Immunological Heterogeneity of Carcinoembryonic Antigen: Purification from Meconium of an Antigen Related to Carcinoembryonic Antigen¹

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ABSTRACT

Two antigens cross-reactive with carcinoembryonic antigen (CEA) and distinct from the nonspecific cross-reacting antigen were identified in meconium by double immunodiffusion with a conventional goat anti-CEA antiserum. These two antigens together competitively inhibited cross-reacting antibodies against them in CEA radioimmunoassay and contributed to the measurement of meconium CEA levels which averaged 6 times higher than that determined with anti-CEA specific antibody. A purification method for one of these antigens, tentatively designated meconium antigen, is described and uses a combination of ethanol fractionation, ion-exchange and molecular sieve chromatography, and adsorption to an immunoadsorbent containing a cross-reactive murine monoclonal antibody to CEA. Preliminary characterization of the purified meconium antigen showed it to be a glycoprotein, migrating as an α-globulin and having a molecular size similar to that of CEA (M, 185,000 versus 200,000). Antigenically, it lacks at least one determinant present on CEA and differs further from CEA by being weakly reactive with concanavalin A and resistant to proteolytic digestion with Pronase E. Although these properties of meconium antigen suggest that it may be nonspecific cross-reacting antigen 2, additional chemical and antigenic studies are required to establish its relationship to CEA and other CEA-related antigens in meconium.

INTRODUCTION

The discovery of CEA3 (5) prompted an extensive examination of the chemical characteristics and diagnostic merits of this tumor-associated antigen (1, 6, 8). During the course of these studies, several CEA-like substances have been described mainly as a result of incidental observations with antisera raised in different animal species against CEA or colon tumor extracts that contained cross-reacting antibodies (31). Among the latter antigens, the first to be described was NCA, a glycoprotein with a molecular weight of 60,000 present in normal human tissues at levels higher than CEA, especially in lung and spleen (31, 32). Several other substances have been described that are most probably NCA based on their immunological cross-reactivity with this antigen. In addition, several antigens in meconium have been described that are most probably NCA based on their immunological cross-reactivity with this antigen. These include normal glycoprotein (16), CEA-associated protein (4), colonic CEA-2 (30), colonic carcinoma antigen-III (21), and tumor-associated antigen (11). NCA and CEA can be distinguished immunologically since they express individually distinct determinants.

A second group of CEA-like antigens in meconium and adult feces was identified independently by Burtin et al. (2) and Matsuoka et al. (17). The NCA-2 characterized by Burtin and coworkers was slightly lower in molecular size than CEA and expressed a determinant not shared with either NCA or CEA (3, 31). NCA-2 appears more closely related to CEA than NCA since, in addition to a common determinant shared among the 3 antigens, a second epitope is shared only between NCA-2 and CEA (3). The NFA in adult feces (17) has been separated recently into 3 molecular species, all of which are immunochemically different from NCA-2, NCA, and CEA (14, 19).

Clarification of the chemical, antigenic, and development interrelationships between CEA-like antigens in meconium and CEA is needed since they may have a role as tumor markers themselves or contribute to the measurement of CEA levels in view of their strong immunological cross-reactivity with this antigen. We have reported previously in preliminary form the presence of 2 NCA-unrelated, CEA-like antigens in meconium that were recognized with cross-reactive antibodies in a conventional CEA antiserum (22). This paper reports the purification of one of these antigens, provisionally designated as MA, using a murine monoclonal antibody to CEA, and presents the initial description of some of its properties.

MATERIALS AND METHODS

Purification of CEA and NCA from Tumor Tissue. The preparation of CEA used in this study, other than for RIA, was isolated from liver metastases of colon adenocarcinoma according to the procedure of Krupey et al. (19), as modified by Newman et al. (21). Briefly, the concentrated PCA extract was applied to DEAE-cellulose: carboxymethylcellulose (1:1; Whatman, Inc., Clifton, N. J.) equilibrated to 0.1 M ammonium acetate, pH 4.0. Adsorbed substances were eluted with a discontinuous gradient of 0.05, 0.1, and 0.2 M NaCl in the same buffer. Approximately 30% of the initial CEA immunoreactivity in the PCA extract appeared in each of the application buffer (A), 0.05 M NaCl (B), and 0.1 M NaCl (C) fractions as monitored with the Roche CEA assay kit (Roche Laboratories, Nutley, N. J.). Fraction C was subjected to sequential chromatography over Sepharose 6B and Sephadex G-200 (Pharmacia Fine Chemicals, Piscatway, N. J.) in 0.05 M phosphate (pH 5.0):0.15 M NaCl buffer. A final separation step over concanavalin A-Sepharose (Pharmacia) was performed according to the method of Pritchard and Todd (25), whereby adsorbed antigen was eluted with 20% (w/v) α-methyl-D-glucoside at room temperature. The CEA was dialyzed against distilled water, lyophilized, and dried to constant weight over CaCl₂. The purified CEA had a specific activity (neutralizing activity per unit dry weight) of 0.7 as determined in the Roche assay. It migrated in immunoelectrophoresis as an α-globulin compared to normal serum and gave a band of identity with Roche reference CEA in double immunodiffusion. A single band was observed in 7.5% polyacrylamide gels stained for protein or carbohydrate.

