Organ Specific Neoantigens Reactive in the Leukocyte Adherence Inhibition Assay: Affinity Purification of Human Colon Carcinoma Antigen and Its Cross-Reactive Protein Using Monoclonal Antibodies

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ABSTRACT

A monoclonal antibody (mAb) was prepared against a semipurified preparation of an organ specific neoantigen (OSN) reactive in the leukocyte adherence inhibition (LAI) assay. The mAb (LC20.1) induces a positive LAI response when incubated with leukocytes of normal individuals in the presence of OSN derived from either human colon or lung carcinoma cell lines. Absorption of crude OSN preparations from these cell lines on immobilized LC20.1 mAb eliminates all the LAI reactive material suggesting that the mAb recognizes a common determinant on OSN from both colon and lung carcinomas. The LC20 mAb was used to affinity purify the colon cancer OSN as well as a cross-reactive normal protein from the urine of colon cancer patients and healthy donors, respectively. The colon cancer OSN and normal cross-reactive protein display an apparent molecular weight of 29,000, have a similar linear tryptic peptide map, and are indistinguishable by isoelectric focusing analysis. Regardless of the molecular similarity, only the colon cancer OSN preparation could induce a positive LAI when incubated with leukocytes of colon cancer patients. Seven additional anti-colon cancer OSN mAbs were prepared against purified material. These mAbs can be divided into three groups, each of which recognizes a distinct antigenic determinant that is shared by the colon cancer neoantigen and its cross-reactive normal protein.

INTRODUCTION

The demonstration of antitumor immune response in humans is largely dependent on in vitro assays. One of such assays that demonstrate specific antitumor immune response is the LAI assay, which is based on the phenomenon that leukocytes from cancer patients lose their normal ability to adhere to glass when incubated in the presence of specific tumor antigen (1). Since the introduction (2) and modification (3) of the LAI assay, its validity and reproducibility were established (4–7). The adherence of leukocytes of patients with a given carcinoma was inhibited only as a result of their incubation with antigen derived from a tumor of the same organ and histogenesis (8). The mechanism by which the LAI phenomenon operates was recently elucidated (9). A pivotal role is attributed to monococytes "armed" with cytophilic antitumor antibodies. Upon encounter with the specific tumor antigen, cross-linking of the cell bound antibody induces subsequent biochemical events leading to arachidonic acid synthesis. The leukotriene metabolites of arachidonic acid are the actual mediators of the LAI phenomenon (8, 9).

The specific antitumor immune response as revealed by the LAI assay is directed at a common antigen which has been defined as an OSN (8), which is shared by most of the tumors of the same organ and histogenesis. The OSN is an integral membrane protein which can be solubilized by papain digestion and is probably associated with β2-microglobulin (10). OSN molecules which are shed from the tumor cell surface find their way into the circulation and are ultimately filtered into the urine (11). The establishment of the blocking assay of LAI (12) enabled the detection of OSN in serum and urine of cancer patients as well as in spent medium of human tumor cell lines (5, 13).

Although organ specific, the LAI assay is tedious and requires fresh supply of "armed" leukocytes. In attempts to establish more convenient immunoassay and to obtain large amounts of OSN, we describe here the preparation of monoclonal antibodies against a LAI reactive OSN and their usage for the affinity purification and characterization of cancer OSN from the urine of colon cancer patients. In addition, we report the purification of NCP from the urine of healthy donors.

MATERIALS AND METHODS

Source of Antigen. The LAI reactive preparation that was used for immunization and screening was partially purified and characterized by Dr. Aaron Fink in Dr. D. M. P. Thomson's laboratory at McGill University. Protein was extracted from the urine of lung cancer patients by precipitation with 80% saturated ammonium sulfate and was further purified by the following methods: (a) affinity chromatography on blue Sepharose; (b) ion exchange chromatography on DEAE A50; (c) molecular sieve chromatography on Sephadex G-75 superfine; and (d) adsorption chromatography on hydroxylapatite. In each stage, OSN was detected by the LAI assay and the purity of the preparation was determined by chromatography of the radioiodinated proteins on polycrylamide gels containing SDS. The purification procedure is described by Fink (14).

