HHF35, A Muscle-Actin-Specific Monoclonal Antibody

I. Immunocytochemical and Biochemical Characterization

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A monoclonal antibody to muscle cell actin isotypes was produced and characterized. Immunocytochemical analysis of methanol-Carnoy's-fixed, paraffin-embedded human tissue revealed that this antibody, termed HHF35, reacts with skeletal muscle cells, cardiac muscle cells, smooth muscle cells, pericytes, and myoepithelial cells, but is nonreactive with endothelial, epithelial, neural, or connective tissue cells. When assayed by indirect immunofluorescence, HHF35 reacts with microfilament bundles from various cultured mammalian smooth muscle cells, but does not react with cultured human dermal fibroblasts or various epithelial tumor cell lines. In one-dimensional gel electrophoresis immunoblot experiments this antibody detects a 42-kd polypeptide from tissue extracts of uterus, ileum, aorta, diaphragm, and heart and extract from smooth muscle cells. The antibody also reacts with a comigrating 42-kd band of highly purified rabbit skeletal muscle actin. HHF35 is nonreactive on immunoblots of extracts from all tested nonmuscle cell extracts. Immunoelectrophoresis followed by immunoblotting performed in the presence of urea and reducing agents reveals recognition of the alpha isoelectrophoretic variant of actin from skeletal, cardiac, and smooth muscle sources and of the gamma variant from smooth muscle sources. Because HHF35 reacts with virtually all muscle cells, it will be useful as a marker for muscle and muscle-derived cells. (Am J Pathol 1987, 126:51-60)

SEVERAL cytoskeletal proteins such as myosin, actin, and tropomyosin exhibit isotypic variants, the expression of which is a function of tissue type (eg, muscle versus nonmuscle) or state of cellular differentiation (eg, embryonic versus adult).[1-12] Most attention has been given to the myosin isotypes, reflecting differences in the primary structure of both heavy and light chains. The expression of a particular form of myosin is determined by the developmental, physiologic, and hormonal state of the muscle cell.[2-5] Less is known about the actins, which have been divided into six isotypes based on differences in isoelectric point and amino acid sequence.[6] In contrast to myosin, there is no known significant divergence in the biologic activity of isoactins. Yet the microheterogeneity in actin primary structure clearly indicates that the isoactins are products of a multigene family that permits expression of antigenic specificity to cell type or cell state.[6-11] Consequently, the production of monoclonal antibodies specific to actin isotypes can provide a new insight into actin microheterogeneity. We have previously reported the generation of a smooth-muscle-cell-specific monoclonal antibody, designated CGA7, which specifically recognizes the alpha and gamma actins of smooth muscle cells, but which does not recognize cardiac, skeletal muscle, or

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nonmuscle actins.[13] We now describe a second actin isotype-specific monoclonal antibody, designated HHF35, which detects an epitope common to the actin isotypes of all muscle cell types, but not the actin isotypes of nonmuscle cells.

Materials and Methods

Source of Antigen

A surgical specimen of dissected human myocardium from a case of idiopathic hyptertrophic subaortic stenosis was received directly from the operating room at University Hospital, Seattle. A portion of the specimen was washed in Tris-buffered saline (TBS) (155 mM NaCl, 10 mM Tris Cl, pH 7.4) with 0.5 mM ethylenediaminetetraacetic acid (EDTA) and 0.5 mM phenylmethylsulfonylfluoride (PMSF) kept at 0 C. The tissue was then homogenized in 10% vol/wt of 2% sodium dodecyl sulfate (SDS), 100 mM Tris Cl, 5 mM EDTA, and 0.1 mM PMSF at pH 6.8. SDS was included in the extraction buffer to 1) completely solubilize the majority of the polypeptides, 2) increase antigenicity,[14] and 3) facilitate characterization of the monoclonal antibody after its production. (It has been reported that only 11% of monoclonals are able to recognize, via immunoblotting, the SDS denatured primary structure as compared to the native structure.[15]) The homogenate was boiled for 10 minutes and centrifuged at 2500*g* for 10 minutes. Excess SDS, low-molecular-weight contaminants, and lipid were removed by concentrating the protein fraction through the addition of 10% vol/vol of methanol/crude extract and incubation overnight at −70 C. The collected precipitate was used as the immunogen.

Production of Hybridomas

Monoclonal antibodies directed to SDS-extracted protein fractions from human myocardium were generated according to the basic scheme outlined previously.[16] Briefly, a series of three intraperitoneal injections of 400 μg of methanol precipitate suspended in phosphate-buffered saline (PBS) with extensive sonication was given to a BALB/c mouse over a 4-week period. The spleen was then removed and a fusion procedure performed with NS-1 cells as described previously.[16] Hybridoma supernatants were screened with indirect immunofluorescence on acetone-fixed, frozen sections of human myocardium. Only wells producing hybridoma supernatant fluids that discriminated myocardium from stromal endothelial cells and fibroblasts were cloned. The cells in these positive wells were subjected to a two-step cloning procedure, and ascites fluid was generated as described previously.[16] One clone, HHF35, was isolated and characterized.

