Alveolar Cell Carcinoma-like Antigen and Antibodies in Patients with Alveolar Cell Carcinoma and Other Cancers

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SUMMARY

Antiserum was produced in sheep against a particle pellet recovered from human alveolar cell carcinoma (ACC). The antiserum was used for the detection of similar antigens in patients with ACC using the immunodiffusion method. Tumor cell lines were used in the indirect fluorescent technique for the detection of antibodies in the serum of patients with tumors and control serum. The results indicate that similar antigens are present in the serum of some patients with ACC, primary adenocarcinoma of the lung, and Hodgkin's disease. Antibodies develop in some patients with ACC and primary adenocarcinoma of the lung and in sheep inoculated with the agent.

INTRODUCTION

Coalson et al. (1) reviewed the ultrastructure of ACC with special emphasis on the intranuclear and intracytoplasmic virus-like particles within the malignant cells. These particles when put in an in vitro system are able to produce cytopathic effects in a variety of cell lines as reported by Coalson et al. (2). More recent immunological studies have shown these particles to possess specific ACC antigens (4). The same antigens can be induced following infection of continuous commercial cell lines (WI-38 and BHK-21) with these particles. The present study was undertaken to determine the antigenicity of this agent in sheep and to investigate the sera of patients with ACC, those with other tumors, and healthy volunteers for the presence of similar antigens and/or antibodies.

MATERIALS AND METHODS

Cell Cultures. Continuous tumor cell cultures from 2 ACC's and 1 indicator cell line (HeLa-65), supplied by Flow Laboratories, Inc., Rockville, Md., were cultivated at 37° with 5% CO2 on Eagle's minimal essential medium with 10% heat-inactivated fetal calf serum, L-glutamine, and a variety of antibiotics. The methods for the establishment of these continuous tumor cultures have been reported elsewhere (2).

Production of Antiserum. Tumor cultures producing supernatant known to induce cytopathic effects in indicator cells were subcultured to increase medium yields. Pools of culture medium were centrifuged at 2,000 x g, followed by 20,000 x g and 92,000 x g. The final pellet was resuspended in the smallest possible volume of phosphate-buffered saline and emulsified in an equal volume of Freund's adjuvant. The resulting mixture was injected s.c. into young sheep. Booster injections were given i.v. every 30 days. The sheep were bled by external jugular puncture before and 30 days after the initial inoculation and every 30 days thereafter. Absorption of pooled serum was accomplished with lyophilized fetal calf serum, lyophilized tissue culture medium, lyophilized normal human serum, and lyophilized normal human lung. Absorption of pooled serum was accomplished with lyophilized fetal calf serum, lyophilized tissue culture medium, lyophilized normal human serum, and lyophilized normal human lung utilizing the stained red blood cells absorption method.

Tanned Red Blood Cell Absorption Method. The washed erythrocyte suspensions were treated with tannic acid according to the method of Stavitsky (6). Two ml of the washed 2.5% suspension of tanned erythrocytes were added to a mixture of 8 ml of buffered saline, pH 6.4, and 2 ml of the proteins (in NaCl solution) used to sensitize the tanned erythrocytes; the sensitis included lyophilized fetal calf serum, lyophilized tissue culture medium, lyophilized normal human serum, lyophilized normal human lung, and lyophilized normal human liver. After 10 min incubation at room temperature, the cells were centrifuged and washed in 0.2% heat-inactivated Bovine Fraction V and resuspended in 2 ml of 0.2% heat-inactivated Bovine Fraction V to the original volume. The sensitized tannic acid cells were then incubated with the pooled sheep anti-ACC serum at 37° for 15 min. The mixture was gently centrifuged; the cells were discarded and the supernatant was saved.

Antigens. Antigens were obtained from filtered supernatant, from tumor cell cultures, and from filtered supernatant from inoculated indicator cells. Control antigens included supernatant from uninfected indicator cell lines, normal human lung, and Freund's adjuvant.

Preparations for Immunofluorescence. Tumor cell cultures, infected indicator cells, and control cells were grown on coverslips in Leighton tubes with standard growth medium. At various intervals, coverslip preparations of tumor cell cultures, infected indicator cells, and controls were fixed in acetone. Heat-inactivated serum from a variety of patients was layered over the tissue surface and
incubated at 37° for 30 min. The coverslips were repeatedly washed with Difco FA buffer and allowed to dry. Fluorescein-conjugated γ-globulin (supplied by Hyland; goat anti-human γ-globulin) was then applied to the tissue and incubated at 37° for 30 min. This was washed 3 times and mounted in buffered glycerin. These were then viewed on an Orthomat fully automatic UV microscope. Photographs were taken using the Orthomat automatic camera system and Kodachrome II color film.

**Immunodiffusion Studies.** The method for gel diffusion was that described by Ouchterlony (5). Various percentages of Noble agar, plate thickness, and hole patterns were used. The diffusion plates were allowed to run for 7 days at room temperature and washed in phosphate-buffered saline for a minimum of 24 hr. Photographs were taken with a Cordis immunodiffusion Polaroid camera.