Immunization of Mice. BALB/c × C57BL/6 F1 mice were immunized by intrafootpad injections of 10 μg purified OSN per mouse in 50% complete Freund's adjuvant (Difco) on days 0 and 11 and with 10 μg OSN in 50% incomplete Freund's adjuvant (Difco) on day 22. One week following the final boost the mice were bled from the retroorbital sinus and their sera were tested individually for anti-OSN antibodies by RIA. The mouse with the highest titer was chosen for production of mAbs.

Generation of mAb. Four days before fusion the mice received an i.p. injection of 10 μg OSN in PBS. The fusion of P3-Ag4-1-NSO myeloma cells (kindly obtained from Dr. C. Milstein) with spleen cells of immune mouse, maintenance of cultures, cloning of hybridoma, and preparation of ascites fluid were done as described previously (15). Two groups of mAbs were prepared from mice immunized with (a) biochemically purified lung carcinoma OSN (LC series) or (b) immunofluorescence purified (on LC20.1 column) colon carcinoma OSN (CC series).

Solid Phase RIA. Screening of mAb and determination of antibody titers was conducted with a solid phase RIA system. Polystyrene chloride microtiter plates (Dynatech) were coated with affinity purified goat anti-mouse immunoglobulin antibodies (50 μg/ml in PBS; 50 μl/well; 2 h at room temperature) and washed three times with PBS. Hybridoma
supernatant or ascites fluid dilutions (50 µl/well) in duplicate were added, and the plates were incubated for 4 h at room temperature and washed three times with PBS. Radiolabeled OSNs (10⁶ cpm/well in 50 µl PBS plus 0.5% BSA) were then added and the plates were incubated for 16 h at 4°C. Following incubation, the plates were washed three times with PBS and dried under a lamp, and individual wells were cut and counted by a gamma spectrometer.

**Inhibition Radioimmunoassay.** Microtiter plates coated with affinity purified goat anti-mouse immunoglobulin (see above) were incubated with LC20.1 mAb (purified from ascites fluid on DEAE-52 cellulose, 1 µg/ml, 50 µl/well) for 4 h at room temperature. Following incubation, the plates were washed three times with PBS. Serial dilution of inhibitor antigen (50 µl/well) and radiolabeled OSN (10⁶ cpm/well in 50 µl PBS to 0.5% BSA) were then added. After 16 h incubation at 4°C the plates were processed and counted as described above.

**Antibody Cross-Inhibition Assay.** This assay was used to determine whether different mAbs recognized similar or different antigenic determinants. Microtiter plates were coated with anti-mouse IgG as described above. The first mAb was added to the wells (50 µl of hybridoma supernatant) and the plates were incubated for 4 h at room temperature. Following incubation the mAb was removed and the plates were incubated with normal mouse serum (50 µl of 1/100 dilution in PBS, 1 h at room temperature) and then with BSA (0.1 ml of 0.5-mg/ml solution in PBS for 30 min at room temperature). Following incubation the solution was removed and the second mAb (50 µl of hybridoma supernatant) and radiolabeled antigen (10⁶ cpm in 50 µl of PBS plus 0.5% BSA) were added to the wells. The plates were then incubated for 16 h at 4°C and washed three times with PBS, and individual wells were cut and counted. Assay was done in duplicate. Percentage of inhibition of the binding of the radiolabeled OSN to the plastic bound mAb was calculated from the maximal binding obtained by using PBS plus 0.5% BSA instead of the inhibitor mAb.

**Analysis of Radiolabeled OSN on SDS-Polyacrylamide Gels.** The OSN was radiolabeled by the chloramine-T method (16). The specific activity of the radiolabeled OSN was routinely around 2-3 µCi/µg. Radiolabeled OSN was separated on SDS-polyacrylamide gels under reducing conditions as described previously (10). The gels were dried and autoradiographed on a CURIX PP-2 X-ray film (Agfa) at −70°C.