Cell Culture

Aortic medial smooth muscle cells were prepared from normal rabbit or nonhuman primate by the method described by Ross.[17] Human fibroblasts were explanted from foreskins. A human epidermoid carcinoma cell line, A431, was obtained from American Type Culture Collection, Rockville, Maryland. A human hepatocellular carcinoma cell line, Hep3B, was originally obtained from Dr. David Aden and Barbara Knowles, of the Wistar Institute (Philadelphia, PA). A melanoma cell line, Mel-1, was established from a portion of an axillary lymph node containing pigmented melanoma metastasis.[18] The cells were cultured in RPMI-1640 (GIBCO Laboratories) medium supplemented with 15% fetal calf serum, L-glutamine (1 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml; GIBCO Laboratories).

Sample Preparation

Fresh tissue samples were obtained and stored at −70 C. until used. Cells collected from culture were detached in TBS containing 10 mM EDTA, 20 mM benzamidine, and 0.1 mM PMSF by gentle incubation at 20 C for 10 minutes. Fifty-milligram samples for isoelectric focusing (IEF) were first minced and then homogenized in 10% vol/wt of 18.75% glycerol with 2.75% Triton X-100, 10 M urea, 10 mM benzamidine, 4 mM EDTA, 200 μM sodium orthovanadate, and the following mixture of ampholytes (Pharmolyte; Pharmacia Fine Chemicals): 1.25% 3–10 and 6.25% 4–6.5 (IEF sample buffer) and 1 mM dithiothreitol unless otherwise noted. After homogenization the samples were sonicated for 2 minutes and then clarified in a microfuge. Samples for SDS polyacrylamide-gel electrophoresis (SDS-PAGE) were either homogenized directly into 10% vol/wt of SDS sample buffer (100 mM Tris Cl, pH 6.8, with 2% SDS, 20% glycerol, 10 mM benzamidine, 5 mM EDTA, 200 μM sodium orthovanadate, and 2% β-mercaptoethanol), or an equal volume of SDS sample buffer was added to crude extracts of samples prepared for IEF. All SDS samples were then heated to 100 C for 5 minutes and then clarified at 2500*g* for 5 minutes. Loads of either 10 μl of the SDS sample buffer and IEF sample buffer crude extracts or 20 μl of the SDS-IEF sample buffer extracts were applied per slot.

One Dimensional SDS-PAGE

The samples were electrophoresed as described[19] on 14 × 16-cm slab gels 1.5 mm thick in the presence of 0.1% SDS at 15 C with an 8% or 8.75% separating gel and a 5% stacking gel. Pyronin Y was used as the tracking dye, and electrophoresis was stopped when it reached 1 cm from the bottom of the gel. Resolved proteins were visualized with Coomassie brilliant blue R-250[20] or immediately immunoblotted.[21]

IEF Gels

Gels in the presence of urea were prepared as described[22] with the following modifications: ampholytes (Pharmalyte; Pharmacia Fine Chemicals) were adjusted to 0.95% 3–10, 3.84% 4–6.5, and 5.69% 5–6 for optimal resolution of the actin isotypes. The gels were prerun with 500 V and 15 mA maximums per 14 × 0.075-cm x-sectional area for 60 minutes at 15 C. The samples were then focused under the same conditions for 18.5 hours. Bromphenol blue, owing to its optimal pI, was used as a tracking dye, and electrophoresis was stopped when the migratory band, through gradient spreading, reached 2 cm from the bottom of the gel.

Immunoblotting

Immunoblotting was performed[23–25] immediately upon completion of the SDS-PAGE and after pH determination with a flat surface electrode (Markson Science Inc., J-1208) in the case of IEF-PAGE. The nitrocellulose strips were then washed in TBS for 10 minutes and blocked for 15 minutes at 37 C with 5% bovine serum albumin in TBS containing 4% horse serum.[25] The strips were then incubated with HHF35 ascites fluid diluted 1:200 in 5% bovine serum albumin in TBS for 15 minutes at 37 C, washed four times each for 2 minutes in TBS at 20 C, and then incubated with glucose oxidase-conjugated goat anti-mouse IgG and IgM (Accurate Chemical and Scientific) diluted 1:200 in 5% bovine serum albumin in TBS as for HHF35. After an additional wash the strips were developed in 50 mM Tris Cl, pH 8.1, containing 75 mM glucose, 1.65 mM nitroblue tetrazolium, and 0.82 mM phenazine methosulfate. Development was stopped with a water rinse followed by air drying.

Immunocytochemistry on Fixed, Embedded Tissue (Light Microscopy)

Muscle tissue was immersion-fixed overnight at room temperature in the following fixatives: 4% paraformaldehyde in phosphate-buffered saline (PBS), 2% paraformaldehyde in PBS, 1% paraformaldehyde in PBS, Bouin's fixative,[26] Carnoy's fixative (60% ethanol, 30% chloroform, 10% glacial acetic acid), methanol–Carnoy's fixative (60% methanol, 30% chloroform, 10% glacial acetic acid), 0.1% glutaraldehyde with 2% paraformaldehyde in PBS, 0.5% glutaraldehyde with 3% paraformaldehyde in PBS, 1% glutaraldehyde with 3% paraformaldehyde in PBS.

