ABSTRACT

Murine monoclonal antibodies against carcinoembryonic antigen (CEA) derived from a colonic tumor were analyzed by radioimmunoassay for reactivity with CEA and the CEA-related antigens, meconium antigen (MA) and nonspecific cross-reacting antigen. Antibody-antigen binding profiles revealed three classes of hybridomas. The Class I antibody, NP-1, recognized an epitope shared among all three antigens, and its affinity for CEA and MA was high but low for nonspecific cross-reacting antigen. The Class II antibodies reacted with sites shared between CEA and MA, while those of the Class III type only bound CEA. The Class III antibody, NP-4, bound less than 50% of the CEA molecules recognized by goat specific anti-CEA antibody and the other classes of monoclonal antibodies. Two Class II antibodies, NP-2 and NP-3, bound similar amounts of CEA and MA with moderate but different affinities for CEA. Studies using labeled monoclonal antibodies for CEA epitope blocking revealed that NP-2 and NP-3 recognize two separate epitopes on CEA within the Class II category. Thus, monoclonal antibodies to CEA can differentiate at least four antigenic sites on colonic cancer CEA. One is shared by CEA, MA, and nonspecific cross-reacting antigen; two others are shared by CEA with MA; and a fourth appears specific for a subpopulation of CEA molecules.

INTRODUCTION

Since the discovery of CEA (11), the definition of this antigen has been based on the molecule fulfilling certain physicochemical, immunological, and even biological criteria. As originally described, CEA was restricted to fetal and malignant gastrointestinal tissue and was an M, 200,000 β-glycoprotein, soluble in perchloric acid and having a particular amino acid and carbohydrate composition (12, 16). The identification of this substance was solely dependent upon the ability of an antiserum to recognize a specific immunodominant grouping on the glycoprotein as that detected by the original antiserum of Gold and Friedman (11). It has been subsequently shown that CEA exhibits extensive heterogeneity in its physicochemical and immunological properties (reviewed in Refs. 3, 8, 10, 25 and 30). A major question that has permeated many investigative studies of CEA, including its clinical application, has been the specificity of the various methods used to detect this antigen. Conventional antisera raised against CEA characteristically contain antibodies that react with a group of substances closely related to CEA (36). At present, the major members of this family of CEA-related antigens consist of the NCA which shares a similar tissue distribution with CEA (37), the NCA-2 (6) and NFA-2 (18) found in meconium and adult feces, and the biliary glycoprotein I present in normal bile (35). Although the clinical utility of some of these antigens is unknown and it remains to be clarified whether they represent cleavage products of CEA, CEA precursor molecules, or truly different gene products, their identification has resulted in the delineation of at least 3 epitopes on CEA that are unequally shared between the related antigens (7, 18, 26).

Recent efforts to improve the immunospecificity of CEA detection and measurement have centered on the development and comparison to conventional assays of monoclonal antibodies against CEA (5, 9, 24, 32). However, it is anticipated that cross-reactivity with CEA-related antigens will be encountered with monoclonal antibodies to CEA, analogous to their conventional counterparts. This paper describes the development of monoclonal antibodies to CEA and characterizes their cross-reactivity with NCA and an antigen, MA, that we have isolated recently from meconium (27). This work has appeared in preliminary form elsewhere (22).

MATERIALS AND METHODS

Antigens and Goat Antisera. CEA, used for mouse immunization, and NCA were isolated from liver metastases of a colonic adenocarcinoma, and MA was purified from meconium as described previously (27). All antigens were radioiodinated by the chloramine-T method (13) with 125I (Amersham/Searle Corp., Arlington Heights, Ill.) to a specific activity of approximately 30 Ci/g. Radiolabeled CEA from the Roche CEA assay kit (Nutley, N. J.) was used routinely when it was found to give results similar to those obtained with the CEA used for immunization.

The goat anti-CEA antibody in the Roche kit was used in RIA. Goat anti-NCA antiserum (No. 80) was generously supplied by Edward Newman, Roche Research Center, Nutley, N. J., and, before use, was depleted of antibodies cross-reacting with CEA by passage over a CEA immunoadsorbent.

Binding characteristics of labeled CEA, MA, and NCA with goat antiserum are described elsewhere (27). The Roche kit antibody and a goat anti-CEA antiserum made specific for CEA by absorption with NCA and meconium (27) reacted similarly with labeled CEA. The labeled MA was bound by Roche kit antibody but not by the goat specific anti-CEA antiserum. NCA did not react with either the Roche...
kit antibody or the specific anti-CEA antiserum nor did goat specific anti-NCA antibody bind CEA and MA.