**Construction of Immunoadsorbent and Absorption of OSN.** Coupling of the LC20.1 mAb and normal mouse immunoglobulin to agarose polycryllic hydrazide was performed as described previously (18). Agarose polycryllic hydrazide (Bio-Yeda) was washed four times with H₂O by centrifugation and suspended in glutaraldehyde solution (10% in H₂O) for 4 h at room temperature with slow stirring. Following incubation, the agarose was washed extensively with cold H₂O until no odor of glutaraldehyde was detected. The washed agarose was suspended in the protein solution (10 mg of 45% ammonium sulfate cut of LC20.1 hybridoma ascites fluid per 1 ml of agarose) and incubated for 16 h at 4°C with slow stirring. Following incubation, the nonbound protein was removed and the agarose was suspended in 2 volumes of hydratze hydrate solution (0.05 M in PBS, pH 8.0, fresh) for 1 h at room temperature. Following incubation, the agarose was washed with PBS and stored at 4°C in PBS containing 0.05% NaN₃. Before absorption of antigen, the immunoadsorbent was washed with 2 volumes of 0.2 M glycine-HCl, pH 2.5, and neutralized with PBS.

For absorption of antigen, 0.5 ml of washed agarose was incubated with 0.5 ml of concentrated (×20) spent medium of colon or lung carcinoma cell lines containing OSN for 30 min at room temperature. Following incubation the tubes were centrifuged and the supernatant was collected, diluted 1/10, and tested in a regular LAI assay.

**Immunoadsorption of OSN from Urine.** Urine collected from colon cancer patients and from healthy donors (1–10 liters) was stored at 4°C with 0.05% sodium azide. The urine samples were concentrated by ultrafiltration through a dialysis tube (cutoff, 13–15 × 10⁶ daltons; Spectrapore) to 20 ml and dialyzed against PBS. The antigen from the urine was affinity purified on LC20.1 mAb coupled to agarose. The urine samples were mixed with the conjugated agarose for 2 h at room temperature. The supernatants were discarded with PBS until effluents were free of protein, washed with 1 volume of PBS plus 1% Triton X-100, and finally washed with a large volume of PBS. Elution of the bound antigen was achieved by washing the columns with 2 volumes of 0.2 M glycine-HCl, pH 3.2, at 4°C. The eluate was dialyzed extensively against PBS and stored at −20°C. Each step of the purification was monitored for OSN activity by the inhibition RIA using LC20.1 mAb.

**Peptide Mapping.** OSN was incubated for several h with 8 U m urea and then dialyzed against 1 m urea. OSN in 1 m urea was iodinated by the chloramine-T method. Following iodination the OSN was dialyzed against 0.05 m NH₄HCO₃, pH 8.0. One million cpm of radiolabeled OSN were incubated with trypsin Tosylphenylalanly chloromethyl ketone (Millipore Corporation, 1 ml of 50 µg/ml in 0.05 m NH₄HCO₃, prepared fresh) for 16 h at 37°C and then lyophilized. The trypsin treated OSN was dissolved in acetic acid/formic acid:H₂O (15:5:80, v/v/v) and electrophoresed on cellulose coated thin layer chromatography plates (Merk) in the same buffer. Following electrophoresis the plate were dried at 80°C and autoradiographed on a CURIX PP-2 X-ray film (Agfa) at −70°C.

**IEF.** Radiolabeled OSN preparations were analyzed by the IEF method in 2.5- × 90-mm tubes containing 6% acrylamide, 5% ampholytes (pH interval, 3–10; Pharmacia), and 10% glycerol. The anode electrolyte was 0.01 m r-phosphoric acid, and the cathode electrolyte was 0.02 m NaOH. Following prefocusing of the gels (30 min at 1 m/A gel) the samples (in 10% sucrose) were loaded on top of the gels and focused for 4.5 h at room temperature. The pH gradient in the gels was monitored by cutting two gels into 0.5-cm slices, incubating each slice in 1 ml of distilled water (for 24 h at room temperature), and measuring the pH in each tube. In order to detect the focused proteins, the gels were cut into 2-mm slices and the radioactivity in each slice was measured by a gamma counter.

**Leukocyte Preparation.** Venous blood from patients was drawn into a 20-ml plastic syringe containing heparin, immediately transferred into a sterile glass tube, and incubated at 37°C for 45 min. The leukocyte rich fraction was aspirated and centrifuged at 2000 rpm for 5 min. The cell pellet was resuspended in 10 ml of isotonic Tris buffered NH₄Cl solution to lyse RBC. The leukocytes were then washed and suspended in medium M-199 to a concentration of 10⁷/ml.

