Effect of Intravesical Bacillus Calmette-Guérin on Detection of a Urothelial Differentiation Antigen in Exfoliated Cells of Carcinoma in Situ of the Human Urinary Bladder

Jeffry L. Huffman, Yves Fradet, Carlos Cordon-Cardo, Harry W. Herr, Carl M. Pinsky, Herbert F. Oettgen, Lloyd J. Old, Willet F. Whitmore, Jr., and Myron R. Melamed

ABSTRACT

Flow cytometry was used to detect and quantify expression of a urothelial differentiation antigen (Om5) and nuclear DNA in exfoliated epithelial cells of the urinary bladder from 15 patients with nonpapillary carcinoma in situ during and after intravesical therapy with Bacillus Calmette-Guérin (BCG). Before BCG treatment exfoliated cells reacting with the mouse monoclonal antibody Om5 were found in 13 cases. Following treatment Om5 positive cells were still present in 9 cases but 4 patients who had had Om5 positive cells prior to BCG therapy no longer had detectable antigen-positive cells after therapy. Thus intravesical BCG therapy can alter detection of a urothelial differentiation antigen in exfoliated bladder epithelial cells. It is not certain whether this antigen or other differentiation antigens measured by flow cytometry will advance our present techniques for assessing effects of therapy on carcinoma in situ and other bladder tumors. However, five of nine patients showing persistence of Om5 positive cells after therapy were found to have recurrent tumor by biopsy and two others had positive cytology (median follow-up, 13 months). None of the four without detectable antigen-positive cells after therapy had clinical evidence of tumor by cystoscopy, biopsy, or cytology (median follow-up, 12 months). It now appears feasible and desirable to initiate clinical investigations of this and other differentiation antigens in combination with DNA by flow cytometry of bladder irrigation specimens.

INTRODUCTION

Nonpapillary CIS of the human urinary bladder is typically high grade, potentially aggressive, multifocal, and frequently widespread (1–5). The disease is difficult to delineate cystoscopically, i.e., by transurethral resection and fulguration. Thus reports of a favorable response to BCG and fulguration. Thus reports of a favorable response to BCG and have appeared sporadically in the literature (6–8). The effects of treatment of bladder cancer can be monitored by DNA FCM; patients who respond are identified by a decrease in DNA FCM. We chose to study the effects of BCG on exfoliated cells expressing a cellular antigen recognized by a recently described monoclonal antibody. The mouse monoclonal antibody detecting Om5 was produced by immunization with fresh tissue from a low grade papillary carcinoma of human urinary bladder (15). Om5 has been found in 17 of 20 noninvasive papillary bladder tumors, 6 of 8 CIS, 3 of 15 invasive tumors, and 0 of 6 bladder cancer metastases; it was not detectable in a large number of other epithelial cancers, sarcomas, melanomas, and lymphomas. In a large panel of adult and fetal tissues the Om5 antigen was found in the epithelium of the adult ureter in 2 of 12 cases and in apparently normal bladder urothelium in 12 of 25 cases but not in the fetal ureter in 7 cases studied or any other normal tissue (unpublished data). A similar distribution was recently reported by Fradet et al. Based on these results the Om5 antigen may be considered a highly restricted differentiation antigen of the urothelium, frequently and predominantly expressed on the cells of superficial bladder cancers.

MATERIALS AND METHODS

Fifteen consecutive patients with CIS were studied before and during treatment with intravesically applied BCG. All were patients with recurrent or residual disease following conservative surgery (transurethral resection) for low grade low stage papillary tumors. Following diagnosis and at the time of entry into the study each patient was examined cystoscopically and all visibly abnormal or suspicious mucosa was resected. BCG for 6 consecutive weeks (120 mg in 50 ml normal saline; Pasteur strain, Institut Armand Frappier, Montréal). Three weeks after the last instillation and then at 3 month intervals, the patients were reevaluated by cystoscopy and biopsy. Urine for conventional exfoliative cytology was collected by catheterization prior to each endoscopic procedure.

Sample collection and staining for flow cytometry. Prior to the initial and each subsequent instillation of BCG and at the first cystoscopy after therapy, the empty bladder was irrigated vigorously with 100 ml saline through a 16 French urethral catheter. Five pulses of a piston syringe were used on each collection in an attempt to standardize cell exfoliation. The cells obtained were stained for Om5 by exposure to the mouse monoclonal antibody and a fluoresceinated anti-mouse immunoglobulin, then counterstained with propidium iodide for DNA (16, 17). In brief this was carried out as follows. The suspension of exfoliated epithelial cells was first sieved through a 54 μm nylon mesh to remove large clusters of cells, then divided into aliquots for FCM that were centrifuged and resuspended at 10^6 cells/ml in supernatants of the hybridoma producing the Om5 monoclonal antibody or as a control supernatant of the mouse myeloma fusion partner MOPC-21 NS/1 (18, 19). After incubation on ice for 60 min, the cell suspensions were washed twice, resuspended in 1:40 FITC-conjugated goat anti-mouse immunocytometry.
EFFECT OF BCG ON DETECTION OF ANTIGEN IN BLADDER CARCINOMA

A. PRE-THERAPY

B. AFTER BCG THERAPY

Chart 1. Flow cytometry histograms demonstrating the change in antibody binding to Om5 and in DNA content of exfoliated bladder epithelial cells from a patient with multifocal carcinoma in situ treated with BCG. Note the loss of antibody binding cells in the histogram following BCG (B) which also shows a (normal) tetraploid DNA population and no aneuploid cells.

globulin (Cappel Laboratories, Cochranville, PA), incubated on ice for another 30 min, washed twice, and fixed in 70% ethanol. After 1 h fixation the cells were washed, treated for 30 min at 37°C with 0.2 ml RNase A (5000 units/ml, 1.12% sodium citrate; Worthington Diagnostics, Freehold, NJ). Finally 0.2 ml propidium iodide (50 μg/ml, 1.12% sodium citrate; Calbiochem-Behring Corp., LaJolla, CA) was added to stain nuclear DNA.

