Monoclonal Antibodies to Intermediate Filament Proteins of Human Cells: Unique and Cross-reacting Antibodies

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ABSTRACT Monoclonal antibodies were generated against the intermediate filament proteins of different human cells. The reactivity of these antibodies with the different classes of intermediate filament proteins was determined by indirect immunofluorescence on cultured cells, immunologic identification on SDS polyacrylamide gels ("western blot" experiments), and immunoperoxidase assays on intact tissues. The following four antibodies are described: (*a*) an antivimentin antibody generated against human fibroblast cytoskeleton; (*b*), (*c*) two antibodies that recognize a 54-kdalton protein in human hepatocellular carcinoma cells; and (*d*) an antikeratin antibody made to stratum corneum that recognizes proteins of molecular weight 66 kdaltons and 57 kdaltons.

The antivimentin antibody reacts with vimentin (58 kdaltons), glial fibrillary acidic protein (GFAP), and keratins from stratum corneum, but does not recognize hepatoma intermediate filaments. In immunofluorescence assays, the antibody reacts with mesenchymal cells and cultured epithelial cells that express vimentin. This antibody decorates the media of blood vessels in tissue sections. One antihepatoma filament antibody reacts only with the 54 kdalton protein of these cells and, in immunofluorescence and immunoperoxidase assays, only recognizes epithelial cells. It reacts with almost all nonsquamous epithelium in intact tissue, but fails to recognize squamous epithelium. Thus this antibody identifies an epithelial-specific "cytokeratin" molecule present in only nonsquamous epithelium. The other antihepatoma filament antibody is much less selective, reacting with vimentin, GFAP, and keratin from stratum corneum. This antibody decorates intermediate filaments of both mesenchymal and epithelial cells. The antikeratin antibody recognizes 66-kdalton and 57-kdalton proteins in extracts of stratum corneum and also identifies proteins of similar molecular weights in all cells tested. However, by immunofluorescence, this antibody decorates only the intermediate filaments of epidermoid carcinoma cells. When assayed on tissue sections, the antibody reacts with squamous epithelium and some, but not all, nonsquamous epithelium. Therefore this antistratum corneum antibody and the anti-54-kdalton antibody identify unique epitopes present in the various cytokeratin molecules of epithelial cells. None of the hybridoma antibodies react with neurofilament proteins.

The different patterns of reactivity of these antibodies suggest that many of the immunologically distinct intermediate filament proteins contain common antigenic determinants.

Intermediate filaments, distinctive cytoskeletal components present in virtually all mammalian cells, are distinguished from other cytoskeletal structures such as microtubules and microfilaments on the basis of filament diameter and protein composition. A unique feature of intermediate filaments is that the filaments of different cells are immunochemically distinct. They have been divided into five subclasses based upon biochemical analyses and reactivity with a series of polyclonal rabbit or guinea pig antisera (1). The following classes of intermediate filaments have been defined: (*a*) fibroblast intermediate filaments (FIF; vimentin, decamin), present in a wide range of mesenchymal cells in vivo, but present as well in some epithelial cell lines in vitro (2–4). This protein is composed of a single subunit with a molecular weight in the range of 52–58 kdaltons. (*b*) Keratin (prekeratin, cytokeratin), a complex series of proteins with molecular weights ranging from 40 to 70

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kdaltons, present in skin and a wide range of epithelial cells (5–8). (*c*) Desmin, a 52-kdalton protein characteristic of cells of muscle origin, e.g., skeletal muscle, where it is localized to the Z-disk. It is also present in BHK cells (9, 10). (*d*) Glial fibrillary acidic protein (GFAP), the protein component of intermediate filaments of astrocytes, with a molecular weight of approximately 51 kdaltons (11–14). This molecule is apparently specific to cells of the central nervous system, although its presence in the peripheral nervous system has been reported (15). (*e*) Neurofilaments, intermediate filaments of neurons of the central and peripheral nervous system, composed of three proteins with molecular weights 210 kdaltons, 160 kdaltons, and 70 kdaltons (16). The relationship of these proteins to one another is not clear.

Polyclonal antisera made against a purified intermediate filament protein have been extremely useful in distinguishing among the different molecules. However, monoclonal antibodies afford a much higher degree of specificity than can be obtained with polyclonal sera because they recognize a single unique three-dimensional structure or sequence (epitope). Because the different intermediate filament proteins apparently contain similar as well as unique sequences (17, 18), it should be possible to isolate hybridoma antibodies specific for a single intermediate filament protein, and others able to recognize more than one type of intermediate filament protein (19). A panel of hybridoma antibodies made against each of the filament proteins would therefore be useful in identifying unique and common sequences in these molecules. In addition, the antibodies specific for a single molecule could be used to identify the cellular origin of different neoplasms (7, 20, 21) and therefore would be clinically useful in the practice of pathology.

We report here the characterization of monoclonal antibodies generated against vimentin (decamin), cytokeratin from a human hepatoma cell line, and keratin from human stratum corneum. These antibodies exhibit unique reactivities with the different classes of intermediate filament proteins.

MATERIALS AND METHODS

Cells and Media

The following human cell strains and cell lines were obtained from Dr. Helene Smith, Peralta Institute, Oakland, CA: Hs–0074 Int, normal intestine; Hs–0767 B1, normal bladder; Hs–0700T, metastatic adenocarcinoma; Hs–0766T, metastatic pancreatic carcinoma; Hs–0746T, metastatic gastric carcinoma; Hs–0696T, metastatic adenocarcinoma; Hs–0578T, carcinosarcoma of breast. Human hepatocellular carcinoma cell lines Hep3B and HepG2 were obtained from Drs. David Aden and Barbara Knowles, Wistar Institute, Philadelphia, PA. Human fibroblasts were explanted from foreskins. Rhabdo–1 was derived from a human rhabdomyosarcoma.

All cells, with the exception of Hs–0074 Int, were grown in DME supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). Hs–0074 Int was maintained in Eagle's minimal essential medium (MEM) supplemented with similar antibiotics. Newborn calf serum (10% vol/vol, Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) was used for all cells except Hep3B, and HepG2, which received 5% newborn calf serum, 5% fetal calf serum. All of the lines from Dr. Smith also received 2 μg/ml insulin (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA).

Preparation of Intermediate Filament Proteins

Cells were grown to semi-confluence in 100-mm dishes and extracted with Triton-high salt according to Franke et al. (22). Cell monolayers were washed two times with TNM buffer (10 mM Tris, 140 mM NaCl, 5 mM $MgCl_2$, pH 7.6) and incubated with 1% Triton X-100 in TNM buffer for 1 min at room temperature. The monolayers were washed twice with TNM buffer and exposed to 0.5% Triton X-100, 1.5 MKCl for 30 min at room temperature, after which the cells were washed twice in TNM and scraped off the dish with a rubber policeman. The insoluble material was suspended in TNM and centrifuged at 10,000 rpm for 20 min at 4°C in a Sorvall centrifuge (Dupont Instruments-Sorvall Biomedical Div., Dupont Co., Newtown, CT). The pellet was resuspended in 1% SDS, 50 mM Tris, pH 6.8, and stored at −20°C. The human fibroblast cytoskeletons remained attached to the dish during Triton-high salt extraction. All other cells extracted in this manner detached from the dish during the Triton-high salt incubation. When this occurred, the cell cytoskeletons were centrifuged at 10,000 rpm for 20 min at 4°C and washed with TNM by successive centrifugations.

Keratin was extracted from human stratum corneum from the sole of the foot by the method of Sun and Green (23). Fragments of stratum corneum were extracted overnight at 37°C in 5% SDS, 1% β-mercaptoethanol at a concentration of 4 mg/ml. The extracted tissue was centrifuged to remove the insoluble material and stored at −20°C.

Production of Hybridomas

Hybridoma antibodies to intermediate filament proteins were generated according to the basic outline of Köhler and Milstein (24) as modified by Nowinski et al. (25). Preparations of intermediate filaments as described above from ~10^7 cells were solubilized in 1% SDS and injected intraperitoneally into a 5-wk-old BALB/c mouse; injections were repeated at 2 and 4 wk. 10 100-mm plates of semiconfluent cells were extracted for each injection. For the production of antikeratin antibodies, ~200 μg of solubilized keratin was used for each immunization. 3 d following the third injection, the mouse was sacrificed by overanesthesia with ether. Spleen cells were fractionated into a single cell suspension by vigorous mincing and passage through fine wire mesh and fused to mouse myeloma cells (NS–1) at a ratio of 4:1 (spleen cells:myeloma cells) with polyethylene glycol (PEG). Hybrid clones were grown in RPMI–1640 (Gibco Laboratories) medium supplemented with 15% fetal calf serum, L-glutamine (1 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), streptomycin (100 μg/ml; Gibco Laboratories), and 0.1 mM hypoxanthine, 0.40 μM aminopterin, 0.16 mM thymidine (HAT; Sigma Chemical Co., St. Louis, MO). Cultures were maintained at 7% CO_2 concentration at 37°C. Cells were fed with RPMI/HAT at days 4 and 8; RPMI/HT at day 12; and RPMI–1640 every 4 d subsequent.

