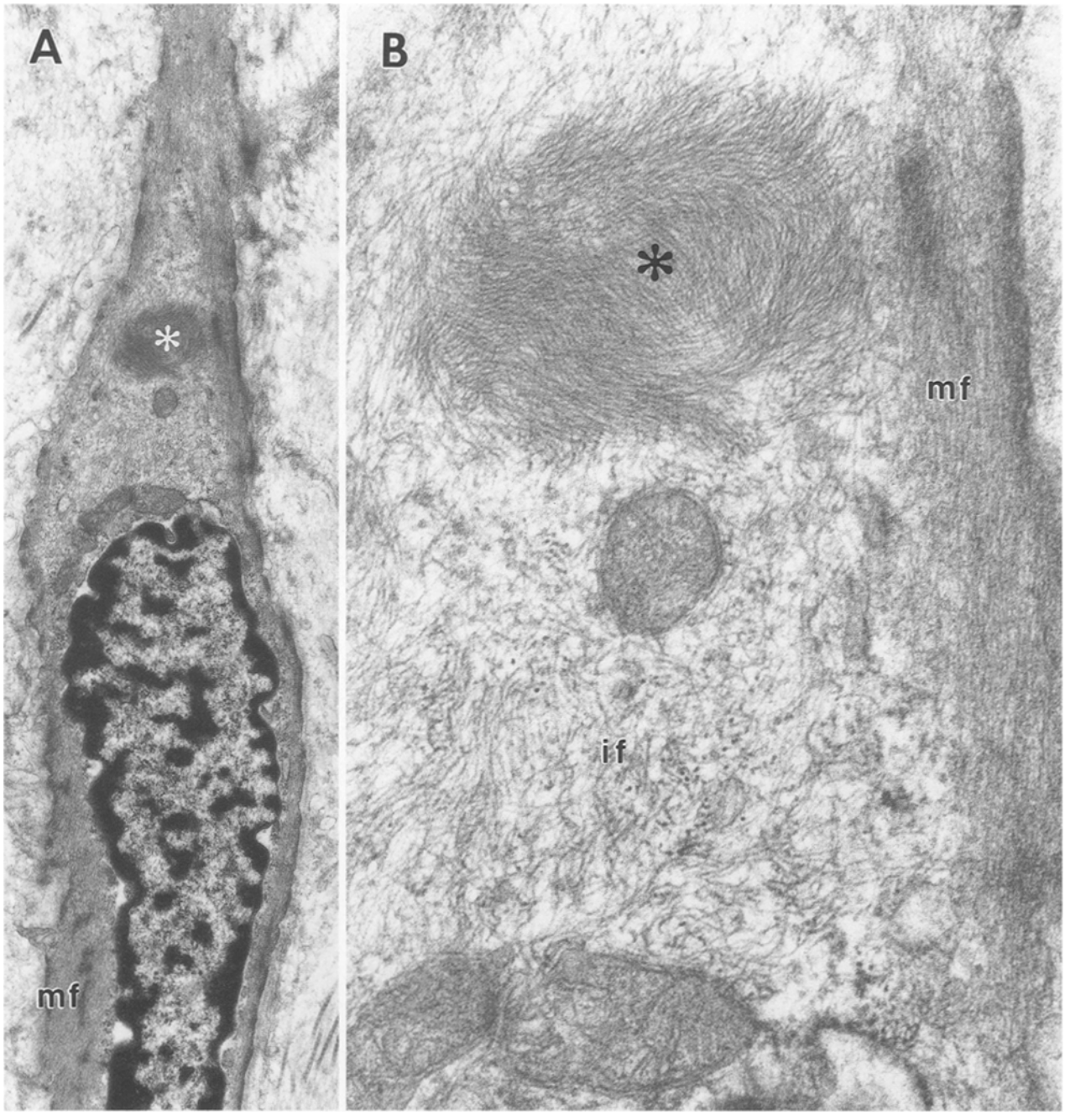

Fig. 5A, B. Normal myometrium – case 9 (48-year-old with menorrhagia and bulky uterus). **A** Myometrial cell showing peripheral myofilaments (*mf*) with focal densities and a paranuclear aggregate of intermediate filaments possessing light and dark (*) areas. ×13000. **B** Loosely organised (*if*) and more compacted (*) areas of the intermediate filament aggregate shown in **B**. *mf*, myofilaments. ×71000

encountered measured 12×15 μm and 9×20 μm (Figs. 3B and 4A respectively). In some cells the aggregates formed a column or cone based on a nuclear pole and were enveloped by the sheath-like mass of myofilaments. Sometimes, intermediate filaments were seen between the peripheral myofilament bundles and the plasmalemma (Fig. 3B). On rare occasions, a very large intermediate filament aggregate occupied almost the entire cell, leaving only a thin surrounding rim of myofilaments (Fig. 4A). The aggregates consisted of ultrastructurally uniform filaments arranged anisotropically (Fig. 4B), with no evidence of the kind of fascicle formation characterising tonofibrils.

Normal myometrium contained intermediate filaments organised in much the same fashion as in the leiomyomata, as described above. As well as comparatively small numbers of filaments dispersed amongst organelles, there were also aggregates (Fig. 5A, B). They resembled those of the leiomyomata (for example, Fig. 3A) except that they were somewhat smaller and fewer, and none were found rivalling in size the giant aggregates illustrated in Figs. 3B and 4A. In one inclu-

sion, intermediate filaments in a discrete mass of increased density – but again lacking the appearance of tonofibrils – were observed within a larger, pale-staining aggregate (Fig. 5A, B), the difference in density possibly being due to the degree of filament packing.