Using the avidin-biotin immunoperoxidase method as described previously,[27,28] we assayed hybridoma ascites fluids on fixed, paraffin-embedded human tissues (liver, lung, kidney, spleen, lymph node, brain, colon, gallbladder, skin, heart, skeletal muscle) obtained from surgical material. Hybridoma ascites fluids were diluted 1:8000–1:12,000 before use.

Immunofluorescence Procedures on Cells in Vitro and Tissue Sections

Indirect immunofluorescence was performed on cells maintained on Teflon-coated slides (Meloy) fixed in −20 C methanol for 5 minutes as described.[16] Hybridoma ascites fluids were diluted 1:100–1:300 before use. Identical methods and antibody dilutions were used on acetone-fixed, frozen sections of various human tissues corresponding to those described above.

Results

We isolated HHF35 in the initial screening of human myocardium and tested further on methanol–Carnoy's-fixed, paraffin-embedded, human tissues and acetone-fixed frozen sections, using as criteria for selection those supernatants demonstrating 1) reactivity on cardiac as well as smooth muscle and 2) ability to recognize antigen in methanol–Carnoy's-fixed, paraffin-embedded sections.

Immunocytochemistry (Light Microscopy)

Identical results were obtained on acetone-fixed frozen sections and paraffin-embedded sections; the latter will be described in greater detail, because cellular details were more clearly discerned. Table 1 lists the various fixatives and results as screened on fixed, embedded sections of vascular (ie, smooth muscle) tissue. All fixatives, with the solitary exception of 3% paraformaldehyde with 1% glutaraldehyde, preserved antigenicity to permit immunocytochemical localization. The most intense reactions, however, were generally obtained with alcohol-fixed (Carnoy's and methanol–Carnoy's) tissue.

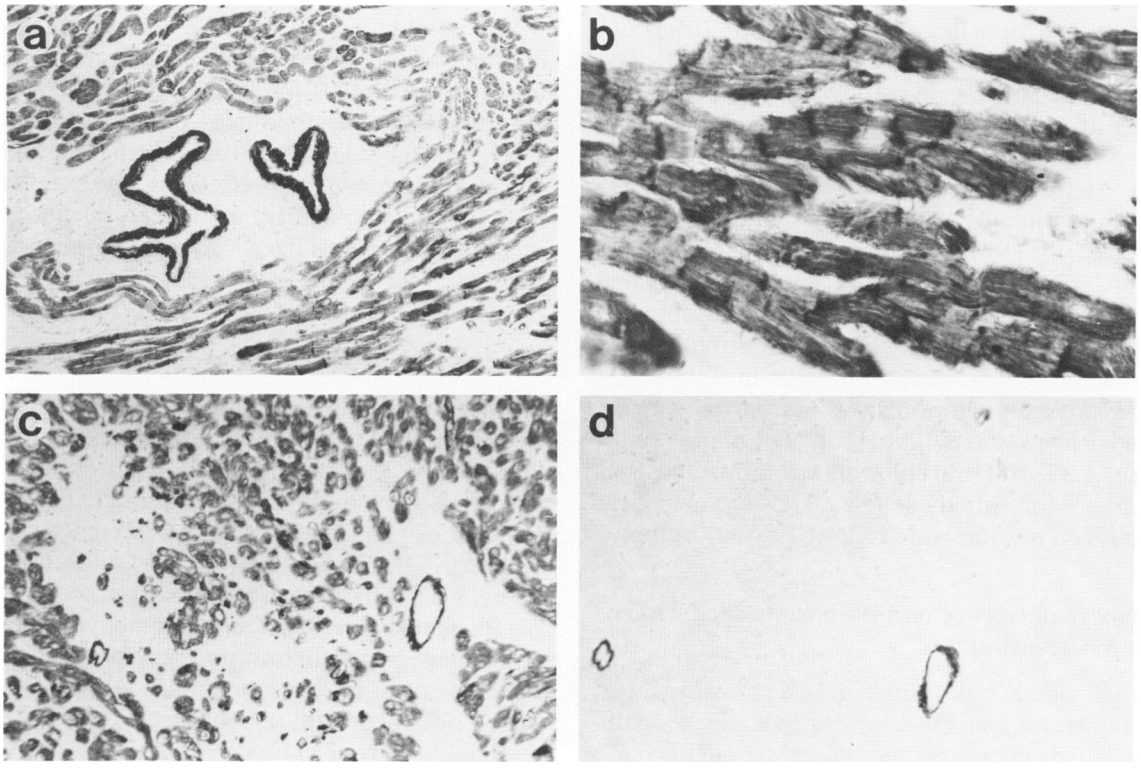
Table 1—Effect of Tissue Fixation of Reactivity of Tissues with Antibody HHF35

Fixative	Reactivity
Acetone	+
Methanol	+
Ethanol	+
4% paraformaldehyde in PBS	+
2% paraformaldehyde in PBS	+
1% paraformaldehyde in PBS + Bouin's fixative	+
Carnoy's fixative	+
Methanol–Carnoy's fixative	+
0.1% glutaraldehyde with 2% paraformaldehyde	+
0.5% glutaraldehyde with 3% paraformaldehyde	+
1.0% glutaraldehyde with 3% paraformaldehyde	−
1% osmium in 0.05 M phosphate buffer	+

