Monoclonal Antibody to Keratin Filaments, Specific for Glandular Epithelia and Their Tumors
Use in Surgical Pathology

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A monoclonal antibody (RGE 53) against keratin intermediate filaments was prepared by fusing myeloma cells with splenic lymphocytes from mice immunized with a HeLa cell cytoskeleton preparation and human callus keratins. The antibody, selected for fibrillar staining in HeLa cells and a negative reaction on human skin, was tested on frozen sections from normal and malignant human tissues. RGE 53 specifically recognizes glandular epithelial cells from female breast, digestive, respiratory, and urogenital tracts, endocrine and exocrine tissues, and mesothelial cells. No reaction is found in stratified squamous epithelia or nonepithelial tissues. Furthermore, RGE 53 can distinguish adenocarcinomas and mesotheliomas, which stain positively in the indirect immunofluorescence technique from squamous cell carcinomas and nonepithelial tumors, which are negative for this antibody.

RGE 53 may, therefore, be a useful tool for differential diagnosis in surgical pathology, especially in those cases in which anaplastic carcinomas have to be characterized.

Additional key words: Vimentin, Adenocarcinoma, Metastasis.

Intermediate-sized filaments (IF) form a substantial part of the cytoskeleton in eukaryotic cells. To a certain extent the proteins that make up the 10-nm filaments are tissue specific (4, 22). In general, epithelial cells contain IF of the keratin type (38), mesenchymal cells contain vimentin (25), IF in muscle are of the desmin type (23), and nervous tissues contain neurofilaments or glial filaments (39). Conventional antibodies directed against the main protein constituents of IF are, therefore, being used in tissue recognition (13, 25, 34) and differential diagnosis in surgical pathology (1–3, 5, 7, 17, 20, 24, 25, 27–33, 35). Carcinomas, sarcomas and lymphomas, rhabdomyosarcomas, and nervous tissue tumors can be distinguished by using specific polyclonal antisera directed against the individual IF proteins or groups of IF proteins as is often the case for the keratins, the proteins specific for epithelial cells and carcinomas. Although the different types of antisera directed against keratins described in the literature (2, 10, 11, 14, 20, 24, 27–29, 30, 31, 36) vary in their reaction with different types of normal and malignant epithelial cells, these conventional sera do not provide tools to further distinguish between the different types of carcinomas. The technique devised by Köhler and Milstein (19) of fusing spleen lymphocytes with a myeloma cell line allows the production of highly specific and homogeneous monoclonal antibodies. These antibodies may increase resolution in immunohistochemical studies of tissue recognition and differential tumor diagnosis. Especially in the case of keratin IF tissue specific antibodies may be prepared, since several reports (6, 10, 12, 13, 16) have shown that tonofilament constituents make up a heterogeneous population of proteins, varying with species and tissue. The present report describes the production and characterization of a monoclonal antibody to HeLa-keratin-filaments (14), which specifically reacts with human glandular epithelia and their malignant derivatives, the adenocarcinomas. It does not react with stratified squamous epithelia or nonepithelial tissues and tumors.

MATERIALS AND METHODS

TISSUES

Fresh tissue was frozen in liquid nitrogen immediately after arrival at the pathology department. Frozen sections (4 to 7 µm thick) were air dried for some hours and fixed in methanol (−20°C, 10 minutes) and thereafter in acetone (−20°C, 5 minutes). These fixed sections were
stored for periods ranging from several hours to several weeks at \(-20^\circ\) C. No significant differences between sections stored for shorter or longer periods were observed after staining by the indirect immunofluorescence technique done as described before (27).

**ANTIBOIES**

Preparation and specificity testing of rabbit antisera to human callus keratins and calf lens vimentin have been described before (27–32).