**Mouse Immunization.** Female BALB/c mice (Harlan-Sprague-Dawley, Indianapolis, Ind.), 3 to 4 months old, were given 3 i.p. injections of 20 μg of CEA in incomplete Freund's adjuvant. The second and third injections were separated from the first by 2 and 8 weeks, respectively. Six months after the initial immunization, 2 mice demonstrating serum antibody against CEA were selected as spleen cell donors and received a final series of CEA injections, 50 μg each in 0.9% NaCl solution, according to the immunization protocol of Stahl et al. (34). At 4 days and 1 day prior to fusion, the CEA was injected i.p., while at 3 and 2 days before fusion it was equally divided for both i.p. and i.v. injections.

**Cell Fusion and Cloning.** Two separate fusions were performed with spleen cells obtained from 2 CEA-immunized mice according to the method of McKeever (19). For each fusion, 5 x 10⁹ Ficoll-Hypaque-separated spleen cells and 5 x 10⁶ P3-X63-Ag8.653 myeloma cells (Salk Institute, San Diego, Calif.) were mixed in a 60-mm culture dish and centrifuged at 250 x g for 5 min. Excess medium was removed and replaced with 1.0 ml of 50% (v/v) polyethylene glycol 1500 (Fisher Scientific Co.) in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, N.Y.) at 37°. After a 30-sec exposure to polyethylene glycol, the cells were washed twice and then incubated overnight in 5 ml of Dulbecco's modified Eagle's medium supplemented with 20% α-gamma horse serum (KC Biologicals, Inc., Lenexa, Kans.), 2 mM L-glutamine, 1 mM sodium pyruvate, 0.55 mM L-arginine, 0.27 mM L-asparagine, and 14 mM folic acid. The cells were dispersed in 30 ml of the latter medium further supplemented with 0.1 mM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine. The cell suspension was distributed into 96-well microwell plates (Costar, Cambridge, Mass.) 100 μl/well. An additional 100 μl of medium minus the aminopterin were added to the wells 7 days later. After 2 to 4 weeks of culture, the medium from wells containing growing cell clones was changed into 24-well plates (Costar) containing 10⁶ irradiated (1400 R) Lewis rat thymocytes per well. Upon reaching confluence, all cultures containing growing cell clones were assayed for CEA antibody by RIA. CEA antibody-positive wells were identified from culture media containing 10% α-gamma horse serum. Briefly, immunoglobulin was precipitated with 50% (NH₄)₂SO₄ at pH 7.0, re-dissolved in distilled H₂O, and reprecipitated with polyethylene glycol 7000 to 9000 at 13° (w/v) final concentration. The polyethylene glycol precipitate was solubilized in 0.02 mM phosphate (pH 5.6) and applied to a carboxymethylcellulose (Whatman, Inc., Clifton, N.J.) column equilibrated in the same buffer. Adsorbed immunoglobulin was eluted with 0.02 mM phosphate (pH 7.8):0.2 mM NaCl, dialyzed against 0.01 mM phosphate (pH 8.0), and applied to a DEAE-cellulose (Whatman) column equilibrated in the latter buffer. Adsorbed antibody was eluted with 0.025 mM phosphate (pH 8.0), equilibrated against PBS, and concentrated by ultrafiltration (Amicon, Danvers, Mass.). The purified monoclonal antibodies were radioiodinated by the chloramine-T method to a specific activity of 5 to 10 μCi/μg. In addition to mouse IgG, the purified antibody contained 10 to 40% horse immunoglobulin as determined by binding of the radioiodinated preparation to solid-phase goat anti-horse IgG. The percentage of monoclonal anti-CEA antibody in the preparation was determined by binding of the radioiodinated antibody to a CEA immunoadsorbent, as described previously (29). Antibody isotype was determined by double immunodiffusion with class and subclass specific antisera (Litton Bionetics, Kensington, Md.).

**RIA.** Two types of RIAs were used, differing in the method of separating bound and free labeled antigen. Assay of supernatant media from hybridoma cultures for anti-CEA and anti-MA antibody activity used the Z-gel method described by Hansen et al. (14). Binding studies of selected monoclonal antibodies with CEA and MA, and all assays for NCA reactivity, used a solid-phase double antibody procedure (23).