Clones were generally visible 5–10 d after the fusion, and supernatants tested when the wells were between one-third and one-half confluent, generally 2–3 wk following the fusion.

Screening of Hybridoma Antibodies

Clones producing antibodies to cytoskeletal preparations were identified by assaying hybridoma supernatant fluids for binding to formalin-, acetone-, or ethanol-fixed monolayers of cells by autoradiography or immunofluorescence techniques. For autoradiography, human fibroblasts or Hep3B cells were grown to confluence in 96–well microtiter plates, washed two times with 1% bovine serum albumin (BSA; Sigma Chemical Co.) in PBS, and fixed for 10 min at 4°C with 3.7% formaldehyde in PBS. The fixed cells were washed four times in 1% BSA in PBS.

50 μl of supernatant were withdrawn from each hybridoma-containing well with a multichannel variable pipette (Finnipette, Labsyskms Oy, Helsinki, Finland) and added to the formalin-fixed cells. Plates were incubated for 45 min at 37°C and washed three times with 1% BSA in PBS. 50 μl of immunoglobulin fraction of rabbit antisera to mouse IgG (Miles Laboratories, Elkhart, IN) diluted 1:400 in PBS were then added to all wells. Following incubation at 37°C for 45 min and three serial washes with 1% BSA in PBS, 50 μl of staphylococcal protein A (Sigma Chemical Co.), labeled via the lactoperoxidase method to a specific activity of 5.51 μCi/μg, was added to each well at a dilution of 10^5 cpm/50 μl. Following a 45-min incubation at 37°C and three serial washes with 1% BSA in PBS, positive wells were identified either by direct visualization following overnight autoradiography of the plates on Kodak XR-5 x-ray film backed by a Dupont Lightning Plus intensifying screen at −70°C, or by punching out individual well bottoms with a hammer and 6-mm diameter steel rod and counting directly in a gamma counter. In the latter method, positive wells were defined as those displaying counts three standard deviations or greater beyond the mean background (wells incubated initially with NS–1 supernatant or PBS). Positive wells identified by this initial screening were then tested by immunofluoresence to identify antibodies to intermediate filaments (see below).

Antikeratin antibodies were assayed by binding to keratin-coated dishes. Solubilized keratin (500 ng per well) was dried overnight on microtiter plates and the wells were washed with 1% BSA in PBS. 50 μl of hybridoma supernatant was added to each well and processed as described above.

Wells that were positive in the initial screening assay and by immunofluorescence were cloned in a two-step procedure described elsewhere (25). In the first "miniclone" step, 3–5 cells/well were seeded into one or two 96-well plates with a feeder layer of 10^6 thymocytes/well from 10-d-old BALB/c mice. The best growing cells, which also produced strongly reactive supernatants, were then

formally cloned by seeding one cell per three wells in three 96-well plates. Cloned cells were transferred to flasks, allowed to grow to high density, and 10^7 cells were injected intraperitoneally into pristane-primed BALB/c mice. 10 d to 2 wk later, ascites fluid (5–10 ml/mouse) was withdrawn. Cell lines producing positive supernatants were frozen and stored at −70°C in 10% DMSO.

Immunofluorescence

Cells were plated in growth medium on Teflon-coated slides containing 12 wells (Meloy Laboratories, Springfield, VA) and allowed to attach overnight. The slides were washed in PBS, fixed in 95% ethanol or acetone (−20°C for 10 min) and air dried. 25 μl of diluted supernatant from hybridoma culture medium or ascites fluid were added to each well and the slides incubated at 37°C for 45 min in a humidified chamber. The slides were then washed in PBS and incubated with 25 μl of FITC-conjugated goat anti-mouse IgG and IgM (1:40 dilution, Tago Inc., Burlingame, CA) for 45 min at 37°C. The slides were washed with PBS, then water, and coverslips were mounted with Aqua-Mount (Lerner Laboratories, New Haven, CT). Fluorescence was observed with a Zeiss fluorescence microscope with appropriate fluorescein excitation and emission filters. Fixation with cold 95% ethanol (−20°C) resulted in the best immunofluorescence pattern for the antivimentin antibodies, whereas acetone fixation was better for the antihepatoma and antikeratin antibodies.

SDS Polyacrylamide Gel Electrophoresis

Slab gel electrophoresis was done according to Laemmli (26). 8% gels were used to analyze whole cell lysates or Triton-high salt-insoluble material. Whole cell lysates were prepared by washing confluent 100-mm plates with TNM buffer and solubilizing the cells with 1 ml of SDS sample buffer. Protein concentration of whole cell lysates was ~0.4 mg/ml. Triton-high salt-insoluble material was dissolved in SDS sample buffer at a concentration of ~0.5 mg/ml. Samples were run with dithiothreitol (7.7 mg/ml). The absence of dithiothreitol in the SDS sample buffer did not affect the migration of the intermediate filament proteins in an 8% gel.

Electrophoretic Transfer of Proteins from Polyacrylamide Gels to Nitrocellulose Paper

To identify the antigen recognized by the hybridoma antibodies, proteins separated on 8% SDS polyacrylamide gels were electrophoresed onto nitrocellulose paper and incubated with test antibodies. The procedure was done according to Burnette (27) using a transfer apparatus similar to that described by Towbin et al. (28). Material tested included SDS lysates of whole cells or the intermediate filament preparations made by Triton-high salt extraction of various cell lines. Pyronine Y (0.05%) was included in the SDS sample buffer to mark the bottom of the gel on the nitrocellulose paper. The SDS polyacrylamide gel was apposed to a sheet of nitrocellulose paper, and transfer was done overnight in 25 mM potassium phosphate, pH 6.5, at 100 mamp. The voltage gradient was ~6 V/cm. Transferred proteins were identified on nitrocellulose by staining with amido black (0.1% in 45% methanol/10% acetic acid) for 3 min and destaining with 90% methanol/2% acetic acid.

Immunologic Detection of Proteins on Nitrocellulose Paper

All procedures were performed at room temperature. Nitrocellulose paper containing the transferred proteins was incubated in 25 ml of 5% bovine serum albumin (BSA) in 10 mM Tris, 0.9% saline, pH 7.4 (TSB buffer), for 90 min with gentle rocking. The antibody-containing ascites fluid was added directly to the BSA solution and incubated for another 90 min. The antibody was removed and the nitrocellulose washed according to the following regimen: (*a*) 50 ml of TSB, 10 min; (*b*) 50 ml of 0.05% Triton X-100 in TSB, 20 min; (*c*) 50 ml of 0.05% Triton X-100 in TSB, 20 min; and (*d*) 50 ml of TSB, 10 min. The nitrocellulose paper was then incubated with 5% BSA containing 5×10^6 cpm of ^{125}I-goat anti-mouse IgG, (IgG and IgM specific [Tago Inc.]). After 60 min, the nitrocellulose paper was washed as above, dried, and exposed to Kodak XR-5 film with Dupont Lightning Plus enhancing filters. Exposure times varied from 6 to 18 h. Ascites fluid dilutions of 1:200–1:1,000 were routinely used, but ascites fluids were active to a dilution of 1:10,000.

GFAP and Neurofilament Protein Preparations

GFAP and neurofilament proteins prepared from rat spinal cord were obtained from Dr. F. C. Chiu, Department of Neurology, Albert Einstein College of Medicine (29). Anti-GFAP sera was a gift from Dr. James Goldman, Department of Neurology, Albert Einstein College of Medicine.

Two-Dimensional Peptide Analysis

Peptide maps were prepared exactly as described by Sage et al. (30). Individual protein bands were cut from gels, iodinated with Bolton-Hunter reagent (250 μCi per incubation, New England Nuclear, Boston, MA), and digested with proteinase K (40 μg/ml, E.M. Biochemicals, Darmstadt, Germany) for 18 h at 37°C. Iodinated peptides (2×10^5 cpm) were spotted onto cellulose acetate plates (10 × 10 cm) and separated by electrophoresis in one dimension and chromatography in the second dimension. The dried plates were exposed to Kodak (BB-5) film with Dupont Lightning Plus enhancing filters for 2–6 h.