The monoclonal antibody used in this study (RGE 53) was prepared essentially as follows: BALB/c mice were immunized intraperitoneally with approximately 50 µg of HeLa cell cytoskeleton, prepared by extracting a cell pellet with Triton X-100 and 1.5 M KCl, and 100 µg of human callus keratins. After 2 weeks they were boosted twice (intravenously, 2 and 4 days prior to the cell fusion). Splenic lymphocytes were isolated and fused with mouse myeloma Sp 2/0 Ag 14 cells in polyethylene glycol-4000 and hybrids grown in 24-well clusters in RPMI 1640 (Dutch modification) containing 15% fetal calf serum. The cells were incubated 24 hours before adding hypoxanthine, aminopterin, and thymidine to the medium, and hybridoma cultures were tested for antibody production 2 weeks later. Cultures showing fibrillar staining when tested on HeLa cell monolayers and a negative reaction on human skin epidermis by the indirect immunofluorescence technique were cloned, tested again for fibrillar staining in HeLa cells, subcloned, and grown according to standard techniques (9). Undiluted culture medium or 1:500 times diluted ascitic fluid from BALB/c mice injected with RGE 53 hybrids were used for tests on frozen sections of human tissues by the indirect immunofluorescence technique. Fluorescein isothiocyanate-conjugated rabbit-antimouse IgG (Nordic, The Netherlands) was used as a secondary antibody. As a control, growth medium from nonproducing hybridomas or hybridomas producing antibodies specific for human epidermis were used.

RGE 53 has been tested for its reaction on cultured cells and in the immunoblotting assay. These experiments have shown that RGE 53 antibodies produce fibrillar staining patterns in cultured HeLa cells, in cultured human hepatocellular carcinoma cells (PLC/PRF/5), F9 mouse teratocarcinoma cells induced to differentiate by the addition of retinoic acid, and in human colon carcinoma cell line (WiDr-218). The fluorescence obtained in these cells demonstrates typical IF cytoskeleton organizations, virtually identical with the filament patterns visualized by the rabbit antisera to human skin keratin (see for example Fig. 1e and f). No reaction was seen in cultured bovine lens cells.

Furthermore, immunoblotting has shown that RGE 53 antibodies interact with a cytoskeleton-associated polypeptide with a molecular weight of approximately 45,000 in both HeLa cells and cultured human hepatoma cells (PLC/PRF/5). This polypeptide corresponds to the cytotkeratin D (no. 18) band typical for these cell lines (13).

RGE 53 is an IgG1 with κ light chains.

**RESULTS**

Table 1 is a list of the human tissues examined by indirect immunofluorescence in this study with antibodies to human callus keratins and calf lens vimentin, in addition to RGE 53. As far as normal human tissues are concerned, it is obvious from the data presented in this table that RGE 53 reacts with glandular epithelia of the gastrointestinal tract, the genital system, and columnar and glandular cells of the respiratory tract and exocrine and endocrine glandular tissues. These cells are also positive with the polyclonal serum to keratin but not with the vimentin antisemur. No significant reaction could be observed with RGE 53 in nonepithelial tissues (some of which are, however, vimentin positive) or in stratified squamous epithelia, either keratinizing or non-keratinizing. These latter epithelial tissues did, however, show a strong reaction with the polyclonal antisemur to keratin but, again, no reaction with the vimentin antibodies. Figure 1 depicts some typical examples of normal human tissues incubated with the glandular epithelium-specific antibody. Strikingly, the basal and intermediate urothelial layers do not stain with RGE 53, whereas the cells of the superficial layer, the so-called umbrella cells, do give a strong positive reaction with the monoclonal antibody (Fig. 1d). Furthermore, myoepithelial cells seem to be negative for RGE 53. We have also noted that within one type of epithelial tissue some cells may be negative, whereas the rest are positive. These negative cells do, however, react with the polyclonal antibody to keratin. Therefore, detailed studies on several tissue types are in progress.

Figure 1e and f show the fibrillar staining patterns that are obtained when cultured epithelial cells are incubated with RGE 53.

The differential findings with the normal human tissues were extended to their malignant derivatives. As can be seen from Table 1, the RGE 53 antibody distinguishes between glandular epithelial tumors (adenocarcinomas), which are strongly positive by indirect immunofluorescence, and tumors derived from stratified epithelia (squamous cell carcinomas), which are completely negative. All nonepithelial tumors examined so far were also negative for RGE 53.

