Secretory Component in Human Mammary Carcinoma

Jeffrey P. Harris, Mae H. Caleb, and Mary Ann South

SUMMARY

Since secretory component is thought to be a normal glandular epithelial cell product, surgical specimens from patients with mammary carcinoma, an epithelial cancer, were studied with antisera to human free secretory component by indirect immunofluorescence microscopy. Normal breast tissue (10 cases) showed fluorescent epithelial cells confined to normal ducts. This was in marked contrast to invasive mammary carcinomas (20 cases), which showed intense staining of tumor cells and stromal cells in addition to the normal ductular epithelium. Metastases in axillary lymph nodes (2 cases) showed intense fluorescence for secretory component, whereas axillary nodes without metastases from 2 patients with breast cancer showed no fluorescence. In both normal and tumor tissue, anti-immunoglobulin A stained only ducts and subepithelial plasma cells, thus establishing that the secretory component in tumor cells was not part of an intact secretory immunoglobulin A molecule. This finding was not restricted to mammary carcinoma, since preliminary studies of colon, lung, and bladder carcinoma also demonstrated tumor cells with cytoplasmic fluorescence for secretory component. In contrast, the tumor cells in 2 cases of sarcoma, a nonepithelial cancer, did not exhibit fluorescence for secretory component.

INTRODUCTION

It has been well established that there exists an antigenically distinct portion of the slgA molecule known as SC. This glycoprotein, with a molecular weight of 58,000, has been localized to glandular epithelial cells by immunohistochemical studies (2, 5). Currently, it is postulated that lamina propria plasma cells secrete dimeric IgA in association with J chain, which then enters glandular epithelial cells. There this complex combines with SC and is subsequently secreted from the luminal side of the cell as an intact 11S slgA molecule (1, 3, 4).

Since SC is thought to be a normal glandular epithelial cell product, we undertook this study to determine whether SC could be demonstrated in an epithelial cancer. We report here the finding of SC in mammary carcinoma by indirect immunofluorescence microscopy.

MATERIALS AND METHODS

Tissue Specimens. Tissue specimens were obtained on the basis of availability and with informed consent from 40 patients undergoing surgery for varying breast lesions. The specimens were placed in cold 0.9% NaCl solution, divided into small pieces, and then snap-frozen on a chuck in O.C.T. (Ames Company, Elkhart, Ind.) embedding media. Serial 4-μm frozen sections were made on an International Harris cryostat and the slides washed for 10 min with 3 changes of PBS. The slides were fixed in acetone for 10 min then rehydrated in PBS for 10 min prior to the application of antisera. Incubation with the antisera was carried out for 30 min in a humidified chamber, and the slides were again washed for 10 min with 3 changes of PBS. Those slides examined with direct staining were mounted in Elvanol (E. I. DuPont de Nemours, and Co., Wilmington, Del.) and stored at 4° until ready for viewing. For slides examined by indirect fluorescence microscopy, a 2nd incubation with antisera was then accomplished, with subsequent washing and mounting. Representative sections were placed in formalin for hematoxylin and eosin staining.

Antisera. Fluorescein isothiocyanate-conjugated goat antisera specific for human α, γ, and μ chains (lots 40334, 09141, and 10941, respectively) were obtained from Meloy Laboratories (Springfield, Va.) and tested for specificity in the following ways: (a) Immunoelectrophoresis was performed reacting normal human serum against anti-normal human serum and the heavy-chain-specific antisera. A single precipitin line elicited between each heavy-chain-specific antisera and normal human serum was shown to correspond to the appropriate immunoglobulin precipitin line from the known immunoelectrophoretic pattern of normal human serum. (b) Preliminary fluorescent antibody studies utilizing these antisera 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. Marked apical staining of glandular epithelial cells with

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1 This study was supported by USPHS Grant CA-05022-18.
2 To whom requests for reprints should be addressed, at Room 8065, The Children’s Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, Pa. 19104.
3 The abbreviations used are: slgA, secretory IgA; SC, secretory component; PBS, phosphate-buffered saline (8.5 g NaCl, 1.07 g Na₂HPO₄, and 0.345 g NaH₂PO₄, H₂O in 1000 ml of distilled water); FITC-GAR Ig’s, fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulins.

