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# *Smooth Muscle Cells Can Express Cytokeratins of "Simple" Epithelium*

## *Immunocytochemical and Biochemical Studies* In Vitro *and* In Vivo

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**Cytokeratins are a set of 19 proteins that together constitute the class of intermediate filament protein expressed by epithelial cells and tumors. Using a panel of 9 different monoclonal anti-cytokeratin antibodies, the authors have performed immunocytochemistry on methanol-fixed, frozen sections and methacarn-fixed, paraffin-embedded tissue of human myometrial specimens. Anomalous cytokeratin expression (ACE) by smooth muscle cells was found in all specimens. Immunoblots of this tissue confirmed the presence of cytokeratin 19, and possibly 8. In addition, immunocytochemical studies demonstrated ACE in human fetal tissues within the intestinal muscularis and the heart, especially in the region of the aortic outflow tract, and in 8 of 19 cases of leiomyosarcoma from adults. Indirect immunofluorescence studies were also performed on cells explanted from myometrial tissue; the overwhelming majority of cells derived from these cultures were smooth muscle cells as verified by expression of muscle actins, and a subpopulation of these cells was found to be cytokeratin-positive. ACE was confirmed *in vitro* by double labeling experiments demonstrating simultaneous expression of muscle actins and cytokeratins within the same cell. The significance of this smooth muscle cell ACE is unknown, but it may be a phenotypic marker of smooth muscle in a proliferative state. ACE could be a source of confusion in the immunocytochemical analysis of poorly differentiated malignancies if a complete panel of antibodies is not employed. (Am J Pathol 1988, 132:223–232)**

INTERMEDIATE-SIZED FILAMENTS are ubiquitous constituents of virtually all mammalian cells. They are composed of a family of related proteins, each displaying a tissue-restricted distribution.[1] Members of this family include: 1) cytokeratins, which consist of a set of 19 proteins restricted to epithelial cells; 2) vimentin, a 58 kd protein present in mesenchymal cells; 3) desmin, a 55 kd protein expressed only by muscle cells; 4) glial fibrillary acidic protein (GFAP), a 51 kd protein expressed only by astrocytes; and 5) neurofilaments, a triplet of 68, 150, and 200 kd proteins, the expression of which is restricted to neurons. This restricted expression as a function of cell or tissue type has generally been found to be maintained in corresponding neoplastic tissues, eg, carcinomas express cytokeratins, sarcomas express vimentin, and gliomas express GFAP.[2,3,4]

Nonetheless, exceptions to the "rules" of intermediate filament protein expression have been documented, especially in cases of human neoplasia. Examples of such exceptions include: the coexpression of GFAP and cytokeratins by the epithelial cells of a particular type of salivary gland tumor (pleomorphic adenoma[5]); the coexpression of vimentin and cytokeratins by many different types of carcinomas[6]; and the coexpression of neurofilaments and cytokeratins by a select group of neuroendocrine carcinomas.[7,8]

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More recently, a further example of "aberrant" intermediate filament expression in normal tissues was suggested by the studies of Huitfeldt and Brandtzaeg,[9,10] who demonstrated reactivity of frozen sections of cardiac and smooth muscle tissue with various monoclonal antibodies to cytokeratins. Very recently, both Brown et al[11] and Norton, Thomas, and Isaacson[12] have verified the original observations of Huitfeldt and Brandtzaeg but were unable to confirm by biochemical methods the actual expression of cytokeratins by muscle cells. Biochemical corroboration of cytokeratin expression in myometrial tissue was, however, demonstrated by van Muijen, Ruiter, and Warnaar.[13] This report extends these findings and offers more conclusive evidence of cytoplasmic cytokeratin expression by smooth muscle cells both *in vivo* and *in vitro*.

## Materials and Methods

### Acquisition of Tissues

Embryonic tissues from aborted fetuses of 8–24 week gestation were obtained through the laboratory of Dr. Thomas Shepard at the University of Washington, within 12–24 hours of the time of abortion. Uterine specimens, as well as specimens of leiomyomas, leiomyosarcomas, and normal colonic mucosa from colectomy specimens, were obtained from the Surgical Pathology service of University Hospital at the University of Washington, Seattle, as described previously.[4]

### Cell Culture

Primary uterine smooth muscle cell cultures were established from pieces of myometrium obtained from hysterectomy specimens received directly from the operating room. Cultures were grown only from pieces of uteri without gross or subsequent microscopic evidence (in adjacent tissue sections) of either adenocarcinoma or adenomyosis. Cross-sectional slices of the uteri were obtained, with the endometrium and serosa removed. Portions of myometrium were rinsed in phosphate buffered saline (PBS), minced into 1–8 cu mm pieces, and maintained in plastic dishes (Falcon) in Dulbecco's Modified Eagles Medium (DMEM, high glucose; GIBCO Laboratories) with 20% fetal calf serum (FCS; Flow Laboratories), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (GIBCO Laboratories). Cultures were kept in a 37 C humidified incubator with 7% carbon dioxide. For immunofluorescence assays, cultures that were 50–100% confluent were trypsinized and seeded into multiwelled glass slides (Meloy Laboratories) at 2000 cells/well. All immunofluorescence studies were performed on cells in the first or second passage.