The basic assay consisted of 2.0 ml of 0.01 M ammonium acetate (pH 6.25), containing 1.0% normal rabbit serum, about 0.5 ng of labeled antigen (30 to 50 μCi/μg), and 0.05 ml aliquots of antibody preparations. Incubations at 45° for 1 hr were used for the detection of CEA and MA antibody activity in hybridoma culture media, while 4-hr incubations at the same temperature were used for the detection of NCA antibody. Labeled antigen binding curves and competitive inhibition determinations were derived after incubation for 4 hr at 45° and 24 hr at room temperature, respectively. After incubation, 1.0 ml of Z-gel or solid-phase antiimmunoglobulin, GAM or DAG, was added, and the tubes were mixed. Tubes receiving Z-gel were centrifuged immediately, while an additional 15-min incubation at room temperature was carried out for tubes receiving GAM or DAG. Tubes were washed once with 2.0 ml of 0.1 M ammonium acetate (Z-gel assays) or PBS (double antibody assays) before counting. Nonspecific binding was 10% and 1% for Z-gel and double antibody assays, respectively. Monoclonal antibody, goat antisera, unlabeled antigen, and labeled antigen were diluted in PBS containing 1% human serum albumin.

**Antibody Affinity Determination.** Antibody affinity was determined by the competitive RIA method of Müller (21). The product of the calculated association constant and antibody concentration was less than 10, as recommended by Jacobsen et al. (15).

**Blocking Assays.** Reciprocal blocking experiments used a solid-phase competitive sandwich procedure. Polyclonal goat anti-CEA serum was bound to vinylidine fluoride powder (Kynar, Grade 301F; Pennwalt Corp., King of Prussia, Pa.) and sensitized with unlabeled CEA by incubation for 1 hr at 45° in 0.01 M ammonium acetate. The amount of CEA used bound 40% of the maximal amount of bindable radiolabeled monoclonal antibody. The CEA-sensitized Kynar, 0.5 ml, was incubated with 0.05-ml aliquots of monoclonal antibody dilutions for 1 hr at 45° and centrifuged, and the pellet was resuspended in 1.0 ml of 0.01 M ammonium acetate containing 1% normal rabbit serum. Radioiodinated monoclonal antibody contained in 0.05 ml was added to the tubes, incubated for 1 hr at 45°, centrifuged, and washed once, and the pellet was then counted. Nonspecific binding to unseparated Kynar of the labeled antibody preparations was less than 5%.

**Erythrocyte Binding.** Erythrocytes from A, B, and O secretor and nonsecretor individuals were combined in various proportions as a 2% or 10% suspension and incubated for 1 hr at room temperature with 50-μl aliquots of hybridoma tissue culture medium. The cells were washed, resuspended in PBS, and incubated for 1 hr at room temperature with radiiodinated affinity-purified GAM having heavy and light chain specificities. After incubation, the cells were washed and counted.

**RESULTS**

**Classes of CEA Hybridoma Clones.** Of 750 wells seeded from the 2 separate fusions, 274 wells (37%) produced growing hybrid cell clones after 2 to 4 weeks. Assay of wells showing cell growth detected antibody against CEA in 95 (34%) of the wells. Hybridomas from positive wells were expanded into 2-ml cultures, 67 of which continued to produce anti-CEA antibody and bound 36% (±1.8 S.E.) of radiolabeled antigen in Z-gel assays. The culture supernatants from the 67 clones were also evaluated for reactivity in RIA against the 2 CEA-related antigens, NCA and MA. Antigen binding profiles revealed that the hybridoma clones could be differentiated into 3 distinct classes based on their comparative immunoreactivity with CEA, MA, and NCA (Table 1). Most of the clones from the first fusion
produced antibody that bound both CEA and MA (Class II), while the clones from the second fusion were about equally distributed between Class II and those showing CEA reactivity only (Class III). Reactivity with all 3 antigens (Class I) was found in 16 and 2% of the clones from the first and second fusions, respectively. None of the CEA-reactive cultures bound to human erythrocytes from both secretor and nonsecretor individuals.

Antigen Binding in RIA of Selected Clones. Four hybridoma clones, one from Class I (NP-1), 2 from Class II (NP-2 and NP-3), and one from Class III (NP-4) were selected and recloned for further characterization. The NP-1, -2, and -3 antibodies were purified from culture medium containing agamma horse serum, whereas NP-4 was used as an ascites fluid. The NP-4 clone produced antibodies of the IgG1 (\(\lambda\)) subclass, while the NP-4 clone produced antibody of the IgG1 (\(\kappa\)) isotope. These 4 monoclonal antibodies failed to precipitate CEA in double immunodiffusion.