Metastatic adenocarcinomas also stain strongly positively with this antibody. The anaplastic carcinoma metastases described in Table 1 seem, therefore, to be of glandular origin. Similar to the reaction with the polyclonal keratin antisemur, RGE 53 also gives a very strong reaction in mesotheliomas. Mesothelial cells in ascitic and pleural effusions were also strongly stained with the monoclonal antibody. In these effusions we were, therefore, with these antibodies not able to distinguish between metastatic adenocarcinoma cells and mesothelial cells.

All epithelial tumors were positive with the polyclonal keratin antisemur, whereas mesenchymal tumors stain with antibodies to vimentin. Also, the stromal fibroblasts accompanying the tumors as well as lymph node tissue stain strongly for vimentin. Figure 2 shows a selection of staining patterns observed in human carcinomas with RGE 53.

**DISCUSSION**

This study describes a monoclonal antibody to HeLa keratin filaments (RGE 53) that specifically recognizes human glandular epithelia, some urothelial cells, and
mesothelia by indirect immunofluorescence on frozen sections and distinguishes them from stratified squamous epithelia and nonepithelial cells.

Several arguments support the interpretation that RGE 53 is directed against keratin (also designated pre-keratin or cytokeratin). First, mice were immunized with a keratin-containing cytoskeletal preparation from HeLa cells and human skin keratins. Then, RGE 53 was selected specifically for filamentous staining in HeLa cells. Moreover, as shown in Figure 1, the antibody stains filamentous networks in cultured hepatocellular carcinoma cells and colon carcinoma cells, as well as in differentiated mouse teratocarcinoma cells. These patterns are virtually identical when the cells are stained by the conventional keratin sera but not when stained by the antiserum to vimentin. On frozen sections, RGE 53 stains exclusively certain types of epithelial tissues but not nonepithelial tissues. By the immunoblotting technique the antibody reacts strongly with a polypeptide band in the 45,000 molecular weight region in cytoskeletal preparations from HeLa cells and human hepatocellular carcinoma cells. This band corresponds to the low molecular weight acidic keratin polypeptide described by Lane (21) and Franke et al. (10–13) to occur in simple epithelia (keratin D, no. 18). On cytoskeletal preparations from bovine eye lens and human uterine