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anti-\( \alpha \) and considerably less 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 antisera used in this study. Rabbit anti-free SC, obtained from Behring Diagnostics (Somerville, N. J.), was produced by a plasmin digestion of the isolated salivary slgA 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 intact 115 slgA molecule so that activity to only those SC determinants present in the free state remained. We then tested this antiserum 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 slgA precipitin line when placed in a well beside antihuman colostrum and opposite a well containing human colostrum. Rabbit anti-slgA (kindly supplied by Dr. Richard Hong, University of Wisconsin) was prepared by isolating salivary slgA, 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 utilized in the fluorescent antibody studies it produced results identical with those obtained with the anti-free SC. These antisera to SC were then counterstained with FITC-GAR Ig's, which was obtained from Behring Diagnostics. Nonimmunized rabbits were bled for a pool of normal rabbit serum. Control slides were made for each experiment. Nonimmune rabbit serum was counterstained with FITC-GAR Ig’s, as a control for rabbit anti-free SC. In addition, FITC-GAR Ig’s was used alone to rule out nonspecific tissue attraction. This antiserum also served as a control for the antisera to \( \alpha \), \( \gamma \), and \( \mu \) chains by direct immunofluorescence.

**Fluorescence Microscopy and Photography.** The microscope 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.

**RESULTS**

**Immunofluorescence.** When surgical specimens from 20 patients with mammary carcinoma were tested with both anti-SC and anti-free SC by indirect immunofluorescence, all were found to have fluorescing cells throughout the tumor (Table 1). The histological diagnoses of these adenocarcinomas included infiltrating ductal, medullary, colloid, and papillary carcinoma. Normal staining of ducts was seen where normal architecture remained; however, intense staining of tumor cells, as well as stromal cells, was also noted (Figs. 1 and 2). This was in marked contrast to 10 control specimens of normal breast that showed staining confined to epithelial cells in normal ducts and no other fluorescing cells (Figs. 3 and 4).

**DISCUSSION**

Based on the preliminary data presented in this study, it appears that SC is present in the cytoplasm of mammary carcinoma cells. It has been postulated that SC is a normal secretory product of glandular epithelial cells. The demonstration of SC in neoplastic cells of epithelial origin strongly supports this postulate. However, immunofluorescence microscopy can only localize the presence of the antigenically distinct molecule and does not indicate whether the fluorescence seen is the result of the local production of SC by the tumor cells or the result of adsorption onto the tumor cells after SC production by normal ductular epithelial cells.

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**Table 1**

*Results of immunofluorescence on tissue specimens stained with antisera to SC*

<table>
<thead>
<tr>
<th></th>
<th>No. of cases with fluorescent cells outside of normal ductal epithelium</th>
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<tbody>
<tr>
<td>Invasive mammary carcinoma*</td>
<td>20</td>
</tr>
<tr>
<td>Axillary node with metastases</td>
<td>2</td>
</tr>
<tr>
<td>Axillary node without metastases</td>
<td>0</td>
</tr>
<tr>
<td>Normal breast tissue</td>
<td>10</td>
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</table>

* The histological diagnoses of these carcinomas included infiltrating ductal, medullary, colloid, and papillary carcinoma.

Metastases to axillary nodes from 2 patients were also examined and found to be positive for SC in contrast to uninvolved axillary lymph nodes from 2 patients with breast cancer, which showed no fluorescence (Table 1). In the nodes with metastases, fluorescence was seen in those areas infiltrated with tumor, and also was seen on many cells in what appeared to be adjacent normal lymph node structures.