Discussion

The ultrastructural appearance of the contractile filament system is well documented in normal human myometrium (Mark 1956; Hashimoto et al. 1960; Laguens and Lagrutta 1964; Ferenczy et al. 1971; Morales et al. 1975; Cole and Garfield 1989; Fujii et al. 1989, 1990) and uterine leiomyomata (Ferenczy et al. 1971; Goodhue et al. 1974; Morales et al. 1975; Cramer et al. 1980; Payan et al. 1980; Konishi et al. 1983; Mazur and Priest 1986; Hyde et al. 1989). The other major components of the cytoskeleton – intermediate filaments and microtubules – have, by contrast, received little attention. The intermediate filament system has been investigated immunohistochemically and by gel electrophoresis in myometrium (Huitfeldt and Brandtzaeg 1985; Brown et al. 1987; Norton et al. 1987; Ramaekers et al. 1988; Turley et al. 1988) and in leiomyomata of conventional (Evans et al. 1983; Brown et al. 1987; Norton et al. 1987; Ramaekers et al. 1988) clear-cell, epithelioid (Hyde et al. 1989) and granular cell (Abenoza and Sibley 1987) types. Vimentin, desmin and cytokeratin are the intermediate filament proteins that have been demonstrated and our observations confirm these findings for both normal myometrium and leiomyomata. Our results also confirm the presence of α-smooth muscle actin in the contractile filament system of the cytoskeleton in both myometrium (Huitfeldt and Brandtzaeg 1985) and uterine leiomyomata (Ramaekers et al. 1988; Hyde et al. 1989).

In contrast to these immunohistochemical studies, the structural identity and organisation of intermediate filaments is poorly documented. Loosely organised filaments in the cytoplasmic matrix around membranous organelles – as in our Fig. 2B – can be seen, though they did not prompt comment, in Morales et al. (1975), while a group of cross-sectioned intermediate filaments in the vicinity of myofilaments were pointed out in a smooth muscle cell of the myometrium in a normal nonpregnant uterus by Cole and Garfield (1989). In the case of uterine leiomyomata, the majority of fine structural studies are early investigations predating the intermediate filament concept, where for this reason intermediate filaments seem not to have noticed. We can find only one study drawing specific attention to the fine structural appearance of intermediate filaments in uterine leiomyomata – that of Warner and Seo (1980). They showed a single micrograph of an inclusion of intermediate filaments in an epithelioid variant, in a paper without immunohistochemical correlation or clinical data. In some other studies, sparse filaments corresponding in size and appearance to intermediate filaments can be seen in electron micrograph figures (e.g. Ferenczy et al. 1971) but, as in myometrium, have not prompted comment.

These ultrastructurally identifiable intermediate filament aggregates clearly correspond to the paranuclear and highly localised rounded or oval concentrations of immunoperoxidase reaction product generated during light microscope immunolocalisation with anti-vimentin and anti-cytokeratin but particularly anti-desmin antibodies. Although initially unexpected, cytokeratin immunoreactivity in normal and neoplastic smooth muscle cells is now well documented (Huitfeldt and Brandtzaeg 1985; Brown et al. 1987; Norton et al. 1987; Ramaekers et al. 1988; Turley et al. 1988; Tauchi et al. 1990) and our findings further confirm this phenomenon while emphasising the additional point that ultrastructurally identifiable features of epithelial differentiation are absent.

The intermediate filament aggregates also clearly represent an abnormality of the cytoskeleton in terms of a disordered equilibrium between synthesis and turnover of filament proteins. In normal cells the intermediate filament system is distributed around the nucleus and into cell processes and it is this dispersed state which permits maximum interaction with other cytoskeletal components such as microtubules and myofilaments, enabling the overall cytoskeleton to function appropriately. The aggregation of any one cytoskeletal element necessarily reduces interaction with other components. While such intermediate filament aggregates are to be expected and are well documented in neoplastic cells (Ghadially 1988a), it is perhaps surprising to find them in normal myometrium. However, large numbers of intermediate filaments are present in normal developing smooth muscle from alimentary and genito-urinary sites in several experimental animals but these, despite being numerous, are dispersed evenly throughout the cytoplasm (Uehara et al. 1971) and only rarely form concentrated juxtanuclear localisations; none were of the size and appearance of the intermediate filament aggregates under consideration here. Uehara et al. (1971) recorded a decrease in intermediate filament number with maturation. These observations are broadly compatible with our data on fetal myometrium where, on the basis of immunostaining, large numbers of intermediate filaments seem to exist. What is not readily explained at present is the persistence of large numbers of intermediate filaments in apparently normal adult myometrium. Although the myometrium examined was grossly and histologically normal, in all cases it was sampled from patients with a physiological abnormality and the possibility therefore cannot be excluded that the physiological dysfunctions and filament accumulation are consequential phenomena.

An alternative possibility, that these filament accumulations may be a consequence of ageing (Ghadially 1988b), is not entirely compatible with two observations: (1) their expression in both myometrium and the leiomyomata which are likely to have different "ages" (all of the leiomyomata will have had a shorter residence time within the uterus compared with the myometrial cells themselves); and (2) the presence of immunostaining inclusions in grossly and histologically normal myometrium from fetuses dying from causes unrelated to

reproductive or genito-urinary tissues. A greater insight into the significance of these intermediate filament inclusions might be obtained from studying myometrium and leiomyomata from patient groups more sharply delineated in terms of clinical features and age.

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