In screens of fixed, embedded human tissue, HHF35 reacted with all human myocardial cells (Figure 1 a–c), skeletal muscle cells (Figure 1e), smooth muscle cells (Figure 1f–i), and myoepithelial cells (Figure 1k). The latter is the only presumptive epithelial cell type positive with the antibody. All other nonmuscle cells were nonreactive; this includes vascular endothelial cells, epithelial cells, lymphoid cells, macrophages, connective tissue, and neural cells. In comparison with the previously described anti-smooth muscle cell actin monoclonal antibody, CGA7,[13] the major difference between antibodies CGA7 and HHF35 was the former's nonreactivity with cardiac and skeletal muscle (Figure 1d). With respect to smooth muscle cells, antibody HHF35 had a similar, though not identical, immunoreactivity distribution as antibody CGA7. Thus, both antibodies reacted with the tunica muscularis of the intestinal tract (Figure 1f and g), the uterine myometrium, the medial layer of all blood vessels (Figure 1h and i), and the stromal (ie, smooth muscle) component of the prostate.[13] Focusing on the normal vascular system, the only difference between antibodies HHF35 and CGA7 is the former's ability to react with a population of pericytes around small arterioles that are nonreactive with antibody CGA7 (Figure 1i and j). Similar immunohistochemical profiles were found in tissues of monkey, cow, rabbit, and rat (data not shown). HHF35 identified a restricted subset of cells in such human pathologic processes such as the smooth muscle-like cells referred to as "myofibroblasts"[29] in healing wounds (data not shown), muscle neoplasms such as leiomyomas (Figure 1l) and rhabdomyosarcomas (data not shown), and lesions of atherosclerosis.[30] This reactivity is described in a separate publication (Tsukada et al, submitted).

In Vitro Immunofluorescence

Indirect immunofluorescence was used on cultured cells for examination of a number of different cell lines and cell strains to identify intracellular struc-