In addition to cytoskeletal extracts taken from samples of normal colonic mucosa, cytoskeletal extracts of MCF-7, a breast adenocarcinoma cell line (American Type Culture Collection), were used as positive control material in the immunoblots (see below). This cell line was grown in RPMI Medium (GIBCO Laboratories) supplemented with 15% FCS.

### Sources of Monoclonal Antibodies

The sources and working dilutions of the various murine monoclonal antibodies employed in this study are outlined in Table 1.

### Immunocytochemistry on Fixed, Embedded Tissue

Immunocytochemical studies employed the avidin-biotin (ABC) immunoperoxidase procedure on methacarn-fixed, paraffin-embedded tissues as described previously.[21] Only anti-cytokeratin antibody 35βH11 was applied to the fetal tissues; the complete anti-cytokeratin antibody panel (Table 1) was employed on the uterine specimens.

### Immunofluorescence Procedures on Cells *In Vitro* and Tissue Sections

For single-labeling studies, indirect immunofluorescence procedures were used as described previously.[27] In addition, a propidium iodide nuclear counterstain (0.02 mg/ml in PBS, for 2 minutes) was added to facilitate cell counting.[28] For cultured cells, the percentage of antibody-positive cells was determined by cell counts in a total of 3 random fields at ×200 magnification.

For double-labeling studies, when 2 primary monoclonal antibodies of different isotype were used (eg, HHF35, a murine monoclonal IgG and 35βH11, a murine monoclonal IgM; see Table 1), the following sequence of steps was performed: incubation with the primary IgG monoclonal antibody; FITC-conjugated anti-murine IgG antibody (Tago Laboratories); IgM monoclonal antibody; RITC-conjugated anti-murine IgM antibody (Tago Laboratories). The fluorescein- and rhodamine-conjugated antibodies were used at dilutions of 1:20 in PBS. In cases where the 2 primary monoclonal antibodies were of the same isotype, ie, both were murine IgG, the anti-muscle actin antibody HHF35 was purified from tissue culture supernatant using Protein A-Agarose (Zymed Laboratories) and

Table 1—Monoclonal Antibodies

| Antibody | Specificity | Source | Reference | Dilution* |
|---|---|---|---|---|
| 34BE12 | CK 1, 5, 10, 14† | Enzo Biochem | 14 | 1:2000 |
| 35BH11 | CK 8 | Enzo Biochem | 14 | 1:500 |
| K8.13 | CK 1, 5, 6, 7, 8, 10, 11, 18 | ICN ImmunoBiologicals | 15 | 1:100 |
| K4.62 | CK 19 | ICN ImmunoBiologicals | — | 1:200 |
| MAK-6 | CK 8, 14, 15, 16, 18, 19 | Triton Biosciences | 16, 17 | 1:50 |
| AE1 | CK 10, 14, 15, 16, 19 | Dr. T-T Sun | 16 | Undiluted‡ |
| AE1/AE3 | CK 1-8, 10, 14, 15, 16, 19 | Hybritech | 16 | 1:200 |
| PKK1 | CK 8, 18, 19 | Labsystems | 18 | 1:250 |
| CK-1 | CK 6, 18 | Dako | 19 | 1:50 |
| CAM5.2 | CK 8, 18, 19 | Becton Dickinson | 20 | 1:10 |
| HHF35 | Muscle actin isotypes | Enzo Biochem | 21 | 1:2000 |
| HMB45 | Melanoma antigen | Enzo Biochem | 22 | 1:1000 |
| – | Vimentin | BioGenex | 23 | 1:10 |
| | | Dako | 24 | 1:500‡ |
| – | Desmin | Oncogene Science | 25 | 1:100‡ |

* Denotes dilutions used in avidin-biotin immunoperoxidase procedures on fixed, embedded tissues and/or immunoblots; dilutions used in indirect immunofluorescence procedures are 10-fold more concentrated. Dilutions are of antibodies as supplied, ie, ascites fluid, supernatant, or purified antibody.

† CK denotes cytokeratin according to nomenclature of Moll et al.[26]

‡ Denotes antibodies used in immunoblot experiments only.

directly conjugated with FITC (Sigma Chemical Company). The following sequence of immunostaining steps was then employed: unconjugated anti-cytokeratin antibody (eg, MAK-6); RITC anti-murine IgG (Organon Teknika-Cappel); a blocking antibody (HMB45; see Table 1) to an irrelevant antigen; FITC-conjugated antibody HHF35.