Immunocytochemistry

Autopsy-derived samples of human pancreas and skin were obtained from the University Hospital of the University of Washington. They were fixed in Carnoy's fixative (60% ethanol, 30% chloroform, 10% acetic acid) for 3 h and embedded in paraffin. 7-μm sections were deparaffinized and endogenous peroxidase activity was blocked with 0.01% H_2O_2 in methanol (30 min). Monoclonal antibodies were applied to slides in dilutions ranging from 1:1000 to 1:8000 (30 min). Following a PBS wash, they were then incubated with biotinylated-anti-mouse IgG (Vector Laboratories, Burlingame, CA) at a dilution of 1:500 (30 min). Again, following a PBS wash, a solution of avidin-biotinylated horseradish peroxidase complex (Vector Laboratories, Burlingame, CA) was then applied (30 min), followed by an additional PBS wash. Peroxidase activity was identified by incubating with a solution of 0.1% 3,3'-diaminobenzidine (DAB) and 0.03% hydrogen peroxide in 0.05 M Tris buffer, pH 7.6. Sections were counterstained with methyl green.

RESULTS

Sources of Antigen from Different Tissues

Cytoskeletal preparations from a variety of human cells were examined to identify sources of different intermediate filament proteins. Fibroblasts, a human hepatocellular carcinoma line (Hep3B), and stratum corneum were chosen. An 8% SDS polyacrylamide gel of Triton-high salt-insoluble material from these cells is shown in Fig. 1. Vimentin (decamin), a 58-kdalton protein, is present in human fibroblast cytoskeletal preparations (lane *a*). Hep3B, the hepatoma line, contains a prominent band at 54 kdaltons and a faint band at 58 kdaltons (land *b*). Additional minor bands are present in the 66–70-kdalton region. A431, a human epidermoid carcinoma line, contains a 54-kdalton band, a 56-kdalton band, and minor bands in the 66–70-kdalton region and at 59 kdaltons and 49 kdaltons (lane *c*). Lanes *a*, *b*, and *c* also exhibit bands of 44 kdaltons which contain actin. Keratin, solubilized from stratum corneum, is predominantly composed of a doublet of 66 kdaltons and single bands at 57 kdaltons, 51 kdaltons, and 49 kdaltons (lane *d*). There is a smear of minor bands in the 66–70-kdalton region and other minor bands scattered throughout the 40–66-kdalton region. Monoclonal antibodies were made to vimentin (lane *a*), cytoskeleton from Hep3B (lane *b*), and keratin from stratum corneum (lane *d*). Table I lists the different antibodies to be discussed and the immunogens against which they are directed.

Fig. 1 also shows preparations containing the neurofilament proteins and GFAP isolated from rat brain (supplied by Dr. F. C. Chiu, Department of Neurology, Albert Einstein College of Medicine). Neurofilaments are composed of peptides of molecular weight 200 kdaltons, 160 kdaltons and 70 kdaltons (lanes *e* and *f*). GFAP (52 kdaltons) is also present in the neurofilament preparation (lane *e*). The high molecular weight neurofilament protein usually migrates at 210 kdaltons. The lower molecular weight in our preparation probably results from partial degradation of the molecule.

Immunofluorescence Studies on Cell Lines and Immunological Identification of Antigens

Four monoclonal antibodies were isolated and characterized (Table I). Immunofluorescence assays were performed on a

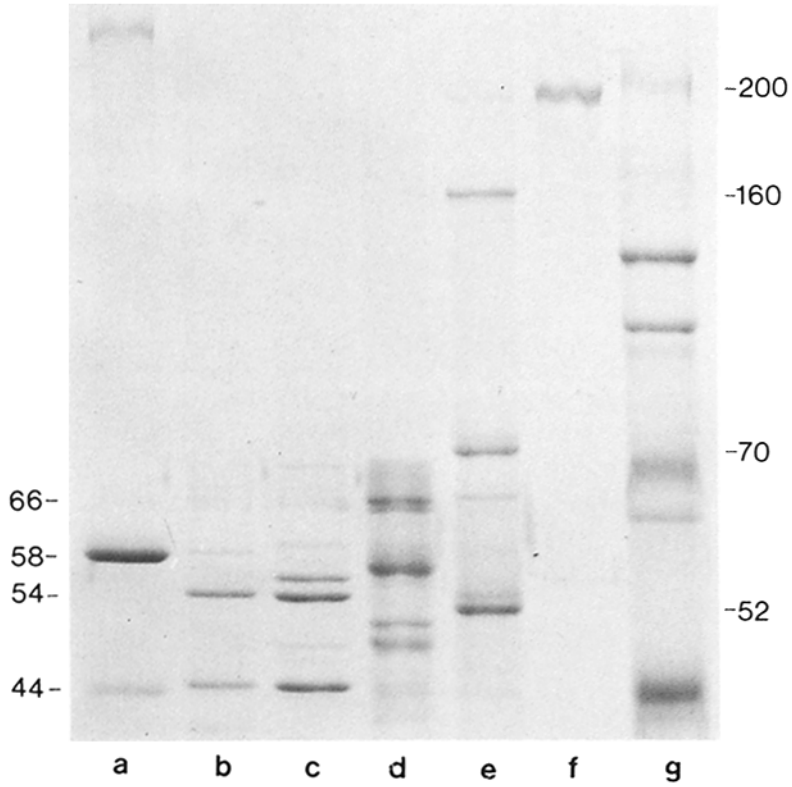

FIGURE 1 SDS polyacrylamide gel (8%) of cytoskeletal preparations from different sources. ~10–20 μg of protein was added in each well. (*a*) Fibroblasts; (*b*) Hep3B (hepatocellular carcinoma); (*c*) A431 (epidermoid carcinoma); (*d*) keratin from stratum corneum; (*e*) rat neurofilament proteins; (*f*) rat 200-kdalton neurofilament protein; (*g*) molecular weight markers of 200 kdaltons, 116 kdaltons, 94 kdaltons, 68 kdaltons, and 43 kdaltons.

TABLE I

Hybridoma Antibodies to Intermediate Filament Proteins

Antibody Name	Antibody generated against
17βG3	Cytoskeleton from fibroblasts
35βH11, 35βD12	Cytoskeleton from Hep3B
34βE12	Keratin from stratum corneum

variety of cultured human cells (both normal and neoplastic) fixed in either −20°C ethanol or −20°C acetone. Results are shown in Fig. 2 and summarized in Table II.

The technique of "western blotting" was used to identify the antigen(s) recognized by each of these antibodies (27). In these experiments, SDS lysates of whole cells were used (Fig. 3, lanes *a–f*), but identical results were obtained using the insoluble residue from Triton-high salt-extracted cells (data not shown). The reactivity of the antibodies with purified neurofilaments (Fig. 3, lanes *g* and *h*), GFAP (Fig. 3, lane *g*) and keratin from stratum corneum (Fig. 3, lane *i*) was also examined.

17βG3: Antibody 17βG3, generated against fibroblast cytoskeleton, reacts with human fibroblasts but not with Hep3B (Fig. 2*a* and 2*b*). In immunoblot experiments, the antibody recognizes vimentin (58 kdaltons) in fibroblasts and Hs–0578T, a breast carcinosarcoma (Fig. 3 *A*, lanes *a* and *b*). All of the cells in Table II that react with 17βG3 contain vimentin (58 kdaltons), even those of epithelial origin (bladder, fetal intestine, and two adenocarcinomas). These results are in agreement with Franke et al. (2), who found that many epithelial cells that normally lack vimentin in vivo contain this protein when cultured in vitro.

Four cell lines, Hep3B, HepG2, A431, and Hs–0700T, fail to react with 17βG3 by fluorescence assays (Table II) and do not contain any immunologically detectable vimentin (Fig. 3*A*, lanes *c–f*). Hep3B contains a minor band at 58 kdaltons (Fig. 1, lane *b*) but this molecule is not recognized by 17βG3. Two-dimensional peptide analyses demonstrate that vimentin from human fibroblasts and the 58-kdalton protein from Hep3B are markedly different (see below).

In some assays, varying reactivity with a protein at 44 kdaltons is seen in cells that either possess or lack vimentin. For example, a 44-kdalton band is recognized by 17βG3 in A431 (Fig. 3 *A*, lane *e*). This reactivity varies from cell line to cell line and from assay to assay. We do not as yet understand this variable reactivity.

The antivimentin antibody cross reacts with GFAP and the high molecular weight bands of keratin (66 kdaltons, 57 kdaltons) (Fig. 3 *A*), but does not recognize the neurofilament peptides. The recognition of GFAP, although weak on this autoradiogram, is stronger in others (data not shown). In addition, we cannot dilute the antibody so that it retains reactivity with vimentin, but no longer recognizes GFAP or keratin. When approximately equal amounts of vimentin, keratin, and GFAP are loaded on a gel, recognition of each of these proteins at different antibody dilutions is identical (data not shown).