**Preparation LAI Reactive Antigen from Tumor Lines.** Human colon carcinoma (HCT-15) and an oat cell carcinoma of the lung (NCI H69) (kindly donated by D. M. P. Thomson) were used. Cell lines were propagated in RPMI medium containing 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid, N-tris(hydroxymethyl)methylglycine, glutamic acid, penicillin, streptomycin, and 10% fetal calf serum. After 3 days of growth the cells were washed twice with PBS and supplemented with RPMI medium without fetal calf serum. Three days later, spent medium was collected and concentrated 20 times on a PM10 membrane (Amicon Corp., Holland). Concentrated medium was centrifuged at 20,000 × g for 20 min, aliquoted, and frozen at −70°C. For use in the assay, samples were quickly thawed and diluted 1/20 with medium M-199. Thawed samples were used once and then discarded.

**Tube LAI Assay.** This assay was essentially performed as described previously (7) with some minor modifications. To triplicate glass tubes (20 ml, 16 × 150 mm) were added 0.2 ml of medium M-199, 0.1 ml of fetal calf serum containing 0.1 mg of protein, 0.1 ml of spent medium containing the tumor antigen, and 0.1 ml of the patient leukocytes (1 × 10⁸ cells). The tubes were well agitated, placed horizontally so that the content covered four-fifths of the length of the tube, and incubated at 37°C in a 5% CO₂ humidified atmosphere. Two h later the tubes were placed upright and the contents at the bottom were gently agitated with a Pasteur pipet. For counting, a sample of cells was aspirated into a hemocytometer and the cells were automatically counted by an image analyzer (Aritek Corp.). The results are expressed as NAI.

\[
\text{NAI} = \frac{A - B}{B} \times 100
\]

where \( A \) is the number of nonadherent cells incubated with the specific antigen and \( B \) is the number of nonadherent cells incubated with unrelated tumor antigen. NAI values lower than 30 as a cutoff point were chosen because more than 95% of control subjects fall below this value (6). All NAI values above 30.0 were considered to be positive (6).

**Induction of LAI on Normal Leukocytes by Immune Complexes.** Immune complexes were prepared in vitro by the addition to 20-ml
glass tubes of 0.15 ml of medium M-199, 0.1 ml of fetal calf serum, 0.1 ml of spent medium of a tumor cell line (diluted 1/20) as an antigen source, or medium M-199 alone, and 0.05 ml of hybridoma supernatant. Triplicate tubes were then incubated for 1 h at 37°C in a shaking bath. Normal PBL, prepared as described above, were then added and the LAI assay was performed as described above.

RESULTS

Establishment of mAb against the LAI Reactive OSN. For the generation of mAbs a mouse which was immunized with purified lung carcinoma OSN and which exhibited the highest titer of anti-OSN antibodies in its serum was chosen for fusion. Spleen cells were fused with the NSO myeloma cell line and hybrid cell growth was apparent in more than 90% of the seeded wells. When screened for antibody activity by the solid phase RIA we found that only one hybridoma secreted anti-OSN antibodies. This hybridoma (denoted LC20) was cloned in semisolid agar, and one clone (LC20.1) was used in this study. The supernatant of hybridoma LC20.1 was titrated by the RIA to an end point titer of 1:1024 and its ascites were titrated to an end point of 1:10⁹.

LC20.1 mAb Recognition of LAI Reactive OSN from Human Colon and Lung Carcinoma Cell Lines. Spent medium of carcinoma cell lines (adenocarcinoma of the colon and oat cell carcinoma of the lung) contained OSN activity which inhibited the adherence of PBL from the respective cancer patients. Table 1 shows the results of 10 experiments in which positive NAIs were obtained when PBL of lung and colon cancer patients were incubated with the appropriate pair of OSNs. The same pair of OSNs incubated with normal PBL resulted in a negative NAI.

To test the specificity of the LC20.1 mAb we used a modification of the LAI assay based on the fact that normal leukocytes demonstrate a positive NAI when challenged with immune complexes. As shown in Table 1, positive NAI was obtained when normal peripheral leukocytes were "armed" by preformed complexes of LC20.1 mAb and OSN present in the spent medium of colon and lung carcinoma cell lines. Conversely, these normal PBL were not able to generate a positive NAI when incubated with either mAb or spent medium alone.