### Gel Electrophoresis and Immunoblotting

For one-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE), extracts of tissue samples with protease inhibitors present were prepared according to the method of Dale et al,[29] with electrophoretic transfer to nitrocellulose and immunoblotting performed using methods identical to those described previously,[21] although peroxidase-anti-peroxidase development system using 4-chloro-naphthol as the chromogen was used.[30]

## Results

### Immunocytochemistry

Nearly identical results were obtained using both acetone- or methanol-fixed frozen sections and methacarn-fixed, paraffin-embedded sections of fetal and uterine tissues. In all studies of the human embryonic tissue, identification of muscle tissue (smooth, skeletal, and cardiac) was verified by reactivity with the anti-muscle actin antibody HHF35.[21,31] Using adjacent sections, colocalization of muscle actins and cytokeratins was seen only in the muscularis of the small bowel and in the heart, especially in the aortic outflow tract region. In general, there was an inverse correlation between cytokeratin expression and the gestational age, with a greater fraction of the more mature fetuses demonstrating cytokeratin expression when the specimens are clustered into 54–115 day and 168–172 day groups (Table 2). In general, it was the inner layers of the developing intestinal muscularis of the small bowel and the esophagus, where cytokeratin expression was best demonstrated: examples of this immunocytochemical positivity are presented in Figure 1A and B.

Nineteen leiomyosarcoma specimens were identified; all had been fixed in methacarn and paraffin embedded. Eight of these demonstrated cytokeratin positivity (Figure 1D), along with the expression of vimentin, muscle actins (Figure 1C), and desmin.[31] There was no apparent relationship between the state of differentiation, patient age, or sex and the expression of cytokeratins (data not shown).

Ten leiomyoma specimens from the uterus and gastrointestinal tract were also identified; only one of these specimens (a uterine leiomyoma) was positive with the anti-cytokeratin antibodies, and its reactivity demonstrated a more limited and focal pattern than the leiomyosarcoma cases (data not shown).

A somewhat more complex pattern emerged from the examinations of the uterine specimens. A total of 20 were examined employing methacarn-fixed, paraf-

Table 2—Cytokeratin Expression in Nonepithelial Fetal Tissues

| | 54–115 day* | 168–172 day* |
|---|---|---|
| Intestinal muscularis | 8/13 | 0/2 |
| Heart (aortic root) | 3/7 | 1/2 |

* Gestational age; figures denote total number of positives/total number of cases examined.

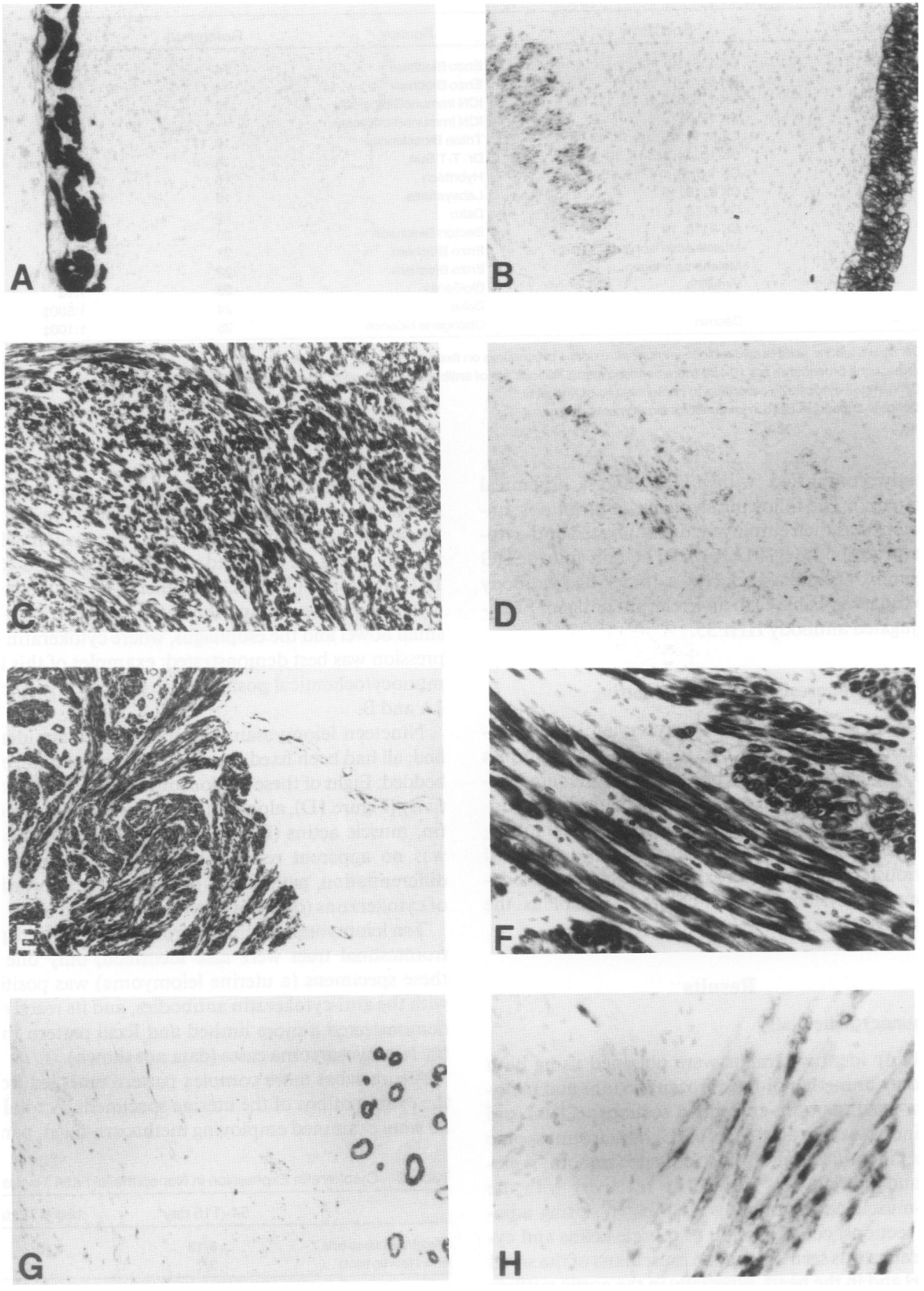
A
B
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**Figure 1**—Avidin-biotin immunoperoxidase localization of cytokeratin in smooth muscle cells. **A and B**—115 day fetal esophagus immunostained with anti-muscle actin antibody HHF35 (**A**) and anti-cytokeratin antibody 35βH11 (**B**). Note uniform cytokeratin expression of overlying epithelium (right) as well as focal cytokeratin expression in muscularis (left). **C and D**—leiomyosarcoma immunostained with anti-muscle actin antibody HHF35 (**C**) and anti-cytokeratin antibody 35BH11 (**D**). **E–H**—normal uterus immunostained with anti-muscle actin antibody HHF35 (**E, F**) and anti-cytokeratin MAK-6 (**G,H**). Note muscle actin expression in myometrium (**E**, left) and in blood vessels of endometrium (right), and cytokeratin expression (**G**) in the endometrial glands (right) and scattered throughout the myometrium (left). Higher magnification demonstrates cytokeratin localization within myocytes (**H**). Original magnifications: **A,B,E,G,** ×250; **C,D,** ×200; **F,H,** ×400