Charts 1 and 2 demonstrate the binding characteristics of the 4 monoclonal antibodies, Roche kit goat anti-CEA antibody, and goat anti-NCA antibody with labeled CEA, MA, and NCA. NP-1, -2, and -3 bound 60 to 70% of CEA and MA, similar to the immunoreactivity of goat anti-CEA antibody with these antigens (Chart 1). NP-4 failed to bind MA and maximally bound only about 30% of the radiolabeled CEA. Normal murine ascites fluid or serum did not react with CEA, and the maximum CEA binding level observed with NP-4 was characteristic of the other Class III hybridomas (data not shown). In addition to binding CEA and MA, the Class I monoclonal antibody, NP-1, reacted with labeled NCA to the same extent as goat anti-NCA antibody (Chart 2). However, 2600 ng of NP-1 were required to bind 30% of the labeled NCA, whereas only 1.1 and 2.5 ng of this antibody were needed to bind the same level of CEA and MA, respectively. Inspection of Chart 1 also reveals that slightly higher quantities of NP-2 were required to bind MA as compared to CEA, while the reverse was true for NP-3.
Since the Class III hybridoma clone, NP-4, maximally bound only 30% of the labeled CEA as contrasted to 70% binding by goat anti-CEA antibody. 2 experiments were designed to show that NP-4 was recognizing a population of CEA molecules also detected by goat antibody. The first experiment tested for additive binding of CEA by mixing NP-4 and goat antiserum together, in quantities at maximal antigen binding level for each, incubating, and then separating bound from free antigen with Z-gel (Table 2). The results of this test showed that the binding of CEA by the combined antibody mixture was no greater than that obtained with goat antiserum alone. If NP-4 was recognizing a population of labeled molecules distinct from those reacted with by goat antiserum, then a mixture of the 2 antibodies should have yielded an antigen binding percentage approximating a summation of the individual binding percentages. The second experiment evaluated the ability of goat antibody to block the reaction of NP-4 with the labeled antigen by sequential incubation of the antigen with goat antibody followed by NP-4, then separating bound from free antigen with solid-phase GAM (Table 2). As expected on the basis of the additive experiment, the goat anti-CEA antiserum completely inhibited the binding of NP-4 to the labeled antigen. Normal goat serum or irrelevant goat antibody against NCA was without effect.

**Competitive Inhibition and Antibody Affinity.** The ability of unlabeled CEA to inhibit the reaction of the 4 hybridoma monoclonal antibodies with labeled CEA was tested in competitive RIA. In this procedure, the quantity of monoclonal or goat antibody used was adjusted to give 50% binding of labeled CEA in the absence of unlabeled antigen. Under these conditions, the 4 monoclonal antibodies generated markedly different antigen inhibition curves (Chart 3). The NP-1 antibody was similar to Roche kit goat antibody in sensitivity to antigen inhibition, whereas the NP-2, -3, and -4 monoclonal antibodies required larger quantities of antigen for inhibition. Using M, 200,000 as the molecular size for CEA, the data obtained from Chart 3 were transformed into the determination of average affinity constants by the method of Müller (21) (Table 3). The K values obtained by this treatment, as well as the ng of CEA per ml needed to inhibit binding by 50%, further illustrate the similarity in antibody affinity between NP-1 and goat anti-CEA antibody and the decreasing antibody affinity of NP-2, -3, and -4.

Since markedly higher levels of the NP-1 monoclonal antibody were required to bind labeled CEA than those needed for labeled CEA or MA, a comparison was made among the abilities of CEA, MA, and NCA to inhibit the reaction of NP-1 with labeled CEA. The quantity of goat or monoclonal antibody used was adjusted to give 50% binding of labeled antigen in the absence of unlabeled antigen. The CEA inhibition of Roche kit goat antibody is depicted.
concentrations of NCA tested would the CEA contaminant begin to contribute to the inhibition induced by NCA. Using molecular weights of 200,000, 185,000, and 60,000 for CEA, MA (27), and NCA, respectively, the affinity of NP-1 was slightly lower for MA and 10 times lower for CEA compared to its affinity for CEA.