<table>
<thead>
<tr>
<th>Diagnosis of leukocyte donor</th>
<th>Antigen A</th>
<th>No.</th>
<th>Cell line</th>
<th>No.</th>
<th>NAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>HCT 15</td>
<td>152 ± 30</td>
<td>NCI H69</td>
<td>93 ± 28</td>
<td>64.4</td>
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<tr>
<td>Normal</td>
<td>HCT 15</td>
<td>94 ± 32</td>
<td>MDA²</td>
<td>90 ± 30</td>
<td>4.4</td>
</tr>
<tr>
<td>Normal</td>
<td>HCT 15 +</td>
<td>159 ± 66</td>
<td>NCI H69</td>
<td>129 ± 48</td>
<td>32.5⁷</td>
</tr>
<tr>
<td>LCC20.1 mAb²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung cancer</td>
<td>NCI H69</td>
<td>131 ± 46</td>
<td>HCT 15</td>
<td>80 ± 50</td>
<td>63.7⁹</td>
</tr>
<tr>
<td>Normal</td>
<td>NCI H69</td>
<td>94 ± 30</td>
<td>HCT 15</td>
<td>91 ± 43</td>
<td>3.2</td>
</tr>
<tr>
<td>Normal</td>
<td>NCI H69 +</td>
<td>170 ± 75</td>
<td>HCT 15</td>
<td>116 ± 43</td>
<td>46.5⁸</td>
</tr>
<tr>
<td>Normal</td>
<td>LCC20.1 mAb²</td>
<td>115 ± 50</td>
<td>NCI H69</td>
<td>118 ± 42</td>
<td>-2.6</td>
</tr>
</tbody>
</table>

² Spent medium recovered from a breast carcinoma cell line.
³ Complexes made between colon adenocarcinoma OSN and LCC20.1 mAb.
⁴ Difference in nonadherent cells is statistically significant (P < 0.05, Student's t test).
⁵ Complexes made between an oat cell carcinoma OSN and the LCC20.1 mAb.

Table 1 Induction of LAI by complexes made between LC20.1 mAb and OSN from colon and lung carcinoma cell lines.

In order to show that the LC20.1 mAb can recognize all the OSN of colon and lung carcinoma cell lines, it was bound to agarose and the spent medium recovered from the colon adenocarcinoma and lung carcinoma cell lines was reacted on this immunoadsorbent. As shown in Table 2, such treatment removed all the LAI activity present in the supernatants, whereas passage through control immunoadsorbent did not reduce the NAI.

Affinity Purification of Colon Cancer OSN and NCP from Urine. Concentrated and dialyzed urine collected from cancer patients and healthy donors were affinity purified on immunoadsorbent made of LC20.1. Each purification step was monitored by the inhibition RIA that could detect 10–1000 ng OSN/ml. The purification was calculated to be 70–75% and we could obtain about 2 mg of purified antigen from 1 liter of urine. The activity of the affinity purified proteins was tested in the standard LAI assay using 1 µg of protein/tube. Results of these experiments are shown in Table 2. The protein that was affinity purified from the urine of colon cancer patients was able to generate a positive LAI response only with leukocytes from colon cancer patients, comparable to that obtained for the same leukocytes when challenged with the spent medium of a colon adenocarcinoma cell line. In contrast, the protein that was affinity purified in a similar fashion from the urine of normal individuals (NCP) was unable to stimulate the leukocytes of colon cancer patients. The LAI activity of the antigen, derived from the urine of colon cancer patients, was colon specific since it could not generate a positive LAI response when incubated with leukocytes from breast cancer patients.

Biochemical Analysis of Affinity Purified OSN and NCP. Colon cancer OSN and NCP were radioiodinated and separated on SDS-polyacrylamide gel electrophoresis under reducing conditions and the gels were dried and autoradiographed. The results (Fig. 1) demonstrate that the purified colon cancer OSN and NCP are homogeneous preparations exhibiting one band with an apparent molecular weight of 29,000.

IEF analysis of the affinity purified and radioiodinated preparations of the colon cancer OSN and the NCP is presented in Fig. 2. Both preparations contain three peaks (pl 6.4, 5.4, and 4.2).