fin-embedded sections and/or snap frozen, methanol-fixed frozen sections. A summary of the data obtained from these specimens is found in Table 3. Briefly, all the monoclonal antibodies (except CK-1; see Discussion) identifying the cytokeratins of "simple" epithelium (generally cytokeratins 8, 18, and 19, according to the Moll catalog[26]) were positive, although the immunostaining intensity and relative number of positive cells varied from antibody to antibody. Distinctly different patterns were identified: some specimens manifested a gradient of positivity decreasing toward the deeper portions of the myometrium, while others displayed greatest positivity in the middle or outer third of the myometrium. In general, only the myometrial cells were positive, although occasionally smooth muscle cells within the walls of large blood vessels deep in the myometrium were also positive. Examples of immunostaining of myometrial specimens are given in Figure 1E–H.

### Gel Electrophoresis and Immunoblotting

In immunoblot preparations of myometrium using various anti-cytokeratin antibodies, expression of cytokeratin 19 (40 kd) was demonstrated clearly, as indicated in Figure 2A. The molecular weight of the cytokeratin 19 band (Figure 2A, lanes 4 and 6) is clearly different from that of the intermediate filament proteins desmin (Figure 2A, lane 2) and vimentin (Figure 2A, lane 1), and of actin (Figure 2A, lane 3) in their respective immunoblots on the same extract. In some immunoblots of myometrial extracts there was also a fainter band seen corresponding to cytokeratin 8 (53 kd). There was a great variation, however, in the ability of the different monoclonal antibodies to identify this latter cytokeratin in myometrium (Figure 2B) relative to that obtained in the positive control lanes, which contained extracts of the breast carcinoma cell line MCF-7. No other cytokeratins were identified in immunoblot experiments.

### *In Vitro* Studies

Smooth muscle cell growth was obtained from all of the myometrial explants, with the cells growing to confluence in primary culture in approximately 3 weeks. Cells had the typical morphology of smooth muscle cells, growing in a "hill and valley" pattern on confluence.[32] In addition, the smooth muscle cell nature of the overwhelming majority of the explanted cells was confirmed with the anti-muscle actin isotype antibody HHF35, which does not react with macrophages, fibroblasts, or endothelial cells.[21] The results of 1 experiment using a panel of monoclonal antibodies are summarized in Table 4. Briefly, all the cells were vimentin-positive, and a subpopulation of cells was cytokeratin-positive, with this fraction ranging from 2–40%, depending on the choice of anti-cytokeratin antibodies (Figure 3). These latter antibodies, when positive, decorated intracytoplasmic filaments that had the typical basket-weave appearance of intermediate-sized filaments and were often predominantly perinuclear in their distribution (Figure 3B). In contrast, the anti-muscle actin antibody decorated straight, stress-type filaments that spanned the entire cell cytoplasm (Figure 3A). Double-labeling experiments demonstrated clearly that muscle actins and cytokeratins could be coexpressed within the same cell (Figure 3C and D).