It is possible that the 52-kdalton band in the neurofilament preparation is not GFAP but, instead, a proteolytic breakdown product of vimentin (31). This possibility is unlikely because polyclonal anti-GFAP sera (supplied by Dr. James Goldman, Albert Einstein College of Medicine) reacts with the 52-kdalton band but does not recognize vimentin (data not shown).

35βH11: Hybridoma 35βH11, made against Hep3B intermediate filaments, decorates filaments of Hep3B but not of fibroblasts (Fig. 2 *c* and 2 *d*). The antibody recognizes a 54-kdalton peptide in Hep3B, HepG2, A431, and Hs-0700T, all epithelial cell neoplasms (Fig. 3 *B*). Hs–0696T and Hs–0746T, both of which are positive by fluorescence, also contain the 54-kdalton protein (data not shown). No peptides are identified in fibroblasts or Hs–0578T, nor in the epithelial cells (bladder, fetal intestine) listed in Table II that are negative in immunofluorescence assays (data not shown). This antibody reacts with

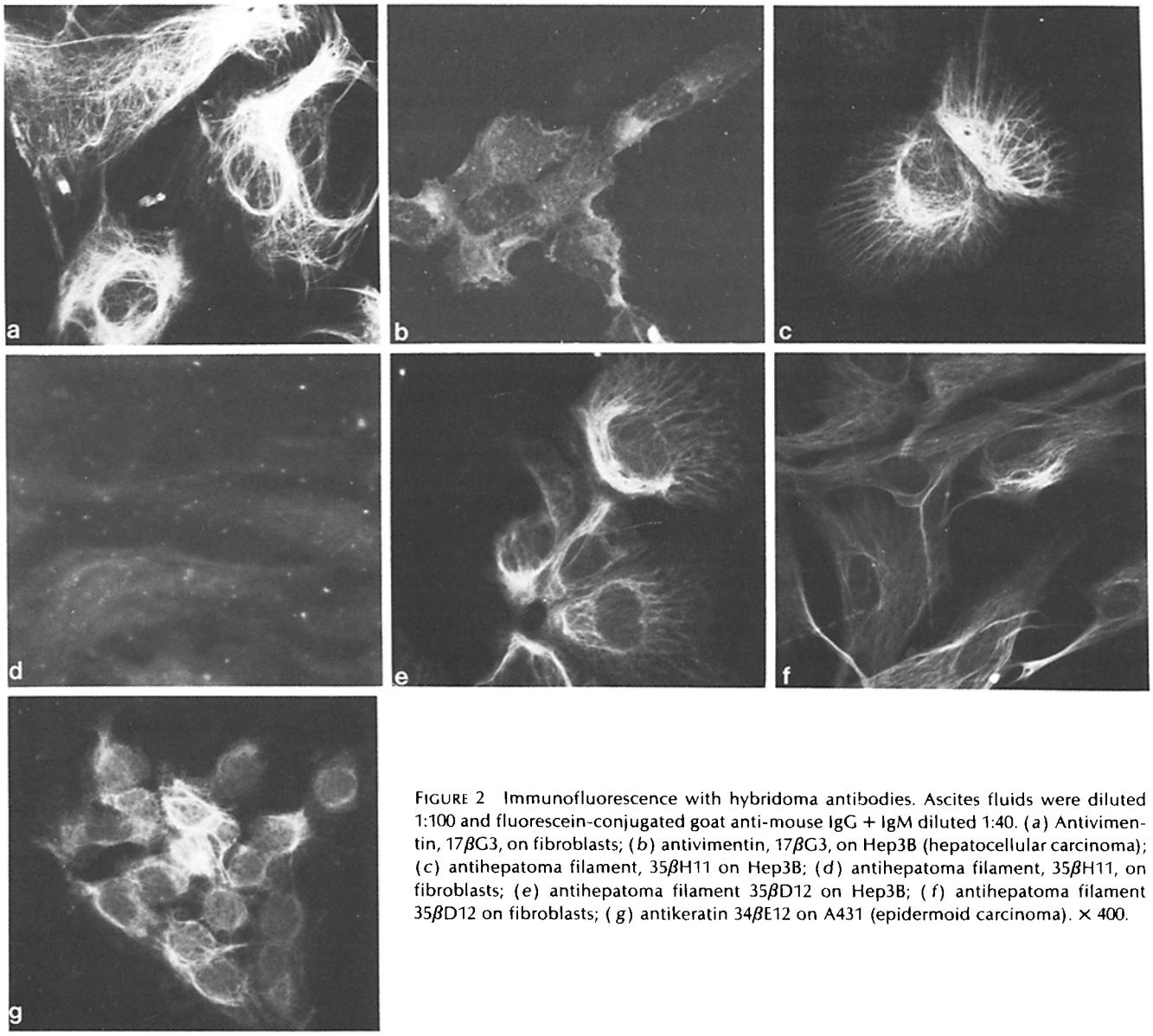

FIGURE 2 Immunofluorescence with hybridoma antibodies. Ascites fluids were diluted 1:100 and fluorescein-conjugated goat anti-mouse IgG + IgM diluted 1:40. (*a*) Antivimentin, 17βG3, on fibroblasts; (*b*) antivimentin, 17βG3, on Hep3B (hepatocellular carcinoma); (*c*) antihepatoma filament, 35βH11 on Hep3B; (*d*) antihepatoma filament, 35βH11, on fibroblasts; (*e*) antihepatoma filament 35βD12 on Hep3B; (*f*) antihepatoma filament 35βD12 on fibroblasts; (*g*) antikeratin 34βE12 on A431 (epidermoid carcinoma). × 400.

TABLE II

Immunofluorescence with Monoclonal Antibodies

Cell line	Origin	17βG3 (vimentin, 58 kdaltons)	35βH11 (hepatoma IF, 54 kdaltons)	35βD12 (hepatoma IF)	34βE12 (stratum corneum)
Fibroblasts	Foreskin	+	−	+	−
Hs0767	Normal bladder	+	−	+	−
Hs0074	Normal fetal intestine	+	−	+	−
Hs0578T	Carcinosarcoma of breast	+	−	+	−
Rhabdo-1	Rhabdomyosarcoma	+	−	+	−
Hep3B	Hepatocellular carcinoma	−	+	+	−
HepG2	Hepatocellular carcinoma	−	+	+	−
Hs0700T	Adenocarcinoma, metastatic to pelvis	−	+	+	±
A431	Epidermoid carcinoma, vulva	−	+	+	+
Hs0696T	Adenocarcinoma, metastatic to bone	+	+	+	−
Hs0746T	Adenocarcinoma, metastatic to muscle	+	+	+	−

Ascites fluids were tested by immunofluorescence at dilutions of 1:100. The filaments listed in parentheses under the antibody number show the immunogen against which the antibodies were generated.

no other intermediate filament proteins (Fig. 3 *B*). It is therefore highly specific, recognizing only a 54-kdalton protein present in many cells of epithelial origin.

35βD12: Antibody 35βD12, isolated from the same fusion as 35βH11, recognizes the intermediate filaments of both Hep3B and fibroblasts (Fig. 2 *e* and 2 *f*) and reacts with all