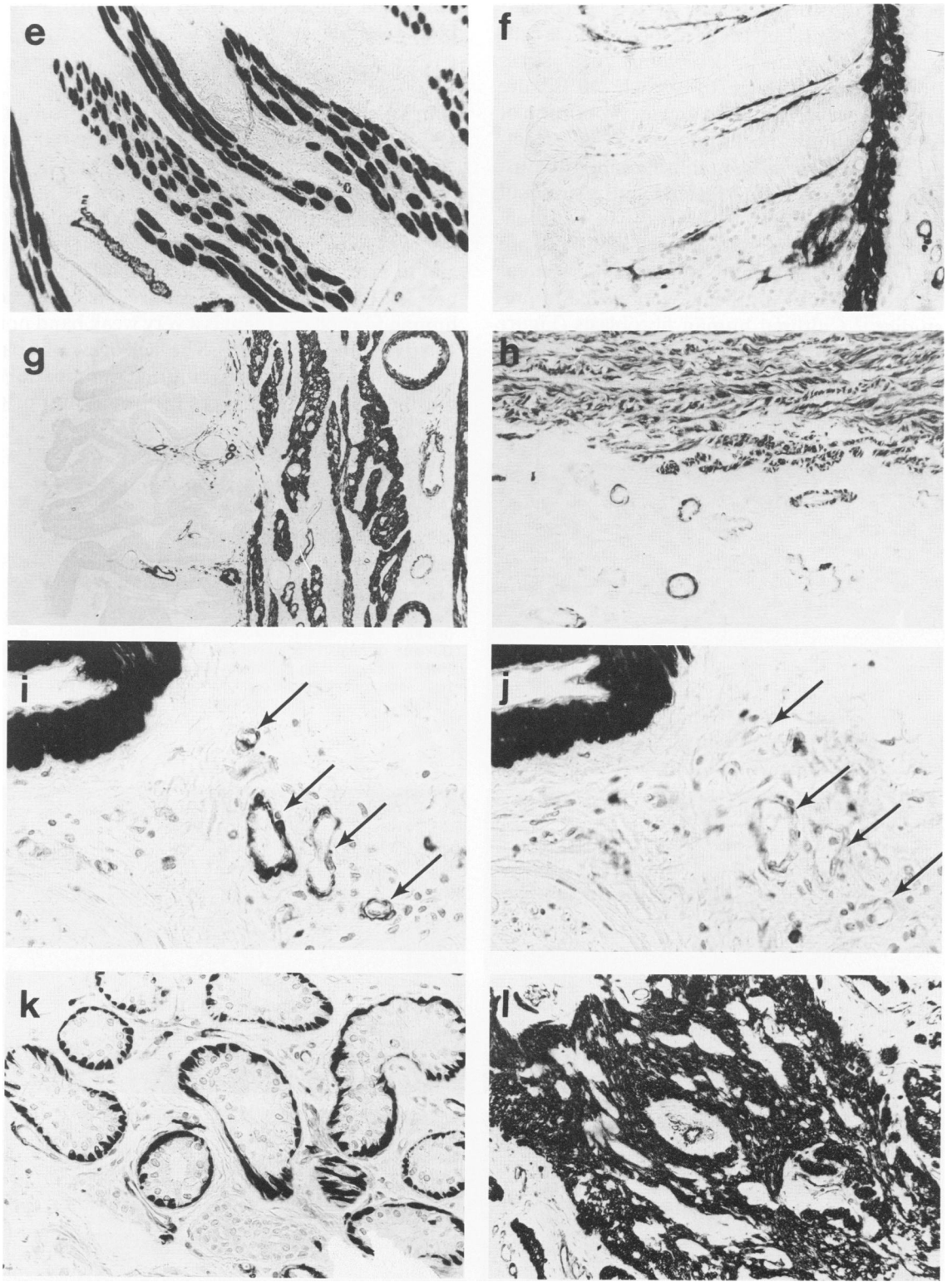


Figure 1 — Avidin–biotin immunoperoxidase staining of methanol-Carnoy's fixed paraffin embedded tissue. **a–d** — Myocardium. **a** — HHF35; note the positive medial layer of coronary vessels *(center)*. (×90) **b** — HHF35. (×625) **c** and **d** — Serial sections stained with HHF35 **(c)** or CGA7 **(d)**. Note cardiac muscle cells positive with HHF35 **(c)**, but negative with CGA7 **(d)**. Both antibodies stain medial smooth muscle cells of vessel wall. (×125) **e** — Skeletal muscle, HHF35. (×62) **f** — Colon, HHF35. (×156) **g** — Gallbladder, HHF35. (×62) **h** — Abdominal aorta, HHF35. Note positive deep medial wall and vessels in the adventitia. (×180) **i** and **j** — Serial sections of breast stained with HHF35 **(i)** or CGA7 **(j)**. Note pericytes *(arrows)* positive with HHF35 **(i)** but negative with CGA7 **(j)**. (×625) **k** — Breast, HHF35. Note positive myoepithelial cells at the periphery of the glands. (×280) **l** — Leiomyoma, HHF35. (×100)

tures recognized by this antibody. HHF35 decorated cultured monkey or rabbit aortic smooth muscle cells in an "actin-like" pattern, decorating linear stress fibers running the entire length of each cell (Figure 2a). HHF35, however, stained a greater proportion of cultured smooth muscle cells than did CGA7, reacting with virtually all cells in established smooth muscle cell lines, whereas CGA7 reacted with a subset of the cultured cells (Figure 2b and c). In addition, HHF35 reacted with more advanced (8th) passage monkey aortic medial smooth muscle cells, whereas CGA7 failed to react with them, as indicated in previous studies.[13] Cultured human fibroblasts (Figure 2d), alveolar macrophages, and a variety of human epithelial neoplastic cell lines, including a hepatoma cell line (Hep3B) (Figure 2e) and an epidermoid carcinoma cell line (A431), were nonreactive with antibody HHF35.

Gel Electrophoresis and Immunoblotting

In Western blot experiments, HHF35 recognizes a 42-kd protein in preparations of purified rabbit skeletal muscle actin[31] and extracts of monkey aorta, uterus, diaphragm, and heart (Figure 3A), but does not recognize the counterpart 42-kd protein in extracts of two epithelial cell lines (A431 and Hep3B) and in extracts of a melanoma cell line (Mel-1) (Figure 3B, Lanes 6–8). In immunoblots of extracts of human fibroblasts, there is a very weak band noted at 42 kd (Figure 3B, Lane 9). The lack of a 42-kd band in the immunoblots of these cultured nonmuscle cells is not the result of differences in the amount of 42-kd

