Secretory Component

A Glandular Epithelial Cell Marker

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Secretory component (SC) has been demonstrated to be produced by both normal and malignantly transformed glandular epithelial cells. By an indirect immunofluorescent technique, this study surveys tumors of varied cellular origin in order to determine the reliability of SC as a marker for tumor cells derived from glandular epithelium. Both primary and metastatic tumors of glandular epithelial origin demonstrated SC fluorescence, while nonglandular epithelial tumors did not. This observation was extended to live single-cell preparations, which demonstrated intense cell-surface fluorescence only when glandular epithelial tumors cells were examined. Additionally, fixed, cytocentrifuged, single-cell preparations of glandular epithelial tumors demonstrated cytoplasmic SC fluorescence. When breast carcinoma was examined, all cases demonstrated SC, regardless of the degree of differentiation. This assay appears to have useful clinical application in that the finding of SC provides indication of the glandular epithelial origin of a malignantly transformed cell. (Am J Pathol 1981, 105:47–53)

SECRETORY COMPONENT (SC) is a normal glandular epithelial cell product. This glycoprotein combines with dimeric IgA in association with J chain to form intact 11S secretory IgA molecules. Immunohistochemical studies have demonstrated SC in the normal glandular epithelial cells lining the body's mucosa.[1,2] Previously, we reported the demonstration of SC by immunofluorescence microscopy in primary and metastatic adenocarcinomas.[3] Additionally, we and others have demonstrated the endogenous production of SC by the incorporation of ^{14}C-labeled amino acids by these epithelial tumor cells and the subsequent appearance of ^{14}C-labeled SC.[4-9] It was, therefore, the purpose of this study to survey various tumors for the presence of secretory component and, by doing so, to substantiate the usefulness of SC as a reliable marker for the glandular epithelial origin of primary or metastatic tumor cells.

Methods

Tissue Specimens

Fresh and frozen tissue specimens were obtained on the basis of availability from surgical procedures and postmortem examinations from either the Department of Pathology, University of Pennsylvania (Philadelphia, Pa) or the Office of Program Resources and Logistics, National Cancer Institute (Bethesda, Md). The fresh tissues were placed in either cold 0.9% NaCl or complete RPMI (RPMI-1640 with 10% fetal calf serum, 200 U/ml penicillin, and 200 μg/ml streptomycin added [Grand Island Biological Company, Grand Island, NY]) and were processed immediately. Tissues received frozen were kept at −70 C until ready for study.

Frozen Sections

Fresh tissues were divided into small pieces (approximately 1 cm × 1 cm × 0.5 cm) and snap frozen at −20 C on a chuck in OCT (Ames Company, Elkhart, Ind) embedding medium. The tissues received already frozen were directly embedded on a chuck of OCT medium. Serial 4-μ frozen sections were made

on an International Harris Cryostat kept between −30 C and −20 C. These sections were then transferred to a warm slide and allowed to air dry for 10–20 minutes.

Fluorescent Antibody Staining

Air-dried 4-μ frozen sections were washed for 10 minutes with three changes of phosphate-buffered saline (PBS) (8.5 g NaCl, 1.07 g Na_2HPO_4, 0.35 g NaH_2PO_4 • H_2O in 100 ml of distilled water). The slides were fixed in acetone for 10 minutes, and then rehydrated in PBS for another 10 minutes prior to the application of antiserums. Incubation with the antiserums was carried out for 30 minutes in a humidifier chamber, and the slides were washed again for 10 minutes with three changes of PBS. Those slides examined with direct staining were mounted in Elvanol (Dupont Company, Wilmington, Del) and stored at 4 C until ready for viewing. For slides examined by indirect fluorescence microscopy, a second incubation with antiserum was then accomplished, with subsequent washing and mounting. Representative sections were placed in formalin for hematoxylin and eosin (H&E) staining.