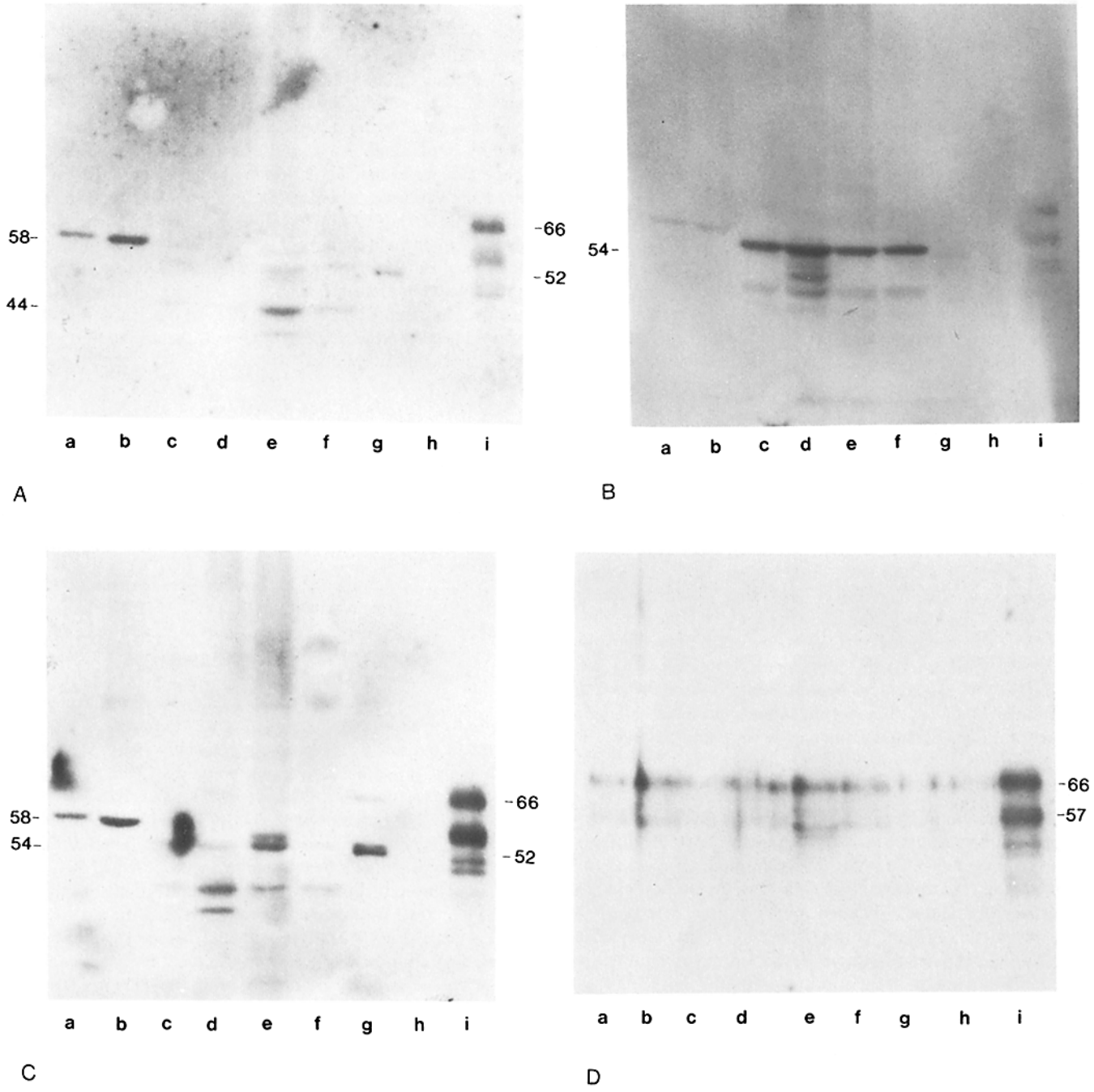

FIGURE 3 Nitrocellulose blotting of different cells with hybridoma antibodies. Cells were lysed in SDS sample buffer, electrophoresed on an 8% SDS polyacrylamide gel, and electrophoretically transferred to nitrocellulose paper. Nitrocellulose strips were incubated with the different hybridoma ascites fluids. The different cells tested are listed in Table II. Lane *a*, fibroblasts; lane *b*, Hs-0578T; lane *c*, Hep3B; lane *d*, HepG2; lane *e*, A431; lane *f*, Hs-0700T; lane *g*, rat neurofilament preparation; lane *h*, rat 200 kdalton neurofilament protein; lane *i*, keratin from stratum corneum. ~10–20 μg of protein was added per well. (*A*) Antivimentin, 17βG3, 1:200 dilution; (*B*) Antihepatoma filament 35βH11, 1:500 dilution; (*C*) Antihepatoma filament 35βD12, 1:200 dilution; (*D*) Antikeratin, 34βE12, 1:200 dilution. The large irregular spot in 3 *C*, lane *c*, is an artifact.

cells tested (Table II). In immunoblot experiments, it reacts with several types of intermediate filament proteins (Fig. 3 *c*). It binds to vimentin (58 kdaltons) in fibroblasts (lane *a*), the 54-kdalton and 56-kdalton proteins of A431 (lane *e*), GFAP (lane *g*), and all the major keratin bands (lane *i*). It does not, however, recognize the different neurofilament proteins (lanes *g* and *h*). The antibody also recognizes a 44-kdalton and 40-kdalton molecule in HepG2, and 44-kdalton proteins in A431 and Hs-0700T. The reactivity with these lower molecular weight bands is highly variable, similar to the variable reactivity of 17βG3 with the 44-kdalton protein of A431. This antibody therefore appears to recognize a common epitope in many different intermediate filament proteins.

The antibody does not recognize the 54-kdalton proteins of the hepatoma lines or Hs-0700T even though these lines are positive in immunofluorescence assays. One explanation for this observation is that the antibody cannot recognize the epitope in the SDS denatured protein from these cells, but can

recognize the SDS denatured protein from A431. This implies that the 54-kdalton proteins of these different cells are not identical; two-dimensional peptide maps show that these peptides are indeed different (see below).

34βE12: Hybridoma 34βE12, generated against keratin from stratum corneum, is only positive by immunofluorescence on A431, the epidermoid carcinoma (Fig. 2 *g*). It is negative on fibroblasts, Hep3B, and all other cells (Table II). This antibody binds to the 66-kdalton and 57-kdalton proteins in stratum corneum and reacts less strongly with the 51-kdalton and 49-kdalton molecules (Fig. 3 *D*, lane *i*). Interestingly, the antibody identifies proteins of 66 kdaltons and 57 kdaltons in all cells tested, even those negative in immunofluorescence assays. These peptides are very minor components of the Triton-high salt-insoluble material from cells. Thus, although specific in immunofluorescence assays on tissue culture cells and immunoperoxidase tests on intact tissue (see below), the antibody recognizes similar molecular weight peptides in all cells in immunoblot experiments.

The immunoautoradiograph of the antikeratin antibody always has this "smeared" appearance, which may be related to the degree of reduction of the proteins. If the gels are run without reducing agent, the antibody does not recognize any bands on the nitrocellulose paper either in the position of the keratin bands or elsewhere (data not shown). The keratin preparation used as immunogen was reduced with β-mercaptoethanol, so we believe the antibody may recognize only the reduced protein. Increasing the amount of reducing agent causes the 57-kdalton band to decrease in intensity, but does not affect the 66-kdalton peptide (data not shown). This hybridoma recognizes none of the other intermediate filament proteins in fibroblasts, hepatoma cells, or neural tissue.

Two-Dimensional Peptide Maps

The immunofluorescence and "western blot" results raise questions about similarities and differences among the different intermediate filament proteins. We performed two-dimensional peptide analyses to answer the following three questions: (*a*) are vimentin and the 58-kdalton protein in Hep3B related? (*b*) Are the 54-kdalton peptides of Hep3B and A431 identical? and (*c*) Does the 66-kdalton protein from stratum corneum resemble the 66-kdalton molecules from Hep3B and A431?

VIMENTIN VS. THE 58-KDALTON PROTEIN FROM HEP3B: The peptide maps of vimentin and the 58-kdalton protein from Hep3B are quite different (Fig. 4 *a* and 4 *b*). Additionally, the maps of the 54-kdalton proteins from Hep3B (Fig. 4 *c*) and A431 (Fig. 4 *d*) differ from that of vimentin, as does the map of the 44-kdalton band from A431 (Fig. 4 *e*). Thus, vimentin and these other proteins are clearly different.

54-KDALTON PROTEINS OF HEP3B AND A431: "Western blot" experiments demonstrate that antibody 35βD12 recognizes the 54-kdalton protein from A431 but not Hep3B. The peptide maps show both similarities and differences in the peptides (Fig. 4 *c* and *d*). The more hydrophobic, less-charged spots are almost identical, but the more-charged peptides show many differences. We conclude that the variation in reactivity with 35βD12 results from structural or sequence differences in the two molecules.

66 KDALTONS FROM STRATUM CORNEUM VS. 66 KDALTONS FROM HEP3B AND A431: Antibody 34βE12 recognizes a 66-kdalton band in preparations of stratum corneum and a protein of similar molecular weight in all cells tested. The 66-kdalton protein from stratum corneum (Fig. 4 *f*) differs from the 66-kdalton molecules of Hep3B (Fig. 4 *g*) and A 431 (Fig. 4 *h*), whereas these latter two proteins are practically identical. The cell-derived 66-kdalton proteins probably share a common epitope(s) with the molecule from stratum corneum.

The reason for the difference in immunofluorescence staining by 34βE12 on A431 and Hep3B remains unclear. The peptide maps of the 66-kdalton molecules from these two cells are identical, yet antibody 34βE12 decorates the intermediate filaments of only A431. Possible explanations include very subtle sequence differences in the molecules, different intercellular localizations of the proteins in the two cells, or a difference in the way the molecules associate with the intermediate filaments. For example, in Hep3B the 66-kdalton protein may be surrounded by other proteins such that the antibody does not have access to the epitope.

Reactivity with Intact Tissues

To further characterize these antibodies, we examined their ability to react with tissue obtained from autopsy and surgical specimens. Samples of skin and pancreas were fixed in Carnoy's solution, and reactivity was demonstrated by immunoperoxidase techniques. A more detailed analysis of reactivity is in preparation.