## Discussion

These results confirm and extend those reported by Huitfeldt and Brandtzaeg[9,10] and more recently by

Table 3—Summary of Immunocytochemical Studies Demonstrating Cytokeratin Expression in Myometrium

| | Fraction of cases with antibody-positive myometrial cells | |
|---|---|---|
| Antibodies | Methacarn-fixed paraffin-embedded sections* | Methanol-fixed frozen sections* |
| 35βH11 | 19/19 | 3/3 |
| 34βE12 | 0/10 | 3/3 |
| MAK-6 | 4/4 | 3/3 |
| AE1/AE3 | 4/4 | 2/2 |
| K8.13 | 4/4 | 2/2 |
| K4.62 | 4/4 | 2/2 |
| PKK1 | 4/4 | 2/2 |
| CK-1 | 0/4 | 0/2 |
| CAM5.2 | 6/6 | 1/1 |

* Number of cases positive/total number of cases examined.

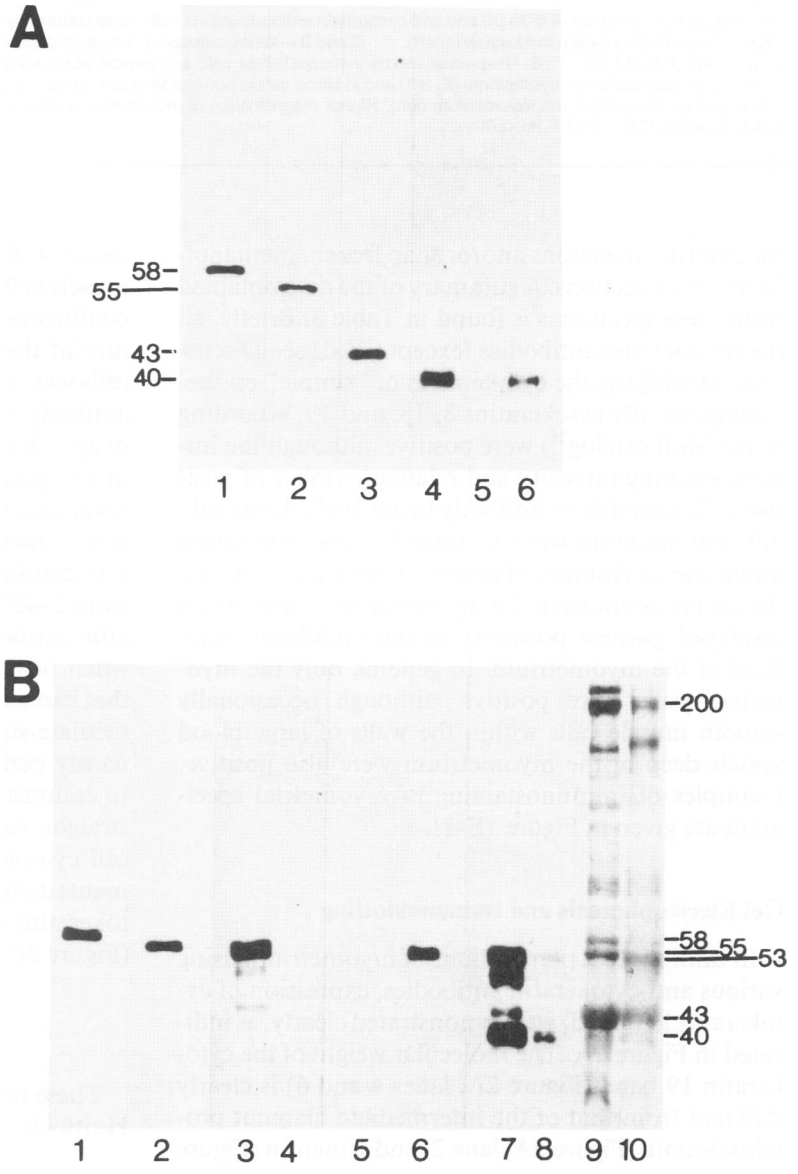


**Figure 2**—Immunoblots on nitrocellulose transfers of one-dimensional SDS-PAGE of myometrium and controls. **A**—Myometrium, lanes 1–3 and 6; MCF-7 breast carcinoma cells, lane 4; blank, lane 5. Anti-vimentin, lane 1; anti-desmin, lane 2; anti-muscle actin antibody HHF35, lane 3; anti-cytokeratin antibody AE1, lanes 4–6. Cytokeratin 19 (40 kd) is identified in both MCF-7 cells and myometrium; control antibodies (lanes 1–3) identify their respective proteins which are distinct from cytokeratin 19. **B**—Myometrium, lanes 1,2,4,5,8,9; colonic mucosa, lanes 3,6,7,10. Anti-vimentin, lane 1; anti-desmin, lane 2; anti-cytokeratin antibody K8.13, lanes 3,4; anti-cytokeratin antibody CAM5.2, lanes 5,6; anti-cytokeratin antibody MAK-6, lanes 7,8; amido black stains, lanes 9,10. Anti-cytokeratin antibody MAK-6 (lane 8) identifies both cytokeratin 19 (40 kd) and, much more weakly, cytokeratin 8 (53 kd) in the myometrium, in contrast with its strong reactivity with cytokeratin 8 in the colonic mucosa extract (lane 7). In contrast, antibodies CAM5.2 (lanes 5,6) and K8.13 (lanes 3,4) identify cytokeratin 8 (53 kd) in the colonic mucosa but not in the myometrium.

Brown et al[11] and Norton, Thomas, and Isaacson.[12] In these previous studies, anti-cytokeratin monoclonal antibodies were found to react with sections of myocardium, uterine myometrium, leiomyomas, leiomyosarcomas, and fetal intestinal muscularis. The results of these biochemical and *in vitro* studies confirm the tentative conclusions of these previous investigators regarding smooth muscle expression of cytokeratins.