FIG. 1. Immunofluorescence of human tissues and cell cultures incubated with the monoclonal antibody to HeLa-keratin-filaments, specific for glandular epithelia (RGE 53). a, Endocervical glandular epithelium; b, ectocervical stratified squamous epithelium; c, liver hepatocytes; d, urinary bladder epithelium. Note the positive reaction in superficial cells (umbrella cells) and negative reaction in intermediate and basal cells; e, cultured human hepatocellular carcinoma cells (PLC/PRF/5) showing a typical pattern of keratin filaments when incubated with RGE 53; f, cultured human colon adenocarcinoma cells (WIDR-218) showing a fibrillar staining pattern. Figure 1a, ×600; b and f, ×500; c and d, ×250; e, ×550.
<table>
<thead>
<tr>
<th>Localization/diagnosis</th>
<th>RGE 53</th>
<th>Anti keratin</th>
<th>Anti vimentin</th>
<th>Localization/diagnosis</th>
<th>RGE 53</th>
<th>Anti keratin</th>
<th>Anti vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratified squamous epithelium of the skin (3×)</td>
<td></td>
<td>+</td>
<td>-</td>
<td>Moderately well-differentiated squamous cell carcinoma of the esophagus (2×)</td>
<td></td>
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<tr>
<td>Sebaceous glands and hair follicles in the skin (2×)</td>
<td></td>
<td>-</td>
<td>+</td>
<td>Poorly differentiated squamous cell carcinoma in esophagus and trachea</td>
<td></td>
<td>-</td>
<td>+</td>
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<tr>
<td>Ductal portion of sweat glands</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Metastasis of a poorly differentiated squamous cell carcinoma in the mandible</td>
<td></td>
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<td>-</td>
</tr>
<tr>
<td>Sweat gland acini (3×)</td>
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<td>+</td>
<td>-</td>
<td>Scirrhous carcinoma of the stomach</td>
<td>+</td>
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<tr>
<td>Moderately well-differentiated keratinizing squamous cell carcinoma of the penis</td>
<td></td>
<td>-</td>
<td>+</td>
<td>Anaplastic carcinoma of the stomach</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Squamous cell carcinoma of the penis with focal keratinization</td>
<td></td>
<td>-</td>
<td>+</td>
<td>Metastasis of an anaplastic gastric carcinoma in the omentum</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Well-differentiated keratinizing squamous cell carcinoma of the vulva (2×)</td>
<td></td>
<td>-</td>
<td>+</td>
<td>Lymph node metastasis of a poorly differentiated gastric adenocarcinoma</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Poorly differentiated non-keratinizing squamous cell carcinoma of the vulva</td>
<td></td>
<td>-</td>
<td>+</td>
<td>Well-differentiated adenocarcinoma of the colon</td>
<td>+</td>
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<tr>
<td>Melanotic melanoma</td>
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<td>-</td>
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<td>Moderately well-differentiated adenocarcinoma of the colon (5×)</td>
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<td>+</td>
</tr>
<tr>
<td>Amelanotic melanoma</td>
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<td>-</td>
<td>+</td>
<td>Metastasis of a colon carcinoma in the omentum</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>Digestive tract</td>
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<td>+</td>
<td>+</td>
<td>Moderately well-differentiated adenocarcinoma of the colon</td>
<td>+</td>
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<tr>
<td>Acini in the parotid gland</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Metastasis of an adenoid cystic carcinoma in the liver</td>
<td>+</td>
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<tr>
<td>Stratified squamous epithelium of the esophagus</td>
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<td>-</td>
<td>+</td>
<td>Metastasis of a moderately well-differentiated adenocarcinoma in the liver</td>
<td>+</td>
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<tr>
<td>Mucoid cells of gastric glands</td>
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<td>Pancreatic duct carcinoma</td>
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<td>Surface epithelium and chief cells in gastric epithelium</td>
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<td>-</td>
<td>Metastases of pancreatic adenocarcinoma (urinary bladder, omen- tum, lymph node)</td>
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<td>Mucous cells in small intestine (2×)</td>
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<td>-</td>
<td>Kidney (collecting) tubular epithelium (5×)</td>
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<td>-</td>
<td>Glomeruli in the kidney (5×)</td>
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<td>Liver hepatocytes</td>
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<td>-</td>
<td>Parietal epithelium cells of glomerulus capsule (5×)</td>
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<td>-</td>
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<td>Intrahepatic bile duct epithelium</td>
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<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Exocrine acini in pancreas</td>
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<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Keratinizing squamous cell carcinomas of the oral cavity (2×)</td>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Poorly differentiated non-keratinizing squamous cell carcinomas of the tongue</td>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Well-differentiated partly keratinizing squamous cell carcinomas of the tongue</td>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Malignant fibrous histiocytoma in the tongue</td>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Pleomorphic adenoma (3×)</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*+, positive staining; -, negative staining; +/-, staining with varying, but mostly negative; numbers in parentheses, numbers of preparations examined.
<table>
<thead>
<tr>
<th>Localization/diagnosis</th>
<th>RGE 63</th>
<th>Anti keratin</th>
<th>Anti vimentin</th>
<th>Localization/diagnosis</th>
<th>RGE 63</th>
<th>Anti keratin</th>
<th>Anti vimentin</th>
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<tr>
<td>Superficial transitional epithelial cells of ureter and urinary bladder (umbrella cells)</td>
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<td>+</td>
<td>-</td>
<td>Lymph node metastasis of a poorly differentiated adenocarcinoma from the ovary</td>
<td>+</td>
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<tr>
<td>Intermediate and basal transitional epithelial cells of ureter and urinary bladder</td>
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<td>+</td>
<td>-</td>
<td>Serous papillary cystadenocarcinoma of the ovary (5×)</td>
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<td>Testicular seminiferous epithelium</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>Metastasis of moderately differentiated serous papillary cystadenocarcinoma in the omentum</td>
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<td>Sertoli cells in the testis</td>
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<td>+</td>
<td>Brenner tumor</td>
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<td>Glandular epithelium of the prostate</td>
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<td>+</td>
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<td>Squamous metaplasia of cervix uteri</td>
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<td>Pollicles in the ovary</td>
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<td>Malignant mesothelioma in the omentum</td>
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<td>Mesothelial cells lining the ovary</td>
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<td>-</td>
<td>Breast</td>
<td>Lobuli in breast (3×)</td>
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<tr>
<td>Endometrial epithelium (5×)</td>
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<td>-</td>
<td>Ductal cells in breast</td>
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<td>Endocervical glandular epithelium (10×)</td>
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<td>-</td>
<td>Myoepithelial cells</td>
<td>-</td>
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<td>-</td>
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<td>Ectocervical stratified epithelium (5×)</td>
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<td>-</td>
<td>+</td>
<td>Lobular breast carcinoma</td>
<td>+</td>
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<td>-</td>
<td>Intraductal carcinoma (2×)</td>
<td>+/-</td>
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<tr>
<td>Transitional cell carcinoma (7×) (some cells +)</td>
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<td>+</td>
<td>-</td>
<td>Well-differentiated invasive ductal carcinoma</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lymph node metastasis of an anaplastic/poorly differentiated transitional cell carcinoma</td>
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<td>-</td>
<td>Moderately well-differentiated invasive ductal carcinoma (7×)</td>
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<tr>
<td>Metastasis of an adenocarcinoma in the bladder</td>
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<td>+</td>
<td>-</td>
<td>Poorly differentiated invasive ductal carcinoma (2×)</td>
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<tr>
<td>Anaplastic seminoma</td>
<td>-</td>
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<td>+</td>
<td>Invasive lobular carcinoma (2×)</td>
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<td>Embryonal cell carcinoma in the testis</td>
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<td>-</td>
<td>Small cell invasive lobular carcinoma</td>
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<td>Metastasis of an embryonal cell carcinoma in the omentum</td>
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<td>+</td>
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<td>Combined tumor of invasive ductal carcinoma and colloid carcinoma</td>
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<td>Metastasis of the yolk sac component of a teratocarcinoma in the liver</td>
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<td>+</td>
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<td>Lymph node metastasis of a poorly differentiated duct carcinoma of breast (2×)</td>
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<td>Well-differentiated metastatic adenocarcinoma in the scrotum (unknown primary)</td>
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<td>-</td>
<td>Lymph node metastasis of an invasive lobular breast carcinoma</td>
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<td>Poorly differentiated adenocarcinoma from prostate</td>
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<td>+</td>
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<td>Metastasis in the thorax of a poorly differentiated duct carcinoma of breast</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>Anaplastic carcinoma from prostate</td>
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<td>+/-</td>
<td>-</td>
<td>Respiratory tract: Glands of the subglottis</td>
<td>+</td>
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<tr>
<td>Lymph node metastasis of an adenocarcinoma from the prostate</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Ciliated columnar epithelium of the bronchus</td>
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<tr>
<td>Poorly differentiated adenocarcinoma of the ovary (2×)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Mixed glands in the wall of the bronchus</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>Pulmonary macrophages</td>
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<td>Capillary hemangiomata from subglottis</td>
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<td>Well-differentiated adenocarcinoma of the lung</td>
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<td>Moderately differentiated mucinous adenocarcinoma of the lung (2×)</td>
<td>+</td>
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</table>
smooth muscle, only a faint background reaction was seen in the immunoblotting assay. Finally, RGE 53 does not stain filamentous structures in cultured bovine lens cells, which have been described to contain exclusively IF of the vimentin type next to microfilaments and microtubules as their cytoskeletal components (26).