Antiserums

Fluorescein-isothiocyanate(FITC)–conjugated goat antiserums specific for human alpha, gamma, and mu chains (Lot Nos. 40334, 09141, and 10941, respectively) were obtained from Meloy Laboratories (Springfield, Va) and tested for specificity in the following ways: 1) Immunoelectrophoresis was performed by reacting normal human serum against anti-normal human serum and the heavy-chain–specific antiserums. A single precipitin line elicited between each heavy-chain–specific antiserum and normal human serum was shown to correspond to the appropriate immunoglobulin precipitin line from the known immunoelectrophoresis pattern of normal human serum. 2) Preliminary fluorescent antibody studies made with these antiserums were performed on normal human breast and intestinal tissues, which demonstrated, as expected, a predominance of IgA-producing plasma cells and a few scattered IgG- and IgM-producing plasma cells.[10,11] Marked apical staining of glandular epithelial cells with anti-alpha and considerably less staining for anti-gamma and anti-mu was found, in addition to the cytoplasmic fluorescence of plasma cells. These constituted positive controls for the heavy-chain–specific antiserums used in this study and were employed periodically with each new batch of antiserum.

Rabbit anti-free SC, obtained from Behring Diag-

nostics (Somerville, NJ), was produced by a plasmin digestion of the isolated salivary sIgA molecule, resulting in the exposure of hidden SC determinants normally present only in the free SC molecule. Rabbits were then immunized, and the resultant antiserum was absorbed with the 11S sIgA molecule so that activity to only those SC determinants present in the free state remained. The antiserum was further purified in this laboratory by treatment with cold saturated ammonium sulfate, in order to obtain an immunoglobulin-enriched preparation. This antiserum was then tested for specificity by Ouchterlony analysis. When this antiserum was placed in a well opposite human milk or agammaglobulinemic saliva devoid of IgA, a single precipitin line resulted. When tested against normal human serum, no precipitin reaction occurred. This antiserum showed partial identity with the sIgA precipitin line when placed in a well beside anti-human colostrum and opposite a well containing human colostrum.

Rabbit anti-sIgA was kindly supplied by Dr. Richard Hong (University of Wisconsin, Madison, Wisconsin) and was prepared by isolating salivary sIgA, dialyzing this molecule against urea, pH 2.3, and then immunizing rabbits with this partially degraded molecule. The resulting antiserum was then absorbed, with an excess of normal human serum, until all reactivity to this serum was removed; however, a single precipitin line could still be demonstrated against agammaglobulinemic saliva. This antiserum was designated anti-SC; when used in the fluorescent antibody studies, it produced results identical with those obtained with the anti-free SC and provided a separate source of antibody to further substantiate our findings.

These antiserums to SC were counterstained with fluorescein-isothiocyanate–conjugated goat anti-rabbit immunoglobulins (FITC-GAR Igs), which was obtained from Behring Diagnostics. Nonimmunized rabbits were bled for a pool of normal rabbit serum, which was then treated with cold saturated ammonium sulfate to obtain an immunoglobulin-enriched preparation. This nonimmune rabbit serum was counterstained with FITC-GAR Igs as a control for rabbit anti-free SC. In addition, FITC-GAR Igs was used alone to rule out nonspecific tissue attraction. This antiserum also served as a control for the antiserums to alpha, gamma, and mu chains by direct immunofluorescence.

Fluorescence Microscopy and Photography

The microscope used was a Zeiss Universal with a Ploem-type vertical illuminator, combined with a KP500 exciter filter. Findings were recorded on Kodak Ektachrome film, ASA 400.

Fluorescent Antibody Staining

Fixed Cells

Single-cell preparations of fresh tumor tissues were adjusted to a concentration of 5×10^4 cells/0.5 ml RPMI-1640. The cells were then cytocentrifuged onto slides using a Cytospin (Shandon Elliott, Sewickley, Pa) at 700 rpm for 10 minutes and allowed to air dry for 10–20 minutes. The cells were then washed in PBS for 10 minutes and fixed in acetone for 10 minutes. They were then incubated with antiserums, by the same procedure described for frozen sections, and examined under a fluorescent microscope.