**Sera Studied.** Serum from patients with ACC, adenocarcinoma, and other tumors were studied for the presence of antigens similar to the ACC antigen and antibody similar to the ACC antisera produced in sheep. Control serum consisted of serum from healthy volunteers with no known diseases and sheep inoculated with control antigens. The indirect fluorescent and the immunodiffusion methods described above were used.

**RESULTS**

**Immunodiffusions.** Gel diffusion was performed with absorbed sheep serum, anti-ACC antigens, and serum from patients with ACC, those with adenocarcinoma, and healthy volunteers. Antigens for diffusion were obtained from tumor cell cultures and from infected indicator cell lines. Control antisera was obtained from sheep inoculated with uninfected indicator cell lines, standard growth medium, and normal human lung.

Utilizing the above antigens with patient serum in the gel diffusion system, no precipitin bands were detected in any of 200 patients with known cancers or in serum of any control subject. However, the absorbed serum from sheep inoculated with ACC agent demonstrated precipitin bands while the controls did not. These bands form a band of identity with the antisera as reported by Nordquist (4) (Fig. 1). When the absorbed sheep serum was diffused against the serum of patients with ACC and primary adenocarcinoma of the lung, precipitin bands did develop in 9 of 18 with ACC and 3 of 4 with primary lung adenocarcinoma (Fig. 2). Bands of identity are present with the ACC antigen in Fig. 2B, while no band is seen in Fig. 2F where the antigen is

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**Table 1**

<table>
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<th>Uninfected HeLa cells</th>
<th>Tumor cell culture</th>
<th>Infected HeLa cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC patients</td>
<td>0/13</td>
<td>11/13</td>
<td>11/13</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>0/15</td>
<td>3/20</td>
<td>5/24</td>
</tr>
<tr>
<td>Primary adenocarcinoma of lung</td>
<td>0/5</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Adenocarcinoma of intestine</td>
<td>0/6</td>
<td>0/6</td>
<td>0/5</td>
</tr>
<tr>
<td>Other tumors</td>
<td>0/4</td>
<td>0/4</td>
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normal human lung. Two of 18 patients with Hodgkin's disease also demonstrated precipitin bands.

**Indirect Fluorescence.** The cell lines used for the fluorescent studies included monolayers of uninfected and infected HeLa cells and tumor cell cultures. No fluorescence was seen on the uninfected HeLa cell monolayers when tested with sera from any patient or healthy volunteers (Table 1). The sheep anti-ACC serum produced fluorescence on both the infected HeLa cell monolayers and the tumor cell culture but not on the uninfected HeLa cell monolayers. The sera from 11 of 13 patients with ACC produced fluorescence on both the infected HeLa cell monolayer and the tumor cell culture. The sera from 5 patients were not tested for immunofluorescence. The sera from 3 of 5 patients with primary lung adenocarcinoma produced a uniform fluorescent stain within the cytoplasm of both the infected indicator cell line and the tumor cell line. We were unable to demonstrate any fluorescence in the tumor cell or infected indicator cell line using the serum from primary adenocarcinoma of the intestine or squamous cell carcinoma of the lung (Table 1). Table 1 further illustrates that the serum from a few healthy volunteers produced fluorescence. It is of interest that these are people working on this study and serum from all 5 before the investigation did not produce fluorescence. Fig. 3 illustrates the fluorescent stain within the cytoplasm of the infected HeLa cell monolayer. Also present in some cells are large, bright-staining bodies within the nucleus, which stain with much greater intensity than normal nucleolar fluorescence (Fig. 4). These particular bodies stained only with use of the infected monolayer or tumor cell line with sera from patients whose serum produced cytoplasmic fluorescence.
DISCUSSION

This study was designed to evaluate the sera of patients with various tumors and those of healthy volunteers for serological reactants to the human ACC agent and antiserum prepared against the ACC agent. The anti-ACC serum produced in this study forms a line of identity with the ACC antiserum reported by Nordquist (4). The 9 patients with ACC whose sera demonstrated precipitin bands tended to be those with more widespread tumor. Four of these patients had diffuse involvement of both lungs while the other 5 had evidence (histologically verified) of metastatic ACC involving the brain, bones, or s.c. tissues. This suggests the presence of circulating antigen in these patients, which supports the hypothesis and findings of Currie and Basham (3). The 9 patients with ACC who did not have circulating antigen all had localized pulmonary lesions. Perhaps with a more sensitive test system, antigen could have been detected. The presence of precipitin bands found with serum from 3 patients with primary adenocarcinoma of the lung and 2 of 18 with Hodgkin’s disease suggests the presence of either a similar antigen or nonspecificity of the test. This is at slight variance with the report by Nordquist who found ACC antiserum to be quite specific for ACC without cross-reactivity with other tumor cells. The indirect fluorescent studies suggest that some patients with ACC and primary adenocarcinoma of the lung possess antibodies to the ACC agent. One patient without fluorescent antibodies did not have circulating antigen and disseminated disease; however, the other patient without antibody had localized disease without circulating antigen. The finding of antibodies in personnel working closely with the agent suggests sensitization with the agent.

REFERENCES

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