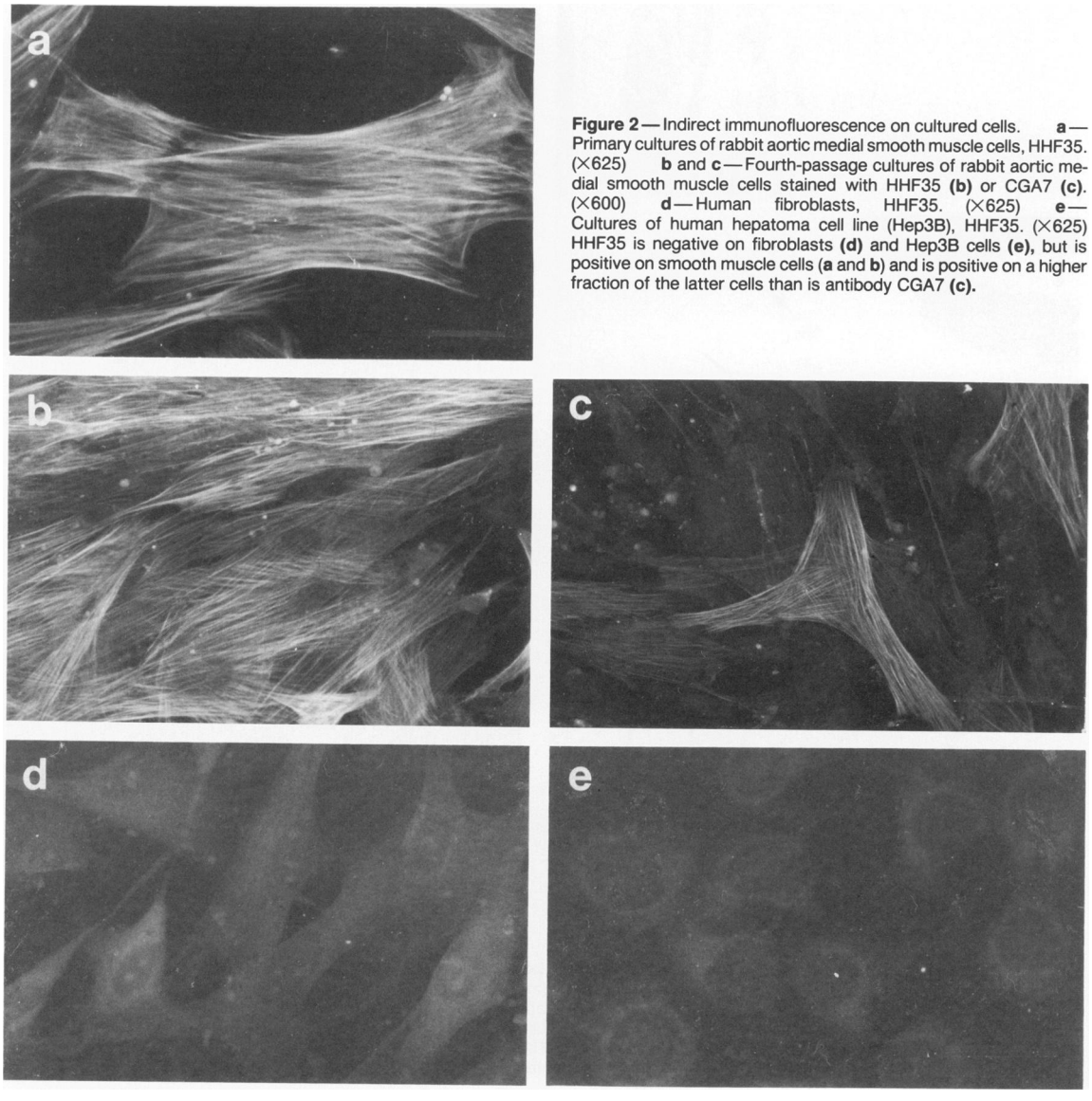


Figure 2—Indirect immunofluorescence on cultured cells. **a**—Primary cultures of rabbit aortic medial smooth muscle cells, HHF35. (×625) **b** and **c**—Fourth-passage cultures of rabbit aortic medial smooth muscle cells stained with HHF35 **(b)** or CGA7 **(c)**. (×600) **d**—Human fibroblasts, HHF35. (×625) **e**—Cultures of human hepatoma cell line (Hep3B), HHF35. (×625) HHF35 is negative on fibroblasts **(d)** and Hep3B cells **(e)**, but is positive on smooth muscle cells (**a** and **b**) and is positive on a higher fraction of the latter cells than is antibody CGA7 **(c)**.