Live Cells

Single-cell suspensions were washed three times in 10% fetal calf serum in PBS (fetal calf serum [FCS]/PBS) in a refrigerated centrifuge (1200 rpm for 6 minutes). The cell suspension was then adjusted to 4×10^6 cells/ml of FCS/PBS and divided equally into test tubes. These test tubes were again centrifuged and aspirated until 0.1–0.2 ml of supernatant remained above the cell button. The cell button was then vortexed gently, and antiserums were added at the appropriate dilutions and at a concentration of 0.15 ml/2 × 10^6 cells. These cells were then incubated for 30–45 minutes at 4 C on a rotator and again washed three times in FCS/PBS. For those cells stained by an indirect immunofluorescent technique, a second incubation with an FITC-conjugated antiserum was performed, with subsequent washing and mounting as previously described for the direct immunofluorescence technique. These slides were kept at 4 C until viewed soon after completion.

Results

Immunofluorescence of Normal and Neoplastic Tissue

Normal Tissue

When normal mucosal tissues were examined for SC, only glandular epithelial cells were found to fluoresce. Lamina propria plasma cells underlying the mucosal epithelium were devoid of SC staining, as were the cellular elements comprising the connective tissue (Figure 1). In Table 1, the normal tissues that were examined in this manner are listed.

Neoplastic Tissues

Tumors received from the National Cancer Institute already frozen and stored at −70 C were found to be uniformly unsatisfactory when examined for SC by these methods. Therefore, the following results were obtained with fresh tissues.
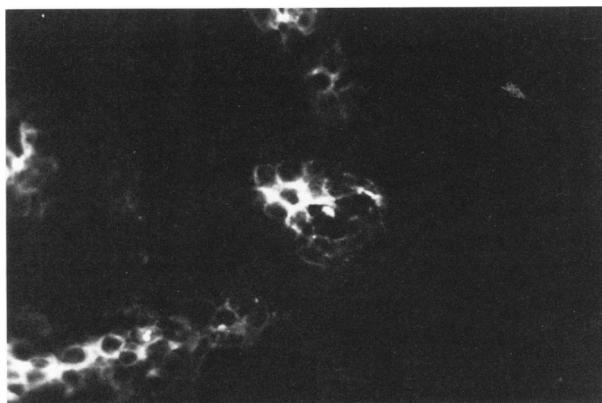

Figure 1—Normal breast tissue stained with anti-SC and FITC-GAR Igs. Notice that fluorescence is confined to the normal ductal epithelium.

When tumors of glandular epithelial origin were examined with antiserums to SC, all were found to have tumor cells with cytoplasmic fluorescence (Table 2). The largest single group of these tumors studied was breast carcinoma (n = 25), the histologic diagnoses of which included infiltrating ductal, medullary, colloid, and papillary carcinomas, which were well to poorly differentiated. In tumor specimens where normal ductal architecture remained, one could observe SC staining in the ductal epithelial cells, in addition to cytoplasmic fluorescence of tumor cells (Figure 2). Also noted in these areas of normal ducts were occasional subepithelial IgA plasma cells. In all but one breast tumor studied, there was no appreciable plasma cell infiltration. The one case of infiltrating papillary carcinoma had marked infiltration of IgA- and IgG-containing plasma cells.

In figure 3, representative sections from a case of infiltrating ductal carcinoma are presented. Figure 3A shows the fluorescent stain with anti-SC and Figure 3B shows the identical section restained with hematoxylin and eosin (H&E). The cytoplasmic SC fluorescence can easily be noticed by comparison with the H&E section. When serial sections of these

Table 1—Results of Immunofluorescence of Normal Tissue Stained with Antiserums to SC

Normal tissues	SC fluorescence
Breast ductal epithelium	Present
Colon epithelium	Present
Bronchial acinar cells	Present
Biliary ductal epithelium	Present
Kidney tubules	Present
Lymph node	Absent
Spleen	Absent
Bone	Absent
Cartilage	Absent
Thyroid	Absent
Skin (squamous epithelium)	Absent
Lamina propria plasma cells (gut)	Absent
Liver parenchyma	Absent

Table 2—Results of Immunofluorescence on Tumors of Epithelial Origin Stained with Antiserums to SC