35βH11: The antihepatoma intermediate filament antibody reacts with pancreatic acinar and duct cells (Fig. 5 *c*) and sweat gland epithelia (data not shown). It fails to recognize epidermis and mesenchymal tissue such as blood vessels and dermis (Fig. 5 *a*). The absence of decoration of squamous epithelia is consistent with the inability of this antibody to recognize keratin from stratum corneum and suggests that the 54-kdalton protein recognized by this antibody is specific for nonsquamous epithelium. In addition, the antibody does not react with skeletal muscle or nervous tissue (data not shown).

34βE12: The antikeratin antibody is specific for epidermis and some but not all nonsquamous epithelium. The full thickness of the epidermis is heavily stained with this antibody (Fig. 5 *b*), as is the epithelium of the sweat glands (data not shown). In the pancreas, the duct cells are positive, but the acinar cells are negative (Fig. 5 *d*). Mesenchymal tissues such as blood vessel, smooth muscle, skeletal muscle, and dermis, as well as nervous tissue, fail to react with this antibody (data not shown). Thus this antibody reacts with squamous epithelium and a subset of nonsquamous epithelium.

17βG3: This antibody most strongly reacts with the media of blood vessels (Fig. 5 *e*) and also manifests a weaker reactivity with dermal fibrous tissue (data not shown). The antibody reacts with none of the epithelial cells in these sections, including epidermis, sweat gland, pancreatic acinar cells, or pancreatic duct cells (data not shown). Thus, this antibody is specific for mesenchymal tissue.

DISCUSSION

Common and Unique Epitopes in Intermediate Filament Proteins

Monoclonal antibodies to different intermediate filament proteins have been isolated and the antigens recognized by these antibodies identified. The reactivity of the antibodies with different intermediate filament proteins is summarized in Table III. Antibody 35βH11 recognizes only a 54-kdalton protein found in epithelial cells, whereas hybridoma 34βE12

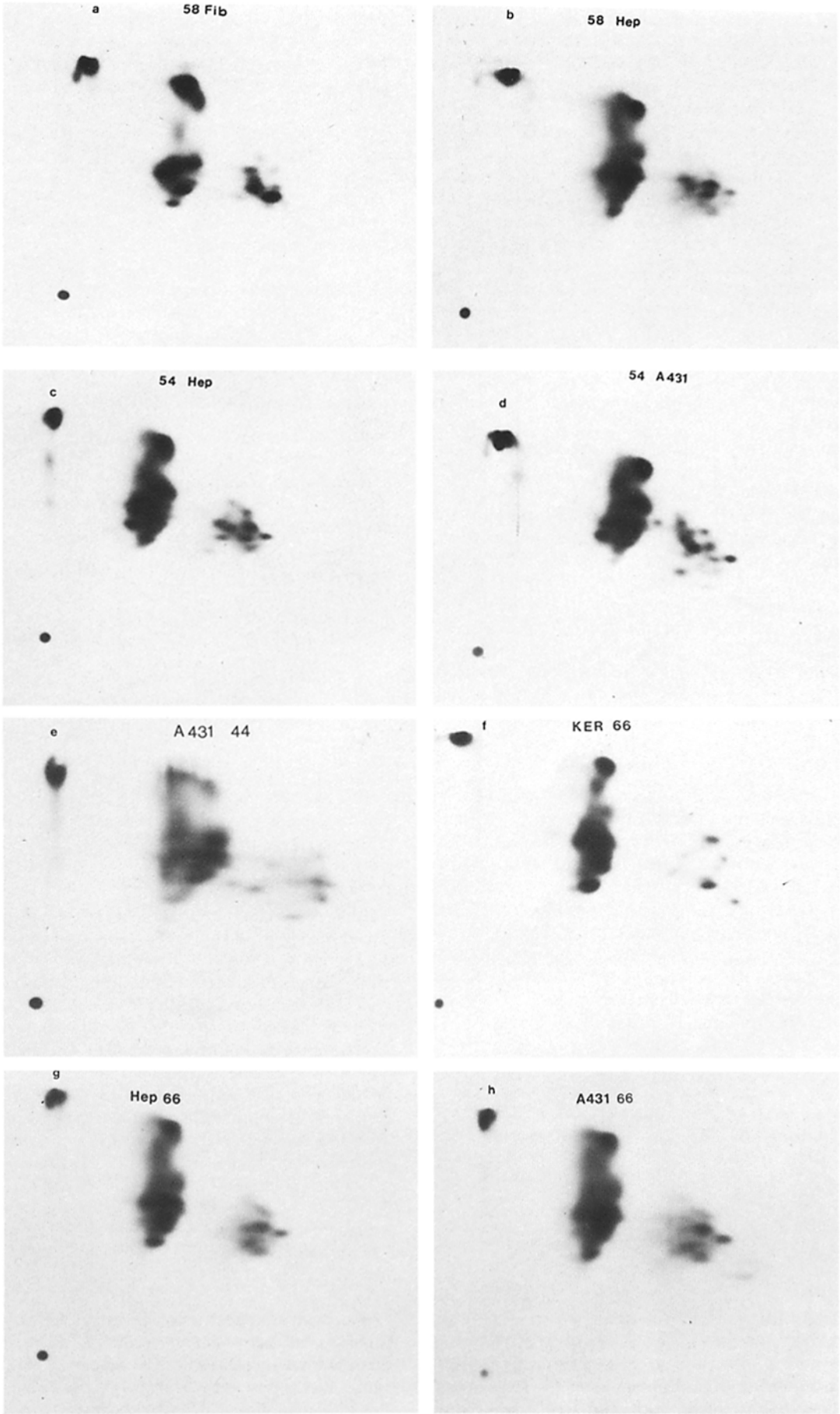

FIGURE 4 Two-dimensional peptide maps of intermediate filament proteins. ~2 × 10^5 cpm of ^{125}I-labeled peptides were applied to cellulose acetate plates. Electrophoresis is from left to right. Chromatography runs from bottom to top. (*a*) 58 *Fib*, fibroblast vimentin, 58 kdaltons; (*b*) *Hep3B*, 58 kdaltons (*c*) *Hep3B* , 54 kdaltons; (*d*) *A431*, 54 kdaltons; (*e*) *A431*, 44 kdaltons; (*f*) keratin, stratum corneum, 66 kdaltons; (*g*) *Hep3B*, 66 kdaltons; (*h*) *A431*, 66 kdaltons.