FCM Analysis. Five thousand cells were measured for each sample on an Ortho-30 Cytofluorograf (Ortho Diagnostic Systems, Inc., Westwood, MA) using fresh human lymphocytes as a diploid control. The green fluorescence of the fluoresceinated antibody and the red fluorescence of the propidium iodide used to stain DNA are separated optically and measured simultaneously for each cell in the cell sample as it passes through the flow cytometer. Three features were measured simultaneously for each sample and recorded on a 2150 Ortho computer: relative DNA content (red fluorescence with propidium iodide staining); nuclear diameter (red fluorescence pulse width); and FITC labeled antigen (green fluorescence).

DNA content and nuclear pulse width were first plotted as a two dimensional scattergram by computer; then cell doublers and larger aggregates were excluded using the nuclear pulse width limits defined by lymphocytes (20). The selected single cells were replotted in a second scattergram according to DNA content and antibody fluorescence. Non-specific binding of the FITC-conjugated antibody was estimated by the fluorescence intensity of the NS/1 stained cells and used as a threshold to determine specific binding of Om5 antibody (Chart 1).

RESULTS

Thirteen of 15 patients had detectable Om5 antigen on exfoliated epithelial cells of the bladder 3–5 weeks following resection of all visible tumor and prior to BCG therapy. Two patients did not have detectable antigen at any time prior, during, or after BCG therapy. Fourteen patients had aneuploid DNA populations prior to BCG; one patient did not. After 2 weeks of BCG therapy 9 of the 13 patients with detectable antigen showed persistent exfoliation of antigen-positive cells, after 4 weeks 7 patients, and at the follow-up cystoscopy or after 6 BCG treatments, 9 patients had detectable antigen (Table 1). Biopsy at the first follow-up endoscopy showed recurrent CIS in 2 of these patients, atypical epithelium in 2, and chronic cystitis in the remaining 5 patients. Six patients had aneuploid or hyperdiploid DNA populations by FCM including one of the patients with CIS, one with atypia, and 4 with chronic cystitis.

Both patients found to have recurrent CIS had positive cyto-
EFFECT OF BCG ON DETECTION OF ANTIGEN IN BLADDER CARCINOMA

Table 1

Patients with CIS of the bladder having persistent Om5 labeled exfoliated cells after intravesical BCG therapy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Condition at time of follow-up cystoscopy (3 mo)</th>
<th>Length of follow-up (mo)</th>
<th>Subsequent condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>Cytology</td>
<td>FCM#</td>
<td>Biopsy</td>
</tr>
<tr>
<td>1</td>
<td>Cystitis Positive</td>
<td>13</td>
<td>CIS</td>
</tr>
<tr>
<td>2</td>
<td>CIS Positive</td>
<td>14</td>
<td>Atypia</td>
</tr>
<tr>
<td>3</td>
<td>Atypia Positive</td>
<td>12</td>
<td>Cystitis Positive</td>
</tr>
<tr>
<td>4</td>
<td>Cystitis Positive</td>
<td>13</td>
<td>Atypia</td>
</tr>
<tr>
<td>5</td>
<td>CIS Positive</td>
<td>13</td>
<td>Atypia</td>
</tr>
<tr>
<td>6</td>
<td>CIS Positive</td>
<td>12</td>
<td>Atypia</td>
</tr>
<tr>
<td>7</td>
<td>Atypia Positive</td>
<td>14</td>
<td>Papilloma</td>
</tr>
<tr>
<td>8</td>
<td>Cystitis Negative</td>
<td>13</td>
<td>No evidence</td>
</tr>
<tr>
<td>9</td>
<td>Cystitis Positive</td>
<td>11</td>
<td>Cystitis</td>
</tr>
</tbody>
</table>

# Positive FCM is defined as an aneuploid stemline or >15% hyperdiploid cells at the time of follow-up cystoscopy after 6 BCG treatments.

Table 2

Patients with CIS of the bladder having lost Om5 labeled exfoliated cells after intravesical BCG therapy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Condition at time of follow-up cystoscopy (3 mo)</th>
<th>Length of follow-up (mo)</th>
<th>Subsequent condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>Cytology</td>
<td></td>
<td>Biopsy</td>
</tr>
<tr>
<td>1</td>
<td>Cystitis Negative</td>
<td>13</td>
<td>Cystitis</td>
</tr>
<tr>
<td>2</td>
<td>NED# Negative</td>
<td>13</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>Cystitis Negative</td>
<td>13</td>
<td>NED</td>
</tr>
<tr>
<td>4</td>
<td>Cystitis Negative</td>
<td>11</td>
<td>NED</td>
</tr>
</tbody>
</table>

# NED, no evidence of disease.

REFERENCES


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