The monoclonal antibody described here seems to have some properties in common with the antibodies directed against PtkI keratin filaments as described by Lane (21) and those described recently by Debus, Weber, and Osborn (8). These monoclonal antibodies, when tested on rabbit and human tissues, respectively, only stained the "simple epithelia," including several columnar and glandular epithelial tissues, but did not react with stratified squamous epithelia. Strikingly, however, in SV 40-transformed cultured keratinocytes, tonofilaments were stained by the antibodies described by Lane (21; see also reference 37). It seems unlikely that the expression of simple epithelial antigenic determinants in keratinocytes is a result of carcinogenesis, since all squamous cell carcinomas examined so far (Table 1 and Fig. 2) seem not to express this antigenic determinant. It will, therefore, be important to study epithelial cell lines derived from skin, ectocervix, larynx, etc., with these antibodies to determine whether the expression of simple epithelial keratin is induced in these cells upon culturing. That expression of IF protein types can change upon culturing of cells has been described previously by several investi-
Fig. 2. Immunofluorographs of human carcinomas incubated with RGE 53. a, Moderately well-differentiated adenocarcinoma of the colon. Note normal glandular structures positive for RGE 53 (upper right corner); b, scirrrous carcinoma of the stomach; c, anaplastic carcinoma of the stomach; metastasis on the omentum; d, metastasis of a malignant mesothelioma on the omentum; e, well-differentiated invasive duct carcinoma from female breast; f, lobular breast carcinoma; g, poorly differentiated adenocarcinoma of the ovary, metastasis in a lymph node; h, squamous cell carcinoma of the penis. Figure 2a, ×60; 5, g, and h, ×300; c, d, and e, ×325; f, ×250.
gators (6, 11, 15, 16). Monoclonal antibodies like RGE 53 may, therefore, be useful in *in vitro* studies of differentiation and dedifferentiation of epithelial cells.