**Blocking of Monoclonal Antibody Binding.** The 2 monoclonal antibodies, NP-2 and NP-3, were initially selected for study on the basis of incubation buffer ionic strength influence on CEA binding. Table 4 shows that appreciably greater quantities of goat, NP-1, and NP-2 antibodies are needed to obtain 30% binding of labeled CEA in 0.1 M ammonium acetate, being 36 times greater for NP-2. By contrast, antigen binding by NP-3 and NP-4 in these 2 buffers was very similar. The possibility that NP-2 and NP-3 recognize separate epitopes within the Class II category was analyzed by reciprocal blocking experiments. In these studies, solid-phase polyclonal goat anti-CEA, sensitized with CEA, was used in a sandwich system with radiolabeled monoclonal antibody probes. The ability of unlabeled monoclonal antibodies to inhibit the binding of radiolabeled antibodies was evaluated in reciprocal cross-blocking experiments (Table 5). Although belonging to separate class categories, NP-1 and NP-2 were very efficient in blocking one another. NP-3 could not block NP-1 and NP-2 binding at the highest concentrations tested, nor could the latter monoclonal antibodies inhibit the binding of NP-3 to CEA.

**DISCUSSION**

Of the different approaches available to characterize epitope specificity of monoclonal antibodies (33), we have focused on the relative binding activities of monoclonal antibodies to CEA and related antigens. Our study demonstrates that monoclonal antibodies developed against colonic tumor CEA differentiate 3 general classes of antigenic determinants based on their reactivity with the CEA-like antigens, NCA and MA (Chart 5). The smallest percentage of clones produced antibody that recognized epitopes shared among all 3 antigens and belongs to the Class I category. Class II monoclonal antibodies reacted with sites shared between CEA and MA, while those of the Class III type bound only CEA, apparently recognizing determinants unique to this molecule. Thus, the immunogenicity in mice of the CEA cross-reactive epitopes and the spectrum of antibodies they elicit in this animal species coincide with similar antigenic properties of CEA as observed in conventional antisera raised in other species (18, 26, 29, 36).

Several studies have reported on the development of monoclonal antibodies to CEA (2, 9, 17, 20, 31, 33), but consideration of their cross-reactivity with related antigens has received limited treatment. Mitchell (20) found that his monoclonal antibody did not bind labeled NCA either in direct binding studies or by electrophoretic analysis of immune precipitates. Other groups reported that NCA could not inhibit the binding of their monoclonal antibodies to CEA in competitive CEA RIA (1, 32). We observed that the affinity of the NCA cross-reactive monoclonal antibody, NP-1, for NCA was markedly lower compared to its affinity for CEA. This property of NP-1 mimics conventionally prepared antibodies to CEA that also cross-react with NCA (29), suggesting that some of the CEA:MA:NCA common epitopes on NCA are altered in topography and/or chemistry or by electrophoretic analysis of immune precipitates. Other groups reported that NCA could not inhibit the binding of their monoclonal antibodies to CEA in competitive CEA RIA (1, 32). We observed that the affinity of the NCA cross-reactive monoclonal antibody, NP-1, for NCA was markedly lower compared to its affinity for CEA. This property of NP-1 mimics conventionally prepared antibodies to CEA that also cross-react with NCA (29), suggesting that some of the CEA:MA:NCA common epitopes on NCA are altered in topography and/or chemistry compared to those on CEA. It is possible then that the NCA cross-reactive property of a monoclonal antibody to CEA may not be revealed unless adequate quantities of antibody or NCA are used in direct labeled antigen binding or competitive inhibition assays, respectively. This does not appear to be the case for MA, since comparable quantities of NP-1 were effective in binding both CEA and MA, and MA was similar to CEA in inhibiting the reaction of NP-1 with CEA. In the accompanying study (28), we have shown that neutrophils stain positive with NP-1 as expected from their known synthesis of NCA but not CEA (4). Thus, the immunocytochemical staining reaction of neutrophils provides an alternative method of assessing the

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**Table 4**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Amount of antibody for 30% binding a</th>
<th>ISB b ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>1:12</td>
<td>1:2</td>
</tr>
<tr>
<td>NP-1</td>
<td>1.1</td>
<td>15.4</td>
</tr>
<tr>
<td>NP-2</td>
<td>4.0</td>
<td>14.0</td>
</tr>
<tr>
<td>NP-3</td>
<td>158.5</td>
<td>174.4</td>
</tr>
<tr>
<td>NP-4</td>
<td>1:320</td>
<td>1:320</td>
</tr>
</tbody>
</table>

a Dilution or ng of antibody required to obtain 30% binding of labeled CEA in 0.01 or 0.1 M ammonium acetate buffer.

b ISB, ionic strength binding ratio, the ratio of the amount of antibody required to bind labeled antigen in 0.1 M ammonium acetate buffer to the amount of antibody required to give the same level of binding in 0.01 M ammonium acetate.