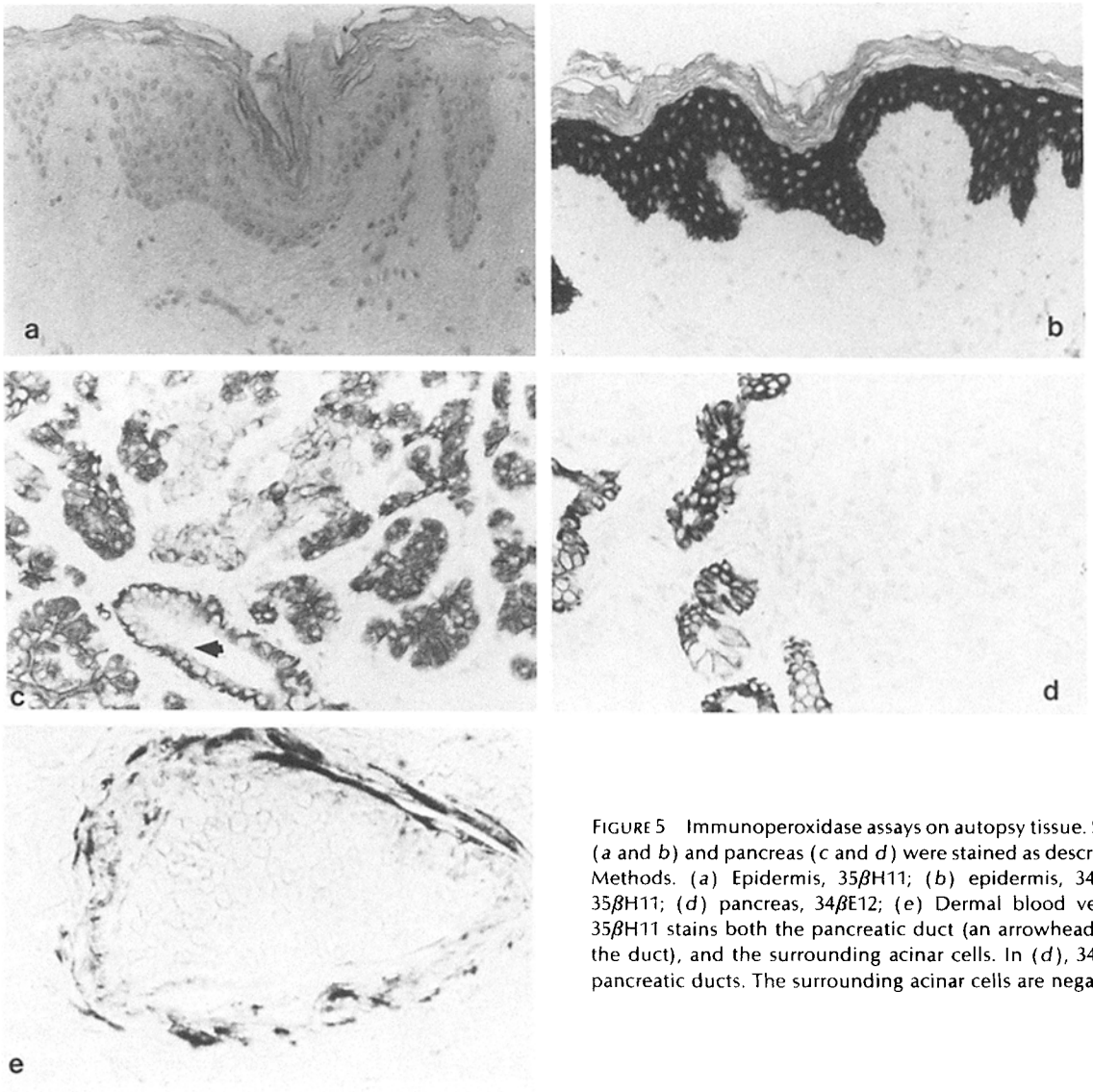

FIGURE 5 Immunoperoxidase assays on autopsy tissue. Sections of epidermis (*a* and *b*) and pancreas (*c* and *d*) were stained as described in Materials and Methods. (*a*) Epidermis, 35βH11; (*b*) epidermis, 34βE12; (*c*) pancreas, 35βH11; (*d*) pancreas, 34βE12; (e) Dermal blood vessel, 17βG3. In (*c*), 35βH11 stains both the pancreatic duct (an arrowhead marks the lumen of the duct), and the surrounding acinar cells. In (*d*), 34βE12 stains only the pancreatic ducts. The surrounding acinar cells are negative. × 200.

TABLE III

Reactivity with Monoclonal Antibodies with Different Intermediate Filament Proteins by Immunoblot Methods

	Reactivity with							
			Keratin from stratum corneum					
Antibody	Vimentin (58 kd)	54 kdalton protein (Hepatoma cells)	66	57	51	49	GFAP	Neurofilament proteins
17βG3	+	−	+	+	±	±	+	−
35βH11	−	+	−	−	−	−	−	−
35βD12	+	+*	+	+	+	+	+	−
34βE12	−	−	+	+	±	±	−	−

* Only in A431.

Summary of immunoblot results.

reacts with the high molecular weight keratins in stratum corneum. Thus these antibodies are highly specific. The anti-vimentin antibody 17βG3 crossreacts with GFAP and the high molecular weight keratins from stratum corneum. Hybridoma 35βD12 is relatively nonspecific in that it binds to vimentin, GFAP, the 54-kdalton protein of epithelial cells, and all the proteins from stratum corneum. The different patterns of cross-reactivity suggest that vimentin, the 54-kdalton protein of Hep3B and A431, GFAP, and multiple proteins in the keratin family share common epitopes. Hybridoma 35βD12 best illustrates this point because it recognizes all of these molecules. The only filament proteins that do not react with these antibodies are the neurofilament peptides. The lack of reactivity with the neurofilament peptides is not due to the inability of the high molecular weight proteins to transfer onto nitrocellulose paper, because the 200-kdalton and 160-kdalton proteins are visible on amido black-stained strips of nitrocellulose, and monoclonal antibodies made to the 200-kdalton protein recognize this protein on "western blots" (data not shown). It is likely that the neurofilament proteins also share antigenic determinants with other intermediate filament proteins because a monoclonal antibody generated against GFAP from spinal cord recognizes all the other intermediate filament peptides (19).

One could argue that the intermediate filament proteins of various molecular weights identified by these antibodies are not truly different, but instead represent different proteolytic fragments of the same molecule (31). This explanation is ruled out by the two-dimensional peptide maps that clearly demonstrate that many of these molecules are unique proteins. The

vimentin map is distinct from that of the 58-kdalton protein from Hep3B, the 54-kdalton epithelial proteins, and the 66-kdalton keratin peptide. This latter molecule and the 54-kdalton epithelial proteins also differ. In addition, the 52-kdalton protein in the neurofilament preparation and fibroblast vimentin probably differ because a polyclonal anti-GFAP sera recognizes this protein and not vimentin (data not shown).

Antikeratin Antibody 34βE12

Hybridoma 34βE12, which recognizes proteins of 66 kdaltons and 57 kdaltons in stratum corneum, yields unexpected results. In immunofluorescence assays performed on a variety of tissue culture cell lines, the antibody decorates only the intermediate filaments of epidermoid carcinoma cells. When assayed on Carnoy's fixed tissue from autopsy and surgical specimens, the antibody decorates squamous epithelium and some, but not all, nonsquamous epithelium (Fig. 5 *b* and *d*). For example, pancreatic duct epithelium reacts with 34βE12, but pancreatic acinar cells do not. Similarly, the antibody recognizes bile ducts in liver but not hepatocytes (data not shown). In immunoblot experiments, the antibody recognizes proteins of 66 kdaltons and 57 kdaltons in all cells tested, including cultured cells negative in immunofluorescence assays and intact tissue negative in immunoperoxidase assays. A protein of 66 kdaltons is identified in immunoblots of spleen and lymph node, neither of which reacts with the antibody in immunoperoxidase assays (data not shown). Therefore, the antibody recognizes peptides of similar molecular weight in all cells examined by "western blots" yet reacts only with specific tissues in immunofluorescence or immunoperoxidase assays.

We do not understand the reason for this apparent discrepancy in reactivity. One could argue that the 66-kdalton proteins of positive and negative cells differ such that the antibody recognizes the native protein of only A431 cells, but recognizes the SDS denatured proteins of all cells. This explanation is unlikely because the peptide maps of the 66-kdalton proteins from A431 and Hep3B are identical (Fig. 4). However, there still may be subtle sequence differences between the two molecules that are not demonstrated by two-dimensional maps. Another explanation is that the 66-kdalton and 57-kdalton proteins are organized in filaments in only A431. These antigens may have a different intracellular location in the negative cells or may be localized in filaments such that the epitope is masked or blocked, thereby preventing antibody binding. A third possibility is that the positive cells may contain more of these proteins than the negative cells. However, we have not consistently seen quantitative differences between positive and negative cells. Finally, it is possible that the "blot" procedure does not identify the correct antigen, but instead shows a serendipitous reactivity with molecules unrelated to intermediate filaments in the cell lysates. Such a phenomenon was observed with monoclonal antibodies to the Thy-1 antigen that also cross-react with vimentin (32). This explanation seems unlikely for the following reasons: (*a*) Pruss et al. (19) have described a monoclonal antibody capable of recognizing all classes of intermediate filaments that also identifies a 66-kdalton protein in many cells, and (*b*) A 66-kdalton peptide is present in cytoskeletal preparations from all cells, including those that do not react with 34βE12 (Fig. 1).

The peptide map data also show that the 66-kdalton protein from stratum corneum is not identical to the 66-kdalton proteins of A431 or Hep3B (Fig. 4). Therefore these three molecules must contain a common epitope(s) recognized by antibody 34βE12.

The 54-kdalton Protein of Epithelial Cells In Vitro: Differentiation of "Cytokeratins" and Keratins from Stratum Corneum

Nonsquamous epithelial cells generally react with antibodies made against keratin from bovine hoof or human skin (5–8). The proteins that comprise the intermediate filaments of epithelial cells have therefore been termed "cytokeratins". Hybridoma 35βH11 demonstrates that a "cytokeratin" from hepatoma cells possesses a unique epitope not found in the family of keratins from stratum corneum. Similar observations have been made by Franke et al. (5), using polyclonal sera raised against bovine hoof keratin, and sera directed against individual intermediate filament proteins from mouse liver. Additionally, Lane (33) has made monoclonal antibodies to cytokeratin that recognize 45-kdalton proteins in PtK_1 cells and also stain nonsquamous epithelium.

35βH11 identifies a 54-kdalton protein in many tissue culture epithelial cell lines and decorates almost all nonsquamous epithelium in Carnoy's-fixed tissue (manuscript in preparation) but does not recognize proteins from stratum corneum and does not react with squamous epithelium (Fig. 5 *a*). Therefore, it appears that the 54-kdalton protein is a major component of the intermediate filaments of nonsquamous epithelium, and antibody 35βH11 can be used to distinguish squamous from nonsquamous epithelium. However, we cannot be certain of this latter statement because A431, a tissue culture line derived from a squamous carcinoma, contains a 54-kdalton protein recognized by 35βH11. It remains to be determined whether the presence of the 54-kdalton protein in A431 is the result of tissue culture, or whether the 54-kdalton protein is present in squamous carcinomas and not in normal squamous epithelium.