As the anomalous cytokeratin expression (ACE) by muscle cells described here appears to violate the "rules" of intermediate filament expression, it is important to rule out any possibility that this smooth muscle immunoreactivity with anti-cytokeratin antibodies represents a phenomenon other than actual cytokeratin expression by myocytes. One theoretic possibility, which was raised previously,[11,12] is that the antibody reactivity on sections represents cross-reactivity with noncytokeratin proteins. The inability of Norton, Thomas, and Isaacson to demonstrate unequivocal immunoblot confirmation of their tissue immunoperoxidase studies would appear to support this hypothesis, and an accompanying editorial underscores this interpretation.[33] Indeed, one might hypothesize that the positive immunostaining represents cross-reactivity with a protein or proteins sharing some homology with the cytokeratins, such as desmin, vimentin, or the nuclear lamins.[34] Based on the results reported here, however, this is an unlikely explanation. First, 7 different anti-cytokeratin monoclonal antibodies, each reacting with a unique subset of cytokeratin proteins and epitopes thereof, demon-

strated positive reactions in smooth muscle tissue, which makes the possibility of fortuitous epitopic cross-reactivity statistically improbable. Moreover, in immunoblot experiments, none of these anti-cytokeratin antibodies reacted with either desmin (55 kd), vimentin (58 kd), or any bands between 60 and 70 kd that might be interpreted as corresponding to the nuclear lamins (Figure 2A).[35] Finally, the characteristic cytoplasmic, wavy filaments decorated by these antibodies in the cells explanted from the uteri suggest strongly that there is true cytokeratin expression in these cells. The difference in the fraction of cells positive with anti-vimentin and anti-cytokeratin antibodies (Table 4) also argues against the possibility that cross-reactivity with vimentin explains the positive anti-cytokeratin antibody immunofluorescence results.

Nonetheless, there are some atypical features of the cytokeratin expression, ie, all the antibodies did not perform equally well in immunoblots, tissue culture, and immunocytochemical studies, despite the uniform ability of these antibodies to react with control tissues such as colonic mucosa. More specifically, cytokeratin 19 was most unequivocally demonstrated to be present in the immunoblots, but other cytokeratins predicted to be present based on immunocytochemistry alone (eg, cytokeratin 8) were not demonstrated consistently on the immunoblots, and, when present, were far less intense than the cytokeratin 19 band. Cu-

Table 4—Immunofluorescence Studies Demonstrating Cytokeratin Expression in Explanted Myometrial-derived Smooth Muscle Cells

| Antibodies | Percentage of cells positive |
|---|---|
| HHF35 | 86 |
| Anti-vimentin | 100 |
| HMβ45 | – |
| 35βH11 | 28 |
| 34βE12 | – |
| MAK-6 | 39 |
| AE1/AE3 | 21 |
| K8.13 | 2 |
| K4.62 | 17 |
| PKK1 | 33 |
| CK-1 | 22 |
| CAM5.2 | 40 |