Tissue samples	Number of samples	Number of samples showing tumor cells with cyto-plasmic fluores-cence for SC
Primary tumors		
Breast	25	25
Lung		
Adenocarcinoma	2	2
Squamous cell carcinoma	2	0
Colon	6	6
Bladder (transitional cell)	1	0
Renal	1	1
Ovarian	2	2
Basal cell of skin	1	0
Metastatic tumors		
Axillary lymph nodes (breast carcinoma)	3	3
Vertebral body (primary unknown)	1	1
Para-aortic lymph node (breast cancer)	1	1
Supraclavicular lymph node		
(adenocarcinoma of lung)	2	2
(squamous cell carcinoma of lung)	1	0
Subcutaneous thigh (adenocarcinoma—primary unknown)	1	1
Subcutaneous abdominal wall (adenocarcinoma of colon)	1	1

Figure 3—Identical sections of invasive breast carcinoma.   A—Section stained with hematoxylin and eosin.   B—Section stained with anti-SC and FITC-GAR Igs.

tissues were stained with antiserums to alpha, gamma, and mu heavy chains, no tumor cell fluorescence could be seen.

In addition to primary breast tumors, metastatic breast carcinoma was also examined (Table 2). Metastases to axillary lymph nodes (n = 3), vertebral body (n = 1), and para-aortic lymph node (n = 1) were studied and found to have tumor cells with cy-
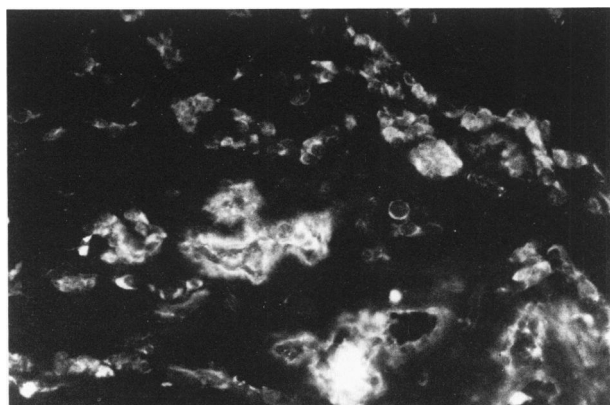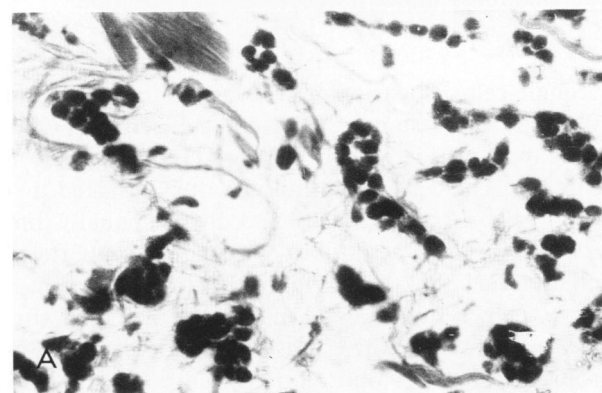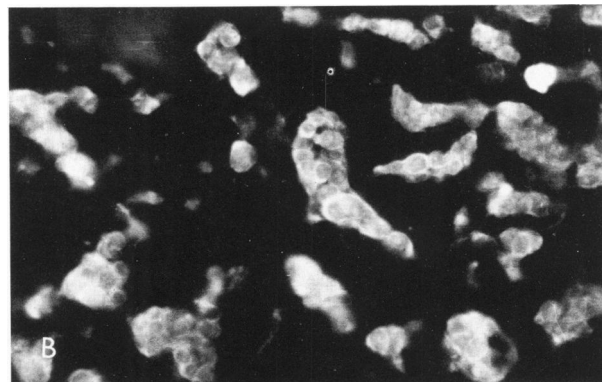

Figure 2—Section of invasive breast carcinoma stained with anti-SC and FITC-GAR Igs. Notice fluorescing tumor cells in addition to normal ductal epithelium.