In an effort to distinguish between the closely related preparations of the colon cancer OSN and the NCP, we have compared their peptide pattern obtained after tryptic digest (Fig. 3). The results demonstrate that, under the conditions used, both the colon cancer OSN and NCP display indistinguishable linear peptide maps.

Binding of mAbs Directed at Different Antigenic Sites to OSN and NCP. Because we did not observe any gross biochemical differences between OSN and NCP and since the LAI assay can distinguish between these preparations, we examined whether additional mAbs might reveal such antigenic differences. These antibodies, raised against either biochemically purified lung cancer OSN (LC series) or affinity purified (on LC20.1 column) colon cancer OSN (CC series), could be divided into three groups according to three clusters of different epitopes that they recognized on the OSN molecule. Thus, mAb LC2, LC4, and CC4 reacted with the same epitope of LC20.1 while mAbs LC5 and CC3 belonged to a second set and LC7 and CC16 belonged to a third group of mAbs. When tested for their ability to bind the colon cancer OSN and the NCP, all of the antibodies, regardless of their fine specificity, could bind similar quantities of both antigenic preparations (data not shown).
AFFINITY PURIFICATION OF ORGAN SPECIFIC NEOANTIGEN

Table 2. LC20.1 mAb eliminates the LAI activity from the supernatant of colon and lung carcinoma cell lines

Leukocytes from colon and lung cancer patients were tested in a regular LAI assay with OSN from the supernatant of colon (HCT 15) and lung carcinoma (NCI H69) cell lines and with the same supernatants that were absorbed on immobilized NMIg or LC20.1 mAb. Results represent the mean ± SD of 4 experiments.

<table>
<thead>
<tr>
<th>Diagnosis of</th>
<th>Cell line</th>
<th>Antigen A</th>
<th>No.</th>
<th>Antigen B</th>
<th>Cell line</th>
<th>No.</th>
<th>NAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>HCT 15</td>
<td>157 ± 35</td>
<td></td>
<td>NCI H69</td>
<td>99 ± 17</td>
<td></td>
<td>58.5*</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>HCT 15 absorbed on NMIg agarose</td>
<td>146 ± 28</td>
<td></td>
<td>HCT 15 absorbed on LC20.1 mAb</td>
<td>98 ± 15</td>
<td></td>
<td>50.0*</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>NCI H69</td>
<td>169 ± 36</td>
<td></td>
<td>HCT 15</td>
<td>108 ± 26</td>
<td></td>
<td>56.5*</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>NCI H69 absorbed on NMIg agarose</td>
<td>155 ± 27</td>
<td></td>
<td>NCI H69 absorbed on LC20.1 mAb</td>
<td>103 ± 11</td>
<td></td>
<td>50.5*</td>
</tr>
</tbody>
</table>

* NMIg, normal mouse immunoglobulin.

** Differences in nonadherent cells are statistically significant (P < 0.05, Student’s t test).

Fig. 1. Analysis of OSN on SDS-polyacrylamide gels. Affinity purified NCP (A) and colon cancer OSN (B) preparations were radioiodinated and separated on SDS containing 10% polyacrylamide gels under reducing conditions.

Fig. 2. IEF analysis of affinity purified OSN. Affinity purified colon cancer OSN and NCP were radioiodinated and analyzed on polyacrylamide gels containing 5% ampholytes (pH range, 3–10). ———, colon cancer OSN preparation; ———, NCP.

DISCUSSION

Although specific tumor antigens have been defined in experimentally induced murine tumors, their existence in spontaneously arising tumors in humans is still debatable. Since the conceptual definition of a tumor antigen should be based not only on its concomitant appearance with the malignant process but also on its immunogenicity in the host, very few assays and antigens can fulfill such strict definitions.