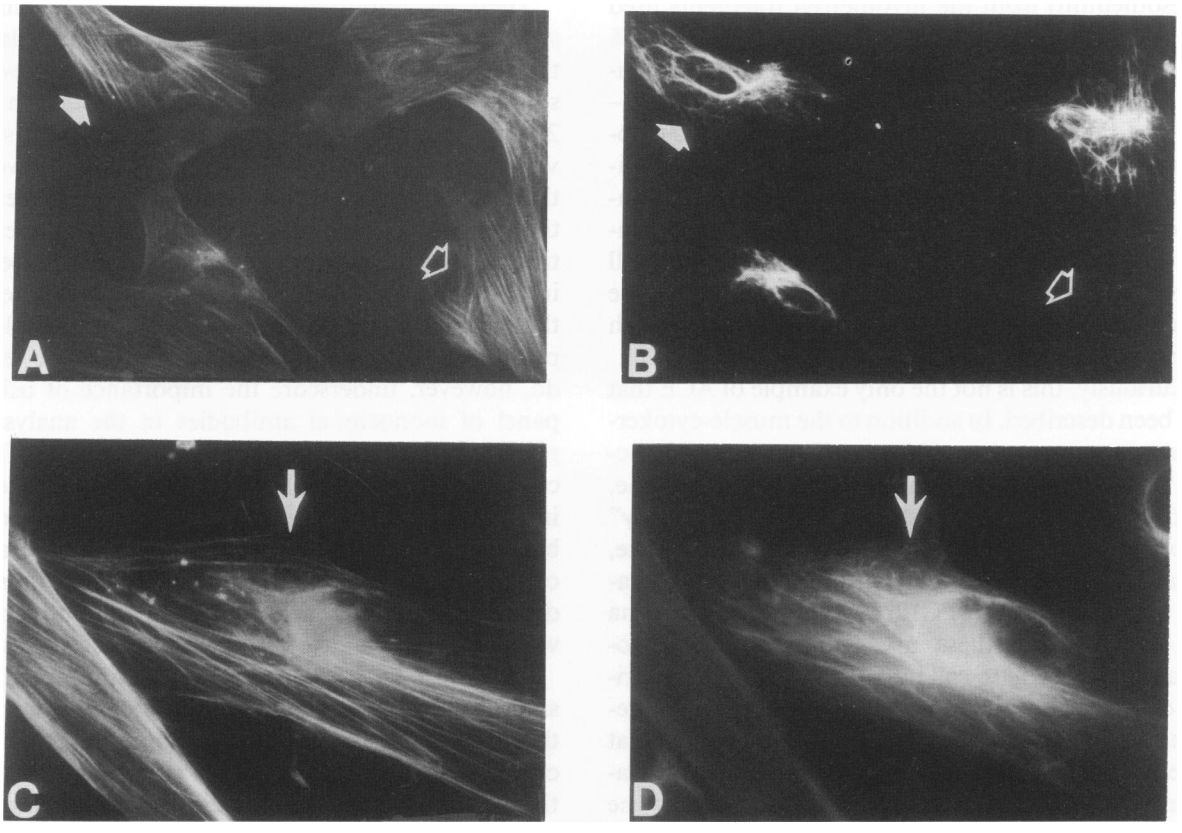


**Figure 3**—Double-label immunofluorescence study of smooth muscle cells explanted from uterine myometrium; see text for methods. **A**—FITC-conjugated anti-muscle actin antibody HHF35 decorating stress-type fibers in cells. Original magnification, ×160. **B**—Same field as in **A** demonstrating reactivity of anti-cytokeratin antibody MAK-6 and RITC-anti-murine IgG. Note that most cells in this field (solid arrow) are positive with both antibodies, although others (open arrow) are positive only with the anti-muscle actin antibody. **C and D**—Higher magnification of a double-labeled uterine smooth muscle cell (arrow) exhibiting straight stress-type fibers decorated by anti-muscle actin antibody HHF35 (**C**) and basket-weave pattern of filaments decorated with anti-cytokeratin antibody 35βH11 (**D**). Original magnification, ×500

riously, van Muijen, Ruiter, and Warnaar[13] recently reported that on 2D-SDS-PAGE immunoblots, cytokeratin 8 could be identified in myometrial extracts in the apparent absence of cytokeratin 19, again suggesting that results can be variable depending on the choice of anti-cytokeratin antibody, presumably reflecting the accessibility of different epitopes. Part of this difference may be explained by masking of epitopes by posttranslational modifications in these anomalously expressed cytokeratins, which result in increased susceptibility to denaturation, as occurs in SDS-PAGE. Differences in sensitivity of immunocytochemistry *vs.* immunoblotting may also explain at least a portion of these differences. The lack of reactivity with anti-cytokeratin antibody CK-1 (Table 3) is probably explained by the fact that the methods used for processing of the tissues did not necessarily conform to those specified for use with this antibody (eg, acetone fixation).