The 10 cases of benign breast lesions included 5 cases of fibrocystic disease and 5 cases of fibroadenoma. In both groups, about one-half showed fluorescence confined to normal ductular epithelial cells, while the remaining specimens also demonstrated weakly positive staining of proliferative stromal cells throughout the specimen. The epithelial and stromal fluorescence seen was never as intense as in the carcinoma specimens.

Staining was also done for \( \alpha \), \( \gamma \), and \( \mu \) chains to determine what class of plasma cell was infiltrating the carcinoma and whether these immunoglobulins were also on tumor cells. Of the 20 cases of carcinoma studied, 19 cases showed no appreciable plasma cell infiltration. However, in subepithelial sites where normal ducts remained, there were scattered plasma cells with cytoplasmic staining for IgA and IgG. In the case of invasive papillary carcinoma there was a marked infiltration of IgA and IgG containing plasma cells. We did not find appreciable staining for \( \alpha \), \( \gamma \), or \( \mu \) chains on the tumor cells in any of the cases studied.
While one cannot rule out the passive adsorption of SC by tumor cells in situ, the finding of SC in tumor cells in lymph node metastases where there are no normal ductular epithelial cells is strong evidence for production of SC by the neoplastic cells themselves. Since the normal axillary lymph nodes from 2 patients with mammary carcinoma failed to show fluorescing cells, it appears that drainage of an involved area is not sufficient to give the fluorescent cellular pattern and that this requires the presence of tumor cells in the section being examined. The finding of cells fluorescing with less intensity in the adjacent normal lymph node structures of cancerous nodes seems best explained by postulating the production of SC in nearby tumor cells and its subsequent adsorption onto these apparently normal cells, and this probably also explains the stromal fluorescence seen in the primary lesions. Since tumor cells do not show fluorescence with application of anti-IgA, it is unlikely that the SC seen in tumor cells is part of an intact sIgA molecule or host response to the tumor.

The finding of weakly fluorescing and nonfluorescing stromal cells in both fibroadenomas and fibrocystic disease is still confusing and inconclusive. Again, the question is raised as to whether the SC is the product of nearby hyperplastic epithelial cells or is endogenously produced by the fluorescing cells. Further study is needed to sort out this problem.

At the outset, the design of this study included examination of all epithelial cancers. Mammary carcinoma proved to be the most readily available lesion and thus is the basis of this series. However, the finding of SC in tumor cells is not restricted to breast cancer. Preliminary studies in our laboratory include 5 colon, 1 bladder, and 2 lung carcinomas, all of which demonstrate SC in their tumor cells. Thus, mammary carcinoma seems to serve as a model for glandular epithelial tumors, and we anticipate finding SC in those epithelial tumors yet to be studied. In contrast, the finding of SC in nonepithelial tumors would be surprising, and in fact we have now examined specimens from 2 cases of sarcoma that did not exhibit SC in their cells. Further study is needed to define the extent of SC in the neoplastic state.

While SC has been intimately associated with the normal secretory immune system, the function of SC is still unclear. In light of this, our finding of SC in the tumor cells from a variety of cancers may have potential significance in understanding regulation of the immune response to these neoplasms.

An obvious clinical application of this finding and one that needs to be evaluated is the use of immunofluorescence microscopy to examine suspected metastases of epithelial cancers for the presence of SC. Hopefully, this technique will prove to be much more sensitive than ordinary light microscopy for the detection of tiny foci of tumor cells.

While the finding of SC in the cytoplasm of tumor cells is suggestive evidence of its endogenous production, isolation of this substance from neoplastic cells themselves is needed to confirm this observation.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Section of invasive mammary carcinoma. H & E. x 640.
Fig. 2. Section identical with Fig. 1 stained with rabbit anti-free SC and FITC-GAR Ig’s. Note tumor cells with cytoplasmic fluorescence. x 640.
Fig. 3. Section of normal breast tissue showing normal ductular epithelium. H & E. x 640.
Fig. 4. Section of normal breast tissue stained with rabbit anti-free SC and FITC-GAR Ig’s. Note fluorescing epithelial cells confined to ducts (section not identical with Fig. 3). x 640.
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