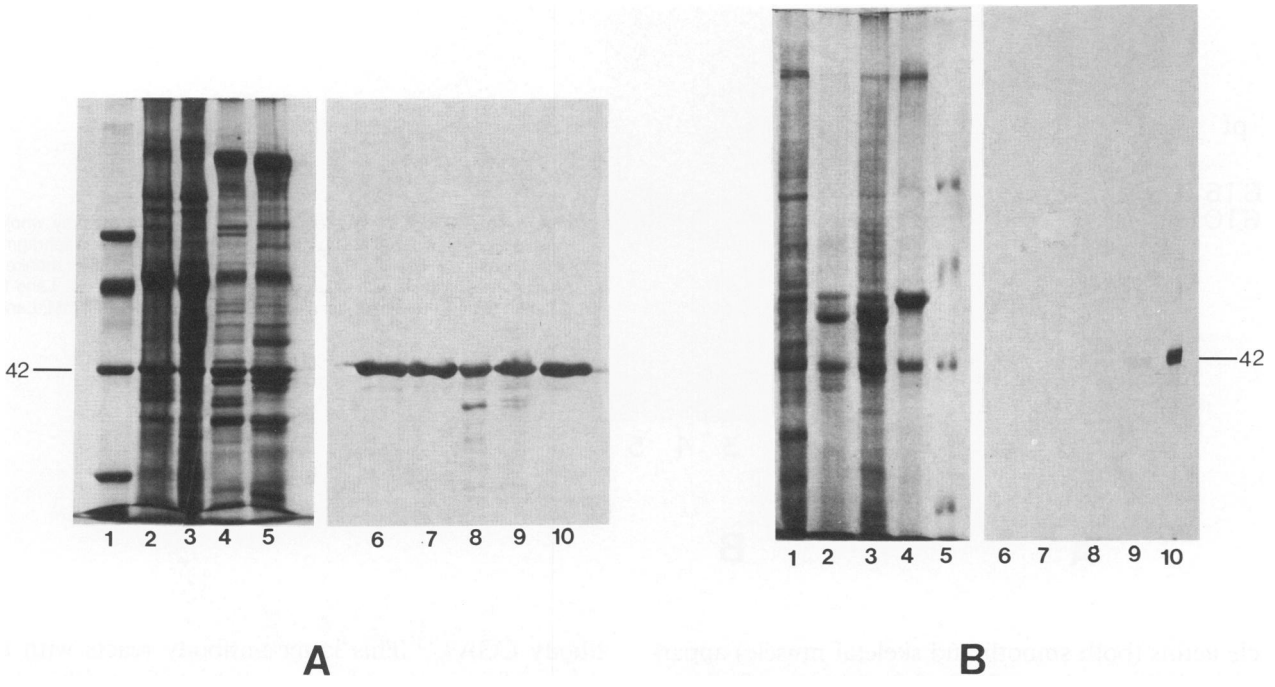


Figure 3A — Monkey whole muscle extracts, 8.75% SDS-PAGE Lanes 1 – 5, Coomassie R-250 protein stain; Lanes 6 – 10, Western blot with HHF35. Lanes 1 and 6, marker proteins. The 42-kd band is of highly purified actin from rabbit skeletal muscle acetone powder. Lanes 2 and 7, aorta; Lanes 3 and 8, uterus; Lanes 4 and 9, diaphragm; Lanes 5 and 10, heart. **B** — Human whole cell SDS extracts from a series of human cell lines, 8.0% SDS-PAGE. Lanes 1 – 5, Coomassie R-250 protein stain; Lanes 6 – 10, Western blot with HHF35; Lanes 1 and 6, Mel-1; Lanes 2 and 7, Hep3B; Lanes 3 and 8, A431; Lanes 4 and 9, fibroblasts; Lanes 5 and 10, marker proteins. The 42-kd band is of highly purified actin from rabbit skeletal muscle acetone powder.

protein present in the gels, because the Coomassie blue-stained nitrocellulose paper demonstrated comparable staining intensities at 42 kd (Figure 3B).

These data suggest that HHF35 recognizes muscle-specific actin and fails to react with nonmuscle actin. HHF35 is nonreactive with beta or non-smooth muscle gamma, because it is nonreactive with actins of nonmuscle cells, which are known to contain beta or non-smooth muscle gamma actins (Figure 3B).[32] In the IEF immunoblot of whole extracts from muscle sources, HHF35 recognizes the alpha actin from skeletal, cardiac, and smooth muscle sources and the gamma actin from smooth muscle sources, but not the beta (nonmuscle) actin which is expressed by "contaminating" cells such as endothelial cells and fibroblasts (Figure 4A). We conclude that HHF35 recognizes muscle-specific alpha and gamma actin isotypes. The ratios of smooth muscle alpha to gamma are reversed in the immunoblots of aorta and uterus (Figure 4A, Lanes 1 and 2). Diaphragm (a skeletal muscle) and heart clearly show the dominant alpha isoelectrophoretic variant (Figure 4A, Lanes 3 and 4). A slight "ghost band" 0.01 pI below each of the bands can also be seen; this phenomenon is induced by differential states of reduction of the actin polypeptide (Figure 4B).

Discussion

Actin is a highly conserved, ubiquitous cytoskeletal protein comprising microfilaments and existing in large amounts in muscle as well as in nonmuscle cells. Different cell types contain immunochemically distinct actins, originally divided into three subtypes (alpha, beta, gamma) based on differences in isoelectric point.[32] It was later found[6] that there are at least six different actins based on amino acid sequence of the amino terminal peptide. Alpha actins from heart, skeletal, and smooth muscle are all unique by sequence. The gamma actin from smooth muscle is far more homologous to the muscle alpha actins, rather than the nonmuscle gamma isotype; that its pI is coincident with that of the nonmuscle gamma actin is probably related to the deletion of an acidic residue from the amino terminus. The presence of distinct actin isotypes in muscle cells, however, suggests that some actin variants are specifically tailored for cell contraction. The monoclonal antibody HHF35 described herein could be an important tool in the elucidation of structural or biochemical correlates of this muscle-specific protein.

Other monoclonal and polyclonal antibodies to actin isotypes have been described. Thus, Lessard et al[33] reported a monoclonal antibody specific to mus-