The data presented here for adenocarcinomas are to a certain extent at variance with data reported by Schiegel *et al.* (33). These investigators conclude, from studies on formalin-fixed, paraffin-embedded tumor tissues and using a conventional antibody to skin keratin, that several types of adenocarcinomas do not contain these tonofilament proteins. We have, however, consistently found positive results with all adenocarcinomas described when using either polyclonal or monoclonal keratin antibodies on frozen sections (31). It is, therefore, very likely that formalin fixation and/or paraffin embedding destroys or masks keratin antigenic determinants. Studies are in progress to determine whether or not the keratin sera described in our study can be used on routine paraffin sections when proteolytic predigestion of the substrate is introduced and/or sensitivity of the immunologic methods is increased. So far, with RGE 53 we have not been able to detect keratin in formalin-fixed, paraffin-embedded tissues.

The monoclonal antikeratin antibody described here can be helpful in differential diagnosis in surgical pathology when used on frozen sections. First, it can distinguish certain types of epithelial tumors from nonepithelial malignancies, for example, in cases in which differentiation between adenocarcinoma metastases and lymphomas is difficult or impossible. Although this can also be achieved by conventional polyclonal sera, monoclonal antibodies have the advantage of homogeneous and standardized quality in almost unlimited amounts. Furthermore, background staining as a result of minor amounts of autoimmune antibodies that may occur in conventional antisera is avoided.

Second, RGE 53 may prove to be an important aid in those cases for which the origin of a metastatic carcinoma is not clear, especially anaplastic tumors, since both treatment and prognosis are different for adenocarcinomas and squamous cell carcinomas.

Some recent publications (8, 18, 21, 30, 38) dealing with preparation and specificity testing of monoclonal antibodies to keratin (cytokeratin) have shown a rather complex pattern of reactivity of these sera, especially since in all of these studies the immunoblotting assay was applied. However, on the basis of their immunohistochemical reaction with tissue frozen sections, mainly four types of monoclonal antikeratin antibodies can be distinguished as follows: (a) broadly cross-reacting antibodies K08.13 (18) and AE1, and AE3 (38), which can be derived after immunization with epidermal keratins; (b) monoclonal antikeratin antibodies LE 61, LE 65 (21), CK4, CK6, and RGE 53, which will react only with simple (glandular) epithelia and are obtained after immunization with keratins from HEaLa or PtK1 cells; (c) monoclonal antibody AE3 (38), which recognizes keratin polypeptides in a restricted number of tissue types. It does, for example, not react with the keratinizing part of the skin but only stains the suprabasal cells of the epidermis; (d) recently, we have prepared the monoclonal antibody RK56 directed against human skin keratin which only reacts with the upper epidermis but not with the basal epidermal cell layer. This antibody stains specifically with keratinizing cells in squamous cell carcinomas (30). The underlying study is one of the first reports of the use of a monoclonal antikeratin antibody in human tumor pathology. Further studies must clarify whether or not other specific monoclonal antibodies to keratin(s) can be prepared and, if so, whether they can be used to increase specificity in surgical pathology diagnosis.

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