Peptides of Similar Molecular Weights in Different Cells

Some cells contain intermediate filament proteins of similar molecular weight that can be distinguished on the basis of reactivity with the different monoclonal antibodies. For example, a 54-kdalton peptide is present in both Hep3B and A431, but in immunoblot experiments antibody 35βD12 reacts with only the molecule from A431 cells. This result suggests that the epitope may be modified or that the arrangement or distribution of the epitope in these two molecules is different. Similarly, antibody 17βG3 does not bind to the 58-kdalton protein in Hep3B, suggesting that this protein and vimentin from human fibroblasts are not identical. The peptide maps demonstrate that these molecules do differ. The 54-kdalton proteins of A431 and Hep3B are not identical, and vimentin and the 58-kdalton protein of Hep3B are markedly different. These results show that much sequence heterogeneity may exist within a specific class of intermediate filament protein, even among those of similar molecular weight.

Clinical Application of these Antibodies

One objective of this project was to generate antibodies specific for different cell types. For tissue culture cells, this seems to have been accomplished. Antibody 17βG3 recognizes vimentin, a mesenchymal cell marker (20, 21). Reaction of 17βG3 with cultured epithelial cells results from the presence of vimentin in these cells (data not shown) (2). Although capable of reacting with keratin in immunoblot experiments, the antibody does not recognize epidermoid carcinoma cells (A431) and does not decorate epithelial cells that normally do

not contain vimentin (Fig. 5). The antihepatoma filament antibody 35βH11 recognizes a 54-kdalton protein present in nonsquamous epithelial cells. The epithelial cells listed in Table II that do not react with this antibody (bladder, fetal intestine) do not contain any immunologically detectable 54-kdalton protein. This antibody also reacts with nonsquamous epithelium in intact tissue (Fig. 5). The antikeratin antibody 34βE12 recognizes squamous epithelium and some, but not all, nonsquamous epithelium. Antibodies 35βH11 and 34βE12 identify two unique cytokeratin epitopes expressed in different epithelial cells and therefore may be useful in distinguishing different carcinomas (neoplasms of epithelial origin). We are beginning to use these antibodies clinically to determine the cellular origin of poorly differentiated neoplasms.

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REFERENCES

1. Lazarides, E. 1980. Intermediate filaments as mechanical integrators of cellular space. *Nature (Lond.).* 283:249–256.
2. Franke, W. W., E. Schmid, S. Winter, M. Osborn, and K. Weber. 1979. Wide-spread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Exp. Cell. Res.* 123:25–46.
3. Hynes, R., and A. Destree. 1978. 10 nm filaments in normal and transformed cells. *Cell.* 13:151–163.
4. Starger, J., and R. D. Goldman. 1977. Isolation and preliminary characterization of 10 nm filaments from baby hamster kidney (BHK-21) cells. *Proc. Natl. Acad. Sci. U. S. A.* 74:2422–2426.
5. Franke, W., H. Denk, R. Kalt, and E. Schmid. 1981. Biochemical and immunological identification of cytokeratin proteins present in hepatocytes of mammalian liver tissue. *Exp. Cell Res.* 131:299–318.
6. Franke, W. W., K. Weber, M. Osborn, E. Schmid, and C. Freudenstein. 1978. Antibody to prekeratin: decoration of tonofilament-like arrays in various cells of epithelial character. *Exp. Cell Res.* 116: 429–445.
7. Schlegel, R., S. Banks-Schlegel, and G. S. Pinkus. 1980. Immunohistochemical localization of keratin in normal human tissues. *Lab. Invest.* 42:91–96.
8. Sun, T.-T., C. Shih, and H. Green. 1979. Keratin cytoskeletons in epithelial cells of internal organs. *Proc. Natl. Acad. Sci. U. S. A.* 76:2813–2817.
9. Gard, D. L., P. B. Bell, and E. Lazarides. 1979. Coexistence of desmin and the fibroblastic intermediate filament subunit in muscle and nonmuscle cells: identification and comparative peptide analysis. *Proc. Natl. Acad. Sci. U. S. A.* 76:3894–3898.
10. Tuszynski, G., E. Frank, C. Damsky, C. Buck, and L. Warren. 1979. The detection of smooth muscle desmin-like protein in BHK21/C_{13} fibroblasts. *J. Biol. Chem.* 254:6138–6143.
11. Dahl, D., and A. Bignami. 1973. Immunochemical and immunofluorescence studies of the glial fibrillary acidic protein in vertebrates. *Brain Res.* 61:279–293.
12. Eng, L., and J. Kosek. 1974. Electron microscopic localization of the glial fibrillary acidic protein and S-100 protein by immunoenzymatic techniques. *Trans. Am. Soc. Neurochem.* 5:160.
13. Goldman, J. E., H. H. Schaumberg, and W. T. Norton. 1978. Isolation and characterization of glial filaments from human brain. *J. Cell Biol.* 78:426–440.
14. Schachner, M., E. T. Hedlye-Whyte, D. Hsu, G. Schoonmaker, and A. Bignami. 1977. Ultrastructural localization of glial fibrillary acidic protein in mouse cerebellum by immunoperoxidase labeling. *J. Cell Biol.* 75:67–73.
15. Yen, S.-H., and K. L. Fields. 1981. Antibodies to neurofilament, glial filament, and fibroblast intermediate filament proteins bind to different cell types of the nervous system. *J. Cell Biol.* 88:115–126.
16. Liem, R. K. H., S. H. Yen, G. D. Salomon, and M. L. Shelanski. 1978. Intermediate filaments in nervous tissue. *J. Cell Biol.* 79:637–645.
17. Geissler, N., and K. Weber. 1981. Comparison of the proteins of two immunologically distinct intermediate sized filaments by amino acid sequence analysis: desmin and vimentin. *Proc. Natl. Acad. Sci. U. S. A.* 78:4120–4123.
18. Steinert, P., W. Idler, and R. Goldman. 1980. Intermediate filaments of baby hamster kidney (BHK-21) cells and bovine epidermal keratinocytes have similar ultrastructures and subunit domain structures. *Proc. Natl. Acad. Sci. U. S. A.* 77:4534–4538.
19. Pruss, R. M., R. Mirsky, M. Raff, B. Thorpe, A. Dowding, and B. Anderson. 1981. All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. *Cell.* 27:419–428.
20. Altmannsberger, M., M. Osborn, A. Schauer, and K. Weber. 1981. Antibodies to different intermediate filament proteins: cell type specific markers on paraffin-embedded human tissue. *Lab. Invest.* 45:427–434.
21. Gabbiani, G., Y. Kapanci, P. Barazzone, and W. Franke. 1981. Immunochemical identification of intermediate sized filaments in human neoplastic cells. *Am. J. Pathol.* 104:206–216.
22. Franke, W. W., E. Schmid, K. Weber, and M. Osborn. 1979. HeLa cells contain intermediate-sized filaments of the prekeratin type. *Exp. Cell. Res.* 118:95–109.
23. Sun, J. J., and H. Green. 1978. Keratin filaments of cultured human epidermal cells. Formation of intermolecular disulfide bonds during terminal differentiation. *J. Biol. Chem.* 253:2053–2060.
24. Köhler, G., and C. Milstein. 1976. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6:511–519.
25. Nowinski, R. C., M. E. Lostrom, M. R. Tam, M. R. Stone, and W. N. Burnette. 1979. The isolation of hybrid cell lines producing monoclonal antibodies against the p15(E) protein of murine leukemia viruses. *Virology.* 93:111–126.
26. Laemmli, U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
27. Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195–203.
28. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U. S. A.* 76:4350–4354.
29. Chiu, F. T., B. Korey, and W. Norton. 1980. Intermediate filaments from bovine, rat and human CNS: mapping analysis of the major proteins. *J. Neurochem.* 34:1149–1159.
30. Sage, H., Pritzl, P., and Bornstein, P. 1981. A new mapping technique for collagen chains. *Collagen Research.* 1:3–15.
31. Nelson, W., and Traub, P. 1981. Properties of a Ca^{2++}-activated protease specific for the intermediate-sized filament protein vimentin in Ehrlich-Ascites-tumor cells. *Eur. J. Biochem.* 116:51–57.
32. Dulbecco, R., M. Unger, M. Bologna, H. Battifora, P. Syka, and S. Okada. 1981. Cross reactivity between Thy-1 and a component of intermediate filaments demonstrated using a monoclonal antibody. *Nature (Lond.).* 292:772–774.
33. Lane, E. B. 1982. Monoclonal antibodies provide specific intramolecular markers for the study of epithelial tonofilament organization. *J. Cell Biol.* 92:665–673.