Common Antigenic Sites on Exfoliated Cells Derived from Cervical Carcinoma and in Tumor Cells of Nonuterine Origin as Demonstrated by Monoclonal Antibodies in Immunoperoxidase Assay

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ABSTRACT

The binding characteristics of monoclonal antibodies produced against a variety of human tumor cells were studied on cervical carcinoma cell lines and on exfoliated cells of cervical smears. The latter included normal epithelial cells, cells derived from cervical intraepithelial neoplasia, and cells from squamous cell carcinoma. Monoclonal antibodies that bound in immunoperoxidase assays to ethanol-fixed smears of cultured human tumor cells but not to normal cervical smears were screened on cervical smears containing malignant cells. Of the six antibodies selected for detailed studies, two each had been produced against bladder carcinoma and melanoma and one each against cervical and gastric carcinoma. Antibody 99-57 stained malignant cells from invasive carcinoma but not normal cervical cells. In cells from intraepithelial neoplasia, staining intensity was highest in severely dysplastic cells. Thus monoclonal antibodies are potentially useful in the detection of malignant cervical cells within a large number of nonmalignant cells, in conjunction with other diagnostic procedures.

INTRODUCTION

The use of the Papanicolaou smear for the detection of cervical cancer resulted in a decreased morbidity and mortality from this disease among a large screened population (18). However, the time-consuming nature of the cytological examinations and the requirement for highly specialized professional personnel for their interpretation have prompted efforts to seek other than morphological criteria for screening cytological smears. Furthermore, despite the value of the Papanicolaou smear test for the early detection of CIN preceding the onset of clinical cancer, the biological behavior of individual cases of these precursor lesions remains unpredictable. Thus a simpler screening procedure and additional parameters to assess the risk for a progressive nature of individual lesions would be of great interest.

In this study we describe the binding characteristics of Mabs to fixed cervical cells. These antibodies were selected from a large panel which included antibodies originally produced against a variety of human tumor cells, such as carcinomas of the colon, rectum, stomach, pancreas, breast, ovary, lung, cervix, bladder, and of melanoma and globlastoma. Of six antibodies initially selected, we studied two in detail for their reactivity with normal cervical epithelial cells, with intraepithelial neoplastic cells and with malignant cells derived from invasive carcinoma.

MATERIALS AND METHODS

Cytological Material Containing Cervical Epithelial Cells. “Touch” preparations of the cervical squamocolumnar junction were obtained at Temple University Hospital, Colposcopy Clinic, by Dr. Parviz Hanjani. Conventional Papanicolaou smears from routine cancer diagnostic procedures were obtained from the file of Temple University Hospital Cytology Laboratory. Additionally, Papanicolaou smears were kindly provided by Dr. Orazio Zumbo, St. Peter’s Hospital, Albany, NY, and by Dr. Louis Gerstley, Wyncote, PA. Cytological material included nonneoplastic cells derived from patients without cancer and neoplastic cells obtained from the patients with CIN or with invasive cervical cancer. Most specimens contained over 200 neoplastic cells per smear, but in three cases as few as 30-40 neoplastic cells were present on a smear.

Cell Lines. Cervical carcinoma cell line SW756 was obtained from A. Liebovitz, Scott and White Clinic, Temple, TX. Cervical carcinoma cell lines SKG-IIa and Ilb (14) were kindly provided by Dr. Shiro Nozawa, Keio University, Tokyo, Japan. Other cell lines used in this study for control purposes have been described elsewhere (3). Before testing cells were scraped from the plastic surface, washed in a potassium chloride-buffered NaCl solution, and fixed.

Cell Fixation. Preliminary examinations in which smears were prepared from cells of cell lines using air drying, 95% ethanol, Cytoprep, cold acetone, or commercial hair spray for fixation showed that cell morphology and antigenic sites were best preserved with ethanol fixation (hair spray gave the poorest results). Therefore all freshly collected touch preparations and Papanicolaou smears obtained from St. Peter’s Hospital were 95% ethanol fixed. However, Papanicolaou smears from the file of the Cytology Laboratory were usually fixed with commercial spray fixative.