toplasmic SC fluorescence. An example of metastatic lesion is presented in Figure 4. Here one can see nests of fluorescing tumor cells lodged in a vertebral body; these cells were believed to have originated from a poorly differentiated primary breast carcinoma. In the axillary lymph nodes with metastases, one could see SC staining of what appeared to be normal adjacent lymphocytes, in addition to the fluorescing tumor cells. The fluorescence seen on these lymphocytes was not cytoplasmic, as in the tumor cells, but instead outlined the cell (rim fluorescence). When 3 cases of uninvolved axillary lymph nodes draining primary breast carcinomas were examined, no SC fluorescence could be seen in any of the normal cellular elements in the lymph node.

As previously reported, approximately half of the cases of benign fibrocystic disease and the fibroadenomas demonstrated weakly positive fluorescence of the proliferative stromal cells, in addition to the strongly positive fluorescence of normal ductal epithelium when stained with anti-SC.[3]

Colon carcinoma provided the next most available tumor (n = 6). These tumors were taken from the rectum, recto-sigmoid, and sigmoid regions of the gut. In all cases, SC fluorescence was seen in tumor
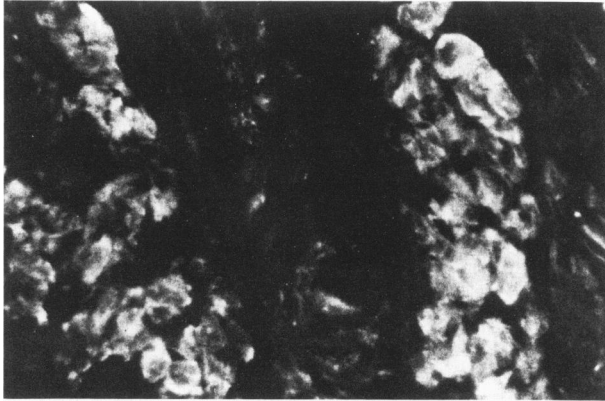
Figure 4—Adenocarcinoma metastatic to vertebral body stained with anti-SC and FITC-GAR Igs.

cells. One case of a colon carcinoma recurring in the subcutaneous tissues of the abdominal wall was also found to have SC fluorescence.

The results obtained from examining cancer of the lung were consistent with presumed cellular origin of the tumor. Adenocarcinoma of the lung (n = 2) showed fluorescence, while squamous cell carcinoma (n = 2) showed none. One case of anaplastic carcinoma of the lung, small cell type, also showed no SC fluorescence.

A case of renal carcinoma and two cases of ovarian carcinoma were also found to be positive for SC, in contrast to a case of transitional cell carcinoma of the bladder and a case of basal cell carcinoma of the skin, which showed no SC fluorescence.

In order to examine the specificity of SC as a marker for cells derived from glandular epithelium, tumors of nonepithelial origin were also studied and are listed in Table 3. No SC fluorescence could be seen in any of these tumor cells, except for one case of Wilms' tumor. This case was interesting in that there were areas of this tumor that histologically resembled a carcinoma, and, clinically, the patient was observed to have an elevated serum level of alpha feto-protein.

Tumors of mixed origin presented a unique opportunity to examine the localization of SC to different cell types in the same section. In a dermoid cyst and in a case of embryonal carcinoma studied with antiserums to SC, only the glandular epithelial cells within the tumor showed fluorescence.

Immunofluorescence of Single Cells

Fixed Cells

Single cell suspensions were cytocentrifuged onto slides, fixed in acetone, and examined for cytoplasmic SC fluorescence. One adenocarcinoma of the colon metastatic to the subcutaneous tissue of the abdomi-

nal wall, and one breast cancer metastatic to a para-aortic lymph node, demonstrated numerous tumor cells with cytoplasmic SC fluorescence. Slides stained with hematoxylin and eosin revealed a predominance of tumor cells in the field with an occasional monocyte and very few polymorphonuclear leucocytes. As controls, single-cell suspensions of tissue from a lymphoma, a squamous cell carcinoma, and a neuroblastoma were cytocentrifuged, and all failed to show fluorescence.