**Table 5**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Blocking (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1 (Class I)</td>
<td>NP-1</td>
</tr>
<tr>
<td>NP-2</td>
<td>NP-2</td>
</tr>
<tr>
<td>NP-3</td>
<td>NP-3 &gt;5000</td>
</tr>
<tr>
<td>NP-2 (Class III)</td>
<td>NP-2</td>
</tr>
<tr>
<td>NP-1</td>
<td>NP-1</td>
</tr>
<tr>
<td>NP-3</td>
<td>NP-3 &gt;5000</td>
</tr>
<tr>
<td>NP-3 (Class III)</td>
<td>NP-3</td>
</tr>
<tr>
<td>NP-1</td>
<td>NP-1 &gt;5000</td>
</tr>
<tr>
<td>NP-2</td>
<td>NP-2 &gt;5000</td>
</tr>
</tbody>
</table>

Summary of blocking antibody binding to CEA.
NCA cross-reactivity of monoclonal antibodies to CEA.

The repertoire of CEA epitopes defined by monoclonal antibodies is only now beginning to be unraveled, and its resolution will require exchange of reagents and standardization of nomenclature. Stähli et al. (33) identified 6 different epitopes on CEA with monoclonal antibodies, although their cross-reactive nature was not established. Reciprocal blocking experiments revealed that the antibodies of Classes I and II, NP-1 and NP-2, respectively, were capable of inhibiting one another, although neither could block the Class II antibody, NP-3, or could be inhibited by NP-3. The reciprocal blocking characteristics between NP-1 and NP-2 suggest that the respective epitopes recognized by them partially overlap or are very close to one another. However, the lack of reciprocal blocking between NP-2 and NP-3 indicates that they recognized 2 separate epitopes within the Class II category. These 2 epitopes, together with at least one determinant within the categories of Classes I and III identified by NP-1 and NP-4, respectively, parallel similar quantitative and qualitative distribution characteristics of CEA epitopes as we have demonstrated previously with a conventional polyvalent anti-CEA antiserum (26, 27). Similar distribution patterns of CEA determinants were disclosed with polyvalent antisera in the studies of Burtin et al. (7) and Kuroki et al. (18), who used the reference antigens, NCA as well as NCA-2 (7) or NFA-2 (18). Our studies with monoclonal antibodies thus confirm these observations made with polyvalent antisera, in that they can differentiate at least 4 antigenic sites on colonic tumor CEA. One is shared by CEA, MA, and NCA (Chart 5, a1), 2 others are shared by CEA and MA (β1 and β2), and a fourth appears specific for CEA (γ1). We are currently examining our other hybridomas developed against colonic tumor CEA for their epitope specificity, and it is expected that additional CEA determinants will be identified within all 3 classes depicted in Chart 5.

The NP-4 monoclonal antibody as well as the other Class III antibodies typically bound less than 50% of the CEA molecules recognized by goat antibody specific for CEA. Kupchik et al. (17) and Rogers et al. (31, 32) also found that CEA was only partially reactive with their monoclonal antibodies to this antigen. The basis for the partial reactivity of Class III antibodies is unclear but may be related to epitope damage as a result of radioiodination, to affinity characteristics of the antibodies themselves, or to their discrimination of subpopulations of CEA molecules.

One major goal for the development of immunospecific monoclonal antibodies to CEA is to stimulate the evolution of refined applications of CEA detection. If various immunological forms of CEA are produced by different individuals or during different disease stages, as already observed by Rogers et al. (32) in their comparison of monoclonal antibody and conventional assays for blood CEA measurement, the ultimate utility of this approach might depend on the creation of a composite of monoclonal antibodies with defined specificities. The incorporation of CEA-related antigens for the determination of monoclonal antibody epitope specificity should facilitate delineation of those antibodies having diagnostic and prognostic relevance for CEA detection. This would be further strengthened by resolving the biological interrelationships between NCA-2, NFA-2, or MA and CEA and the role of the former antigens as tumor markers. Finally, the use of other methods such as immunohistochemistry (28) for the study of monoclonal antibodies to CEA might reveal certain molecular determinants having prognostic potential that would otherwise be unnoticed.

ACKNOWLEDGMENTS

We express our appreciation to Robert Collins, III, for his technical assistance.

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