Monoclonal Antibodies. Of a panel of 60 Mabs, 6 were selected for further study based on the stability of antigenic determinants after fixation in 95% ethanol, and binding to cervical carcinoma cell line SW756 but not to normal cervical epithelial cells. Table 1 summarizes the characteristics of the 6 Mabs. Mabs BL199-157-F11 (99-57) and BL199-136-B4 (99-36) were derived by immunizing BALB/c mice with 3 x 10⁷ bladder carcinoma T24 cells, boosting 5 weeks later with 2 x 10⁶ cells, and fusing splenocytes 4 days later with the nonsecreting mouse myeloma P3X63Ag8 653. Mab CE157-7-61-1 (57.1) was derived from mice immunized with SW756 of cervical carcinoma cells using the same protocol. Four days after the last injection, spleen cells were fused. Growth of hybridomas, cloning, and initial characterization of binding reactivities are described elsewhere (10).

Immunoperoxidase Assay. Fixed cell smears were air dried, rehydrated, and incubated with 1% horse serum for 20 min at room temperature and then tested for binding of Mabs as described elsewhere (1, 6). Briefly, 0.09 ml of undiluted hybridoma supernatant was added and incubated for 30 min at room temperature. The slides were then washed 5-10 min in a buffered solution containing 0.8% NaCl, 0.12% Na₂HPO₄,
0.02% KCl, 0.02% KH2PO4, and 0.1% bovine serum albumin; biotinylated anti-mouse immunoglobulin (heavy and light chains) was then added for an additional 30 min at room temperature, followed by the addition of avidin-biotin peroxidase (*ABC* complex) of Vector Laboratories, Burlingame, CA. Slides were washed in potassium chloride-buffered NaCl solution after each incubation. Binding of Mabs was visualized by incubating slides with 0.05% diaminobenzidine (Sigma Chemical Co., St. Louis, MO) containing 0.01% H2O2 and counterstained with either hematoxylin or Papanicolaou stain. Mouse myeloma P3X63Ag8 supernatant was used as an antibody control.

Stained slides were then examined microscopically for the presence of brown IP pigment in malignant, dyskaryotic, or normal cervical cells. Smears having 5% or more of their neoplastic cells IP reactive were considered positive. Smears with less than 5% of the neoplastic cells IP reactive were considered negative. The relative intensity of the stain was noted (0, 1+, or 2+), as well as the relative number of granules present within the cells (sparse or dense) and the location of the IP stain within the cell (diffuse cytoplasmic and/or perinuclear).

RESULTS

Thirty-three of 60 Mabs initially screened bound to ethanol-fixed smears of cultured human tumor cells but not to normal cervical smears. Six Mabs that showed strong positive IP staining (2+ on more than 75% of cells) of SW756 cervical carcinoma cells were further screened on 84 cervical Papanicolaou smears previously diagnosed as positive for carcinoma. Two Mabs, 31-74 and 99-57, detected malignant cervical cells in Papanicolaou smears. Mab 31-74 stained cells of 8 of 16 cases including 5 of 12 cases of smears with more bizarre malignant cells and 3 of 4 fresh colposcopy touch preparations (Table 2). Mab 99-57 stained 11 of 31 cases with positive Papanicolaou smears. This Mab bound to cells of 6 of 18 smears with CIN. Eighteen smears containing only normal cervical cells were not stained by Mab 99-57. Mab 31-74, on the other hand, did not bind to CIN but did stain cells of 16 of 43 specimens containing normal cells only (Table 2). Mab 99-57 appears to bind in more cases and with higher intensity to cells from CIN II and III than to cells from CIN I (Table 3).

In the 8 cases of CIN that bound Mab 99-57, approximately 15% of neoplastic cells were IP positive, except in one case of CIN II, in which 40% of dyskaryotic cells were positive. The percentage of IP-positive cells seemed to increase in smears of invasive carcinoma treated with Mab 99-57, with 5 of 11 invasive carcinoma smears showing 30% or more IP-positive malignant cells. The remaining 6 carcinoma smears reactive with Mab 99-57 all stained 20% or fewer malignant cells. Intensity of IP staining of individual cells in 13 of 17 smears of CIN and carcinomas generally appeared to correlate with the percentage of dyskaryotic or malignant cells positive, with weak (1+) staining on smears with 10% or fewer cells stained, and strong (2+) staining on smears with 30% or more cells positive. As summarized in Table 3, 2 specimens had strong staining intensity with only a minimal (10% or less) number of positive cells, whereas 2 other specimens showed weak staining but with a higher percentage of positive cells (35 and 55%).