One of the assays that can detect the presence of antitumor immune response in the human host is the LAI assay. Since its inception (2) and modification (3) relatively few investigators have availed themselves of this technique. However, methodological problems have hampered its introduction into routine clinical work. The major difficulty has been the constant need for metastatic liver as a source for antigen extraction and the tedious task of testing each new preparation for activity and specificity. In addition, the manual determination of the LAI results was tedious and subjective. Nevertheless, recent developments such as the standardization of the challenging antigen by the use of spent medium from established human carcinoma cell lines and computerized average analysis made the use of the assay possible on a large scale routine basis (13, 19). The sensitivity of the LAI test, defined as its ability to detect colorectal cancer, decreased with tumor burden and was low in Duke’s stage D cancer (19, 20). However, this problem could be overcome in part by the addition of prostaglandin as modulator of the LAI assay (21, 22). After prostaglandin stimulation, patients with Duke’s stage D in our hands responded positively with subsequent increase in sensitivity to 57% (19). Regardless of the aforementioned technical problems, in the hands of different groups the LAI assay has shown sensitivities of 50–60% in the identification of colorectal carcinoma (6, 19, 20). The specificity of the LAI assay, defined as its ability to correctly identify absence of disease, was found to be around 90% for a normal healthy population and almost 100% considering patients with noncolorectal malignancies (3, 19, 20, 23, 24), implying the specificity of the assay for colorectal cancer. Beyond routine clinical studies, the LAI offers a unified concept for human tumor antigens. It is with the LAI that one can define OSN as proposed by Thomson et al., i.e., the existence of a tumor derived immunogenic protein. Accordingly, it was
expected that it would be possible to isolate a LAI active protein from biological fluids by conventional techniques that would differ from other molecules which are tumor associated but which would not be immunogenic in the human host. Our results with monoclonal LC20.1 seem to comply with this concept.

In the present study, LC20.1 mAb was shown to form a complex with a crude OSN preparation derived from colon and lung carcinoma cell lines. These complexes bound to PBL of healthy donors and resulted in nonadherence of the cells (Table 1). Because the LAI response is triggered by antigen that cross-links cell bound cytophilic antibody molecules, it is not clear how the LC20.1 mAb, which is of the IgG class, induces such effects. Such bridging could occur if the OSN molecule contains more than one epitope that is recognized by the LC20.1 or if the OSN preparation used in this study was aggregated.

Although we were not able to demonstrate organ specificity for LC20.1, it was useful to affinity purify LAI active colon OSN as well as a NCP from the urine of normal individuals. Both colon cancer OSN and the NCP have the same apparent molecular weights, share common antigenic sites, have identical linear tryptic peptide maps and are indistinguishable by IEF analysis. However, although they are very similar biochemically, only the material purified from the urine of a colon cancer patient is reactive in the LAI assay with leukocytes of colon cancer. (Table 2). Our inability to reveal biochemical and immunochemical differences between the OSNs of different tumor patients and NCP, which are readily distinguished by the patient’s immune system, is probably related to either the weak discriminatory power of our methods or to the fact that the patients’ OSN preparation mainly consisted of NCP molecules and only very little of the colon cancer specific OSN. In addition, we could not detect organ specific antibodies in numerous murine polyclonal antibody preparations tested (data not shown). It is likely that the large quantities of NCP in the cancerous OSN preparation interfered with the detection of such antibodies. Alternatively, it is possible that such organ specific determinants are more immunogenic in the autologous human system.

Taken together, our data suggest the existence of at least two major groups of antigenic determinants on OSN, one of which is organ specific and recognized by the LAI assay. The second is common to OSN from other organs and to its normal counterpart (NCP) and is recognized by the immune system in heterologous immunizations. We have shown here, using different sets of mAb, that the OSN antigenic determinants are composed of at least three epitopes.

Currently, we are directing our efforts toward the preparation of additional sets of mAb against affinity purified OSN from different sources in attempts to obtain a mAb that will recognize the cancer and organ specific determinants of OSN. Once prepared, these mAb may be used in order to devise an immunoassay for the detection of colon cancer OSN in the sera of cancer patients. Hopefully, this assay will replace the LAI assay as a sensitive and reliable test for the diagnosis of colon cancer.

ADDENDUM

When this manuscript was in preparation we became aware of a study by D. M. P. Thomson et al. that had been submitted for publication and that reported the affinity purification of LAI reactive proteins from lung cancer cell line using anti-OSN mAb. Unlike the studies reported herein showing that the colon OSN we affinity purified from colon cell line had a molecular weight of 29,000, the proteins isolated by Thomson et al. consisted of a major M, 40,000 protein and minor bands with molecular weights of 32,000, 25,000, and 13,000.

REFERENCES


Cancer Research

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