Live Cells

Suspensions of live tumor cells were examined by indirect immunofluorescence microscopy in order to determine if SC was also present on the membrane of tumor cells shown to have cytoplasmic SC. Two cases of adenocarcinoma metastatic to lymph nodes and one case of adenocarcinoma metastatic to subcutaneous tissue were made into single-cell suspensions, and these live cells were stained with anti-SC. More than 50% of the viable cells examined in these cases demonstrated surface SC fluorescence, characterized by a cell membrane that appeared brightly speckled (Figure 5). Cells that were nonviable could be distinguished by their inability to exclude the antiserums, which gave them a diffuse hazy-green appearance. The viability of these cells ranged considerably (20–60%), depending on the condition of the specimen. Normal peripheral blood lymphocytes separated by a Ficoll-Hypaque gradient were also examined for surface SC and approximately 2% of the cells demonstrated a speckled fluorescent pattern, which is consistent with the observed cross-reactivity of rabbit and goat antiserums with human lymphocytes.[12] In addition, tis-

Table 3—Results of Immunofluorescence on Tumors of Nonepithelial Origin Stained with Antisera to SC

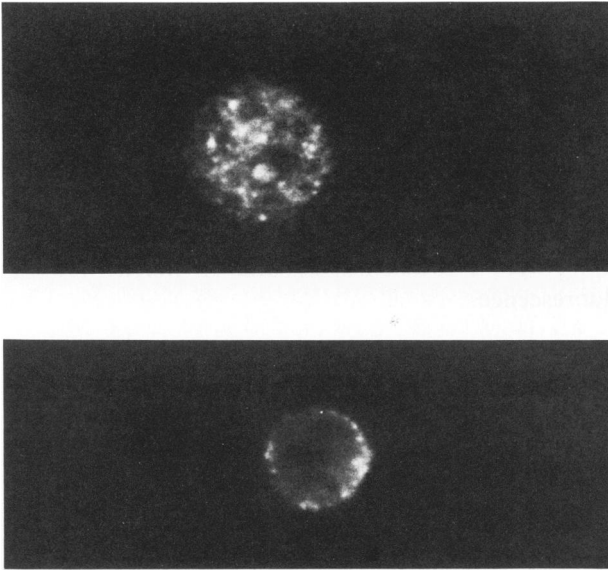| Histologic classifications	Number of samples	Number of samples showing tumor cells with cytoplasmic fluorescence for SC
Hodgkin's disease	3	0
Undifferentiated sarcoma	1	0
Neurofibrosarcoma	1	0
Astrocytoma	1	0
Histiocytoma	1	0
Waldenstrom's macroglobulinemia (lymph node)	1	0
Spindle cell tumor	1	0
Hepatoblastoma	1	0
Wilms' tumor	3	1
Neuroblastoma	1	0

Figure 5—Live metastatic tumor cells stained with anti-SC and FITC-GAR Igs. Notice speckled pattern of fluorescence.

sue from a lymphoma, a squamous cell carcinoma, and an anaplastic (small cell) carcinoma of the lung failed to show surface SC, which corresponded with their failure to show cytoplasmic SC by frozen section.

Discussion

The examination of many tumors of varied cellular origin has confirmed that the specificity that exists for SC in glandular epithelial cells also persists in cells of glandular epithelial origin that have undergone malignant transformation. This specificity is even more obvious in the results, which demonstrated that, while glandular epithelial tumors were SC positive, nonglandular epithelial tumors, such as squamous cell carcinoma of the lung, failed to show SC fluorescence. In addition, the examination of tumors of mixed origin provided an opportunity to confirm this specificity, since only glandular epithelial cells exhibited SC fluorescence, while cartilage, skin, and muscle elements in the same tissue section failed to do so.

Secretory component fluorescence in metastatic lesions served to indicate that the appearance of SC in these cells is not dependent on the specific microenvironment of the mucosa. The discovery of rim fluorescence on the lymph node cells surrounding a metastatic lesion seems best explained by passive adsorption of SC produced by the nearby tumor cells. Since the examination of noninvolved lymph nodes failed to demonstrate SC fluorescence, it appeared that drain-

age of an involved area was not sufficient to produce the fluorescent cellular pattern and that this required the presence of tumor cells in the section being examined.