In 8 specimens of invasive carcinoma that reacted with Mab 31-74 (Table 3), the percentage of positive malignant cells varied from 100% to less than 10% with 6 smears showing 30% or more positive cells. In general Mab 31-74 stained a greater percentage of malignant cells than did Mab 99-57, although the staining of individual cells was weaker. Of the 5 carcinoma smears tested with Mab 31-74 that had weak (1+) staining, 3 showed staining in 30% or more of the malignant cells. In all 3 smears that were strongly stained with Mab 31-74, 70% or more of the malignant cells were positive. There was no apparent correlation between the intracellular location of the stain and the severity of CIN. Figs. 1–4 show representative staining of dysplastic and malignant cervical cells with Mabs 99-57 and 31-74. IP-positive granules are primarily dispersed throughout the cytoplasm of well preserved as well as degenerated malignant cells and are variable in number. Occasionally intracellular IP-positive...
BINDING CHARACTERISTICS OF Mabs TO CERVICAL TUMOR CELLS

Table 3
Reactivity with Mabs 99-57 and 31-74 with CIN and invasive cervical carcinoma

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Cytological preparation</th>
<th>No. of positive specimens/total tested</th>
<th>% of positive neoplastic cells (staining intensity)</th>
<th>No. of positive specimens/total tested</th>
<th>% of positive neoplastic cells (staining intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN I</td>
<td>FCTP</td>
<td>0/2</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>CIN II</td>
<td>FCTP</td>
<td>2/2</td>
<td>40 (2+), 15 (1+)</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>Restained Papanicolaou smears</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>CIN III</td>
<td>FCTP</td>
<td>0/1</td>
<td>5–10 (1+), 5–10 (2+).</td>
<td>4/13</td>
<td>5–10 (1+), 5–10 (1+)</td>
</tr>
<tr>
<td></td>
<td>Restained Papanicolaou smears</td>
<td>0/1</td>
<td>5–10 (1+), 5–10 (1+)</td>
<td>0/7</td>
<td>5–10 (1+), 5–10 (1+)</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>FCTP</td>
<td>3/4</td>
<td>80 (2+), 55 (1+), 40 (2+).</td>
<td>3/4</td>
<td>100 (2+), 80 (2+)</td>
</tr>
</tbody>
</table>

* FCTP, fresh colposcopy touch preparations.

Among nonepithelial cellular components of cervical smears, polymorphonuclear leukocytes usually reacted with Mabs 99-57 and 31-74, but their morphology was sufficiently well preserved to preclude any confusion with neoplastic cells. Histiocytes and lymphocytes which are less frequent in cervical smears were negative.

Table 4 summarizes the binding patterns of 4 additional Mabs to cervical cells. None of the antibodies reacted with cells from intraepithelial neoplasia or with normal cells. Mab 57.1 stained malignant cells in 1 of 6 cases only, whereas Mab 73.3 reacted with 4 of 9 specimens containing carcinoma cells. Mabs 99-36 and ME491 bound to 4 of 11 and 5 of 11, respectively, malignant cell preparations. The percentage of positive malignant cells varied from 10 to 95 for Mabs 73.3 and 99-36 and from 45 to 95 for Mab ME491.

DISCUSSION

Hybridomas derived from mice immunized with different human tumor cells produced Mabs that bound to fixed cells of...
The reappearance of morphologically apparent squamous cell differentiation with the development of keratin in the cells of microinvasive cervical carcinoma has been frequently noted, despite the absence of this differentiation in adjacent areas of CIN (15). Further investigation of Mab 31-74 reactivity could provide evidence for its usefulness in assessing the risk of invasion.

One could also consider the possibility that the variable degree of cellular differentiation of malignant squamous cells is one aspect of their heterogeneity which is reflected in the presence of tumor antigens on the surface of some, but not other neoplastic cells. Alternatively, new clones of neoplastic cells might arise during the development of progressively severe lesions of CIN, some of which express tumor antigens before invasion occurs. Thus it would be extremely valuable to identify these antigens even in a few cells derived from CIN, since their presence may provide the basis for prognosis. The apparent increased binding of Mab 99-57 to cells from CIN II and CIN III as compared with cells from CIN I suggests the presence of such an antigen in increasing quantities on cells from progressive stages of neoplasia. These results are very encouraging in this respect and warrant further investigation.

In another approach to the search for antigenic differences between benign cervical and malignant cells derived from squamous cell carcinoma, several groups (4, 6, 11, 13, 16) have directed their efforts to studying the disappearance of isocytokeratins A, B, and H during the development of cervical carcinoma. These reports differ in their assessment of the precise stage of the disease when loss of these antigens is demonstrable, which again points to the heterogeneity of antigen expression in tested cell populations.

The development of a series of new Mabs specifically directed against cervical carcinoma, which is currently in progress in our laboratories, should aid in determining whether the presence of tumor markers in selected cases of CIN correlates with neoplastic progression.

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**REFERENCES**


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