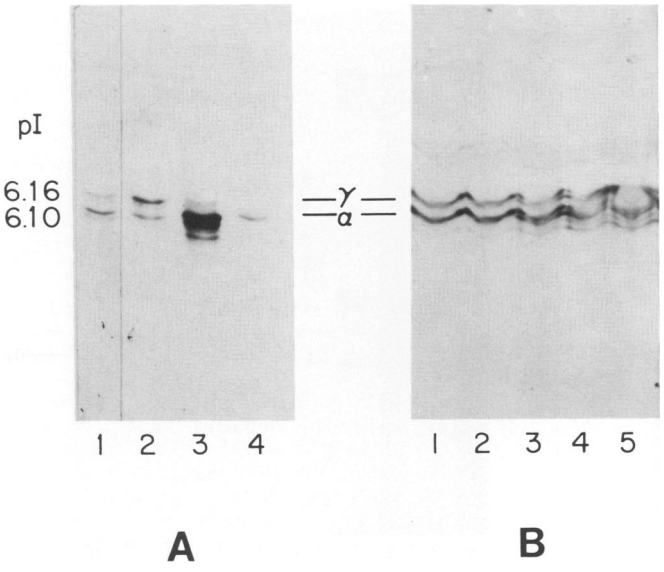


Figure 4A—IEF-PAGE Western blot with HHF35 of monkey whole muscle urea extracts. Lane 1, aorta; lane 2, uterus; lane 3, diaphragm; lane 4, heart. **B**—IEF-PAGE Western blot with HHF35 of monkey uterus whole extracts with differential amounts of dithiothreitol. Lane 1, 4.6 mM; Lane 2, 1.16 mM; Lane 3, 0.29 mM; Lane 4, 0.077 mM; Lane 5, 0 mM.

cle actins (both smooth and skeletal muscle) apparently similar to the antibody described here. Bulinsky et al,[9] using a peptide immunization approach, have reported the generation of polyclonal antibodies specific for skeletal and cardiac muscle alpha actin. Lubit and Schwartz[34] have generated a polyclonal anti-actin antibody, the specificity of which is complementary with antibody HHF35, inasmuch as it is reactive with nonmuscle (beta) actin isotypes. We have previously described a smooth-muscle-cell–specific monoclonal antibody, CGA7, which reacts only with smooth muscle alpha and gamma actin isotypes.[13]

Although excellent correlation between the immunofluorescence and immunoblotting studies on cultured cells was noted, in the case of human fibroblasts, despite negative immunofluorescence results, a weak band at 42 kd was noted in the immunoblots (Figure 3B, Lane 9). This may reflect a low level of muscle actin expression which is below the level of detection of indirect immunofluorescence; expression of smooth muscle actins by non-smooth muscle cells, under certain conditions, has been previously described.[35] Nonetheless, the level of smooth muscle actin expression by fibroblasts is at least an order of magnitude below that of muscle tissue, as can be seen in Figure 3B, in which adjacent lanes have been loaded with comparable amounts of 42-kd protein. One might also speculate that there was smooth muscle contamination of the fibroblast cultures, although one would have expected to find isolated positive cells in the immunofluoresence studies had this been the case.

Several significant characteristics of antibody HHF35 are shared with anti-smooth muscle actin antibody CGA7.[13] This latter antibody reacts with a subset of actins (and hence, cells) positive with antibody HHF35. The major difference is that antibody CGA7 does not recognize the actins of cardiac or skeletal muscle cells. Both antibodies recognize vascular smooth muscle cells in the normal vascular system, but only HHF35 recognizes the pericyte population. HHF35 appears to recognize smooth muscle cells independent of their proliferative state, whereas CGA7 does not react with proliferating smooth muscle cells.[13] The epitope of actin recognized by antibody CGA7, unlike that of HH35, may be masked or modified during cell proliferation, as well as in cells such as pericytes. Smooth muscle cells contain two types of intermediate filaments, vimentin and desmin; an anti-vimentin monoclonal antibody,[16] as well as a polyclonal anti-actin antibody, stain mesenchymal tissues in a totally different fashion from CGA7[13] or HHF35, reacting with such cells as vascular endothelial cells and stromal fibroblasts. Anti-desmin antibodies stain cardiac, skeletal, and smooth muscle cells in a manner similar to HHF35, but fail to recognize myoepithelial cells or pericytes (data not shown). It has also been reported that proliferating smooth muscle cells fail to express desmin.[36] One of our major areas of interest lies in the immunocytochemical analysis of the cellular components of lesions of atherosclerosis; to identify and quantify smooth muscle cells involved in these lesions, they must all be recognized. Antibodies such as CGA7 and those to desmin recognize many, but not all, smooth muscle cells in the intima, perhaps because of phenotypic variations as a function of cell proliferation. Consequently, HHF35 promises to be a useful reagent for analysis of

the cellular content of vascular lesions.[30,37,38] In addition, this antibody should theoretically be of great use to the surgical pathologist in the analysis of the cell of origin of human sarcomas; such work is currently in progress. Finally, it should be noted that both antibodies HHF35 and CGA7 react with a subpopulation of bone-marrow derived cells which have smooth muscle-like features, and are apparently instrumental in the generation of the bone marrow "microenvironment," as described elsewhere.[39]

In summary, HHF35 is a muscle-specific monoclonal antibody which recognizes only muscle actin isotypes and which decorates "stress fibers" in cultured smooth muscle cells. In tissue sections, it stains cardiac, skeletal, smooth muscle cells, and myoepithelial cells, which exactly correspond to the cell types which have been histologically defined as "contractile cells." It also reacts with purified skeletal muscle actin in SDS immunoblots, as well as a band of equal mobility and relative abundance from all muscle sources. In IEF immunoblots, the reactivity of HHF35 matches the published ratios and expected occurrences of muscle actin isotypes from all tissue and cell extracts examined. In subsequent studies, we will demonstrate the utility of this antibody as a cell-specific marker in diagnostic surgical pathology, as well as a marker that is useful in the analysis of the cellular composition of various atherosclerotic lesions.

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