There was an impressive lack of immunoglobulins associated with nearly all the tumors examined. The lack of IgA and IgM staining associated with SC positive tumor cells indicated that the SC seen in these cells was not part of intact sIgA or sIgM molecules that were either being stored in these cells or were part of a host response to the tumor. The demonstration of SC fluorescence in the cytoplasm of fixed, single tumor cells in suspension offered confirmatory evidence that SC fluorescence seen in the cytoplasm of tumor cells on frozen section was not artifactual. These results again confirmed the specificity of SC for glandular epithelial tumors, since those single-cell suspensions of nonglandular tumors were uniformly negative.

The possibility that tumor cells lose their capacity to produce SC with increasing degrees of anaplasia was also examined. The results of the study of poorly differentiated breast carcinomas and one poorly differentiated metastatic adenocarcinoma of undetermined origin indicated that SC production was maintained. It was observed that the pattern of SC fluorescence often varied within a particular tumor. For example, in areas of rather obvious ductlike formation, the fluorescence was localized to the luminal surface of the cell, whereas, in less differentiated areas, the fluorescence was often distributed throughout the cell's cytoplasm. Somewhat at variance with our findings, Poger et al have reported that, while human colonic carcinoma demonstrates secretory component fluorescence, there are some poorly differentiated tumors that failed to do so.[5] This disparity may result from some unrecognized technical difference between the two studies. However, it more likely represents inherent differences between breast cancer and colonic cancer. In normal colons, goblet cells do not produce SC, while columnar cells do. In the poorly differentiated colon cancers, tumor cells derived from goblet cells could not be expected to produce SC. In contrast, adenocarcinomas of the breast, arising from acinar SC-producing cells, would all produce SC, as we have found.

Bussolati et al reported the detection of milk casein in sections of human dysplastic and neoplastic mammary tissues by immunofluorescence.[13] This fluorescence was also noted in metastatic lesions. In addition, two cases of adenocarcinoma of the rectum failed to demonstrate casein fluorescence. These investigators also observed that the majority of poorly differentiated carcinomas were negative for casein. Despite

the obvious similarity reported in their study to a portion of the data presented here, the lack of fluorescence in rectal carcinoma and in those poorly differentiated breast tumors indicates that their antiserums probably did not cross-react with SC. The demonstration of casein, as well as SC, in breast carcinomas appears reasonable and points out the likelihood of finding persistent production of numerous normal proteins by malignantly transformed glandular epithelial cells.

The clinical usefulness of this assay has yet to be fully determined. However, in cases where the cellular origin of a tumor is in question, the discovery of SC would indicate its glandular epithelial origin. Since the sensitivity of immunofluorescence microscopy is far greater than that of ordinary light microscopy, this assay has the additional advantage of being capable of rapidly locating micrometastases in a survey of lymph nodes from a typical lymph node dissection. Since immunofluorescence is easily performed and is less time consuming and less costly than electron microscopy, this assay may provide a useful initial screening procedure for distinguishing carcinomas derived from glandular epithelium from other malignancies.

Additionally, the discovery of SC-positive cells has proven helpful in the cytohistologic diagnosis of pleural, ascitic, or joint effusions. Immunofluorescence microscopy can be performed with cytocentrifugation of aspirated cells. In fact, in one such case, the discovery of SC fluorescing cells established that a patient's pleural effusion was associated with the first recurrence of an old breast carcinoma or a second primary carcinoma, rather than with some unrelated infections or other inflammatory lesion. Occasionally, the evaluation of cells from such an effusion by plain light-microscopic examination can be quite difficult. The fluorecent study for presence of SC offers practical help for this difficulty.

We are currently exploring the possibility of measuring serum SC levels in untreated and treated cancer patients with the hope of establishing a noninvasive assay for tumors derived from glandular epithelial cells.

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