It is conceivable but unlikely that contamination with true epithelial cells explains the anti-cytokeratin positivity. Care was taken to exclude any epithelium that would be present in the endometrium or serosa (mesothelium) from the myometrial fragments used in the explant experiments. Although as much as 14% of the cells might be nonmuscle cell in nature (see Table 4), the range of cytokeratin-positive cells was 2–40%, and the coexpression of muscle actins and cytokeratin was demonstrated by double-labeling experiments (Figure 3). In the *in situ* myometrial and embryonic tissue studies, careful study of the fixed, embedded sections in which the morphology is well preserved leads to the unavoidable conclusion that the anti-cytokeratin immunoreactivity is in the smooth muscle cell population.

Curiously, this is not the only example of ACE that has been described. In addition to the muscle-cytokeratin expression studies of Norton, Thomas, and Isaacson[12] and Brown et al,[11] an erythroleukemia cell line, K562, has been demonstrated to "anomalously" coexpress cytokeratins along with vimentin.[36] Franke, Grund, and Achtstatter[37] recently described cytokeratin expression in the PC12 rat pheochromocytoma cell line. Although these investigators used this cytokeratin expression to call into question the cell of origin of this cell line, it is possible that this, in fact, represents another example of ACE. It is also possible that recent descriptions of cytokeratin expression by melanomas[38] and Ewing's sarcoma cells[39] also fit into the category of ACE.

Furthermore, there are other examples of cells coexpressing epithelial- and muscle-specific proteins. For example, the myoepithelium of the breast and salivary gland is positive with both anti-cytokeratin and anti-muscle actin antibodies.[21] And, in a recent study, the authors have demonstrated the apparent coexpression of cytokeratins and muscle actins by submesothelial stromal cells.[31] The relationship of these examples of coexpression of muscle and epithelial phenotypes to the findings presented here awaits further study.

These studies demonstrate unequivocal expression of cytokeratin number 19, and possibly number 8, in smooth muscle. These are cytokeratins of "simple" epithelium, and are among the cytokeratins expressed in such cells as hepatocytes, pancreatic acinar cells, and colonic mucosa.[40] The authors have not been able to demonstrate smooth muscle cell expression of the cytokeratins associated with more complex epithelium (ductal, squamous). One possible exception is the positive immunoreactivity, on myometrial frozen sections only, of antibody 34$\beta$E12, which in recent immunoblot experiments on squamous epithelium and tumors recognizes cytokeratins 1, 5, 10, and 14 (Gown AM, unpublished observations). Nonetheless, immunoreactivity of this particular antibody in SDS-PAGE immunoblots was not able to be confirmed.

There are important implications of this demonstration of cytokeratin expression by muscle cells and their tumors for the surgical pathologist. In previous studies, cytokeratin expression has been found in only 2 other sarcomas—epithelioid sarcoma[41] and synovial sarcoma.[42] In these latter situations, however, there is corroborative ultrastructural data suggesting that these cells manifest true epithelial differentiation.[43,44] Such differentiation would not be expected in the cases of leiomyosarcomas studied here, although additional ultrastructural studies should be performed with this question in mind. These results do, however, underscore the importance of using a panel of monoclonal antibodies in the analysis of poorly differentiated neoplasms. The use only of anti-cytokeratin antibodies, for example, could lead to the incorrect diagnosis of a carcinoma in an otherwise bona fide case of leiomyosarcoma. The incorporation of anti-muscle specific antibodies, such as those to desmin or muscle actins, into an antibody panel would ensure the correct interpretation of the results.

One can only speculate on the significance of the smooth muscle cell ACE. A feature shared by most of the smooth muscle cells demonstrating ACE (embryonic tissues, neoplasms, cells in culture) seems to be that of proliferation. Although myometrial cells might not appear to fit this description, they are unique in their proximity to cells that are proliferating in response to steroid hormones on a monthly cycle. The gradient of positivity often seen in the myometrium would be consistent with the presence of chemical sig-

nals from the proliferating endometrium. Future studies will investigate the possibility that the pattern of cytokeratin expression (ie, inner *vs.* outer third of myometrium) is a function of time in the menstrual cycle or in the presence of specific exogenous steroids, in the case of postmenopausal patients. The relationship of cytokeratin expression to steroid hormones also could be tested using *in vitro* models. In the studies of embryonal tissue, a greater fraction of the first trimester than second trimester fetal intestinal and myocardial tissues were positive, although the sample size in the latter group was very small. Nonetheless, positivity was not observed with any anti-cytokeratin monoclonal antibodies in resting adult tissue, such as intestinal muscularis, myocardium, or vascular smooth muscle cells outside of the uterus. Although Huitfeldt and Brandtzaeg did report myocardial positivity with anti-cytokeratin antibodies, myocardial cells are unique in also expressing desmoplakins, which are proteins almost always associated with cytokeratins in other cells in which they are found.[45,46] None of these largely morphologic approaches can begin to explain the mechanisms controlling ACE on a cellular level, however, which is best addressed by using an *in vitro* system, such as the myometrial smooth muscle cells described herein. Current studies in the authors' laboratory are addressing this issue in the context of the broader question of control of smooth muscle phenotype.

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