Fraction A from the DEAE-cellulose:carboxymethylcellulose ion ex-
chamber contained NCA and was separated from CEA also present in this fraction by chromatography over a 5 x 90-cm Sephadex G-200 column. The presence of NCA was monitored by double immunodiffusion, and a peak of antigen activity appeared at an elution volume of 1000 to 1200 ml. This fraction was subjected to concanavalin A-Sepharose chromatography, as described for CEA, and the adsorbed antigen was further purified by passage over an anti-CEA immunoadsorbent. NCA was eluted from the immunoadsorbent with 0.2 M glycine-HCl (pH 2.0), neutralized with NaOH, dialyzed against PBS, and concentrated over an Amicon PM-10 membrane (Amicon, Lexington, Mass.). The purified NCA migrated as a β-globulin in immunoelectrophoresis and gave a reaction of identity with reference NCA in double immunodiffusion. NCA was also partially purified from normal spleen by immunoprecipitation, as described previously (24).

**Purification of MA from Meconium.** Meconium, 50 g, was suspended in 300 ml of 0.1 M phosphate (pH 8.0) and mixed overnight at 4°C. After centrifugation for 30 min at 10,000 g, the pH of the supernatant was adjusted to 8.5, and cold absolute ethanol was added to a final 40% concentration. The ethanolic supernatant obtained after centrifugation was dialyzed against 0.02 M phosphate (pH 7.8), mixed with 5.0 g of DE52 (Whatman) equilibrated with the same buffer, and poured into a 2.6 x 20-cm column. Following the elution of unbound substances with the application buffer, adsorbed materials were removed with a discontinuous NaCl gradient in the same buffer. Antigenic activity emerging in the 0.1 M NaCl fraction was dialyzed against 0.05 M phosphate (pH 5.0); 0.15 M NaCl, concentrated over a YM-10 membrane (Amicon), and applied to a 2.6 x 90-cm Sephacryl S-300 (Pharmacia) column. Antigenic activity contained with the 225- to 270-ml elution volume was pooled, dialyzed against 0.1 M phosphate (pH 7.0), and passed over an immunoadsorbent containing goat specific anti-CEA antibody. The unsorbed fraction from the latter immunoadsorbent was then applied to a second immunoadsorbent containing a murine monoclonal CEA antibody, NP-3, described elsewhere (23). On the basis of blocking studies with cross-reactive CEA antibodies from goats (22), NP-3 was shown to recognize a determinant shared between CEA and NCA-unrelated, CEA-related substances in meconium. Antigen bound to the NP-3 immunoadsorbent was eluted as described above for NCA purification. The adsorbed fraction was neutralized, dialyzed against PBS, and concentrated over a YM-10 membrane.

**Animal Immunization and Antiserum Preparation.** Goat anti-CEA antiserum was prepared by the injection of 100 to 200 μg of purified CEA coupled to an equal amount of methylated bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) and emulsified in an equal volume of complete Freund’s adjuvant (Difco Laboratories, Detroit, Mich.). Incomplete adjuvant was used after the initial injection. The goat anti-CEA antiserum, obtained after 21 injections, was repeatedly absorbed with A, B, and O erythrocytes from both Le a and Le b individuals until free of hemagglutinating activity. The antigen was then sequentially chromatographed over normal colon and NCA immunoadsorbents, the unsorbed fraction being used in both cases. Completeness of adsorption was analyzed in double immunodiffusion against normal tissue extracts and NCA. A portion of the NCA-neutralized anti-CEA antiserum was further adsorbed with meconium by adding 250 mg of alcohol-extracted meconium per ml of antiserum. After separation of the immune precipitate, completeness of adsorption was verified in double immunodiffusion against meconium. This NCA- and meconium-absorbed antiserum is hereafter identified as specific anti-CEA antiserum.

Goat anti-NCA antiserum was removed from antibodies cross-reacting with CEA by passage over a CEA immunoadsorbent. The CEA-neutralized antiserum failed to react with CEA in double immunodiffusion or RIA.

**Preparation of Adsorbents.** Our preparation of CNBr-activated Sepharose 4B is described in detail elsewhere (24). Five mg of immunoreactive CEA and 0.44 mg of partially purified spleen NCA were each coupled to 10 ml of gel. A normal colon immunoadsorbent (80 mg/20 ml gel) was prepared with a PBS extract of mucosal scrapings from a normal colon obtained at autopsy of an accident victim. The IgG in 10 ml of CEA-neutralized goat anti-NCA antiserum, 30 ml of goat specific anti-CEA antisemur, and 30 ml of ascorbic acid from mice bearing NP-3 hybridoma cells were separated by DEAE-cellulose chromatography in 0.025 M phosphate buffer (pH 8.0). Individual immunoadsorbents prepared with these antibodies were posttreated with 0.033% glutaraldehyde (12).

**RIA.** The Roche CEA assay kit was used to detect anti-CEA antibody and antigen. Purified NCA from colon tumor and MA were radioiodinated by the chloramine-T method (7) with 125I (Amersham/Searle Corp., Arlington Heights, Ill.) to a specific activity of 30 μCi/μg. Purified NCA was used as standard calibrated on the basis of Lowry protein (15) against bovine serum albumin (Miles Laboratories, Inc., Elkhart, Ind.). Inhibition curves had a range of 0.8 to 16.0 ng NCA. Incubations were carried out at 45°C for 30 min in 5.0 ml of 0.01 M ammonium acetate (pH 6.25), containing 1.0% normal rabbit serum and about 1.0 ng of labeled antigen. Free and bound antigen were separated with immobilized donkey anti-goat IgG (20).

**SDS-PAGE.** Analytical electrophoresis was performed on 7.5% polyacrylamide gels with 1.0% SDS (29). Samples were boiled for 5 min in the presence of 0.2 M mercaptoethanol:1.0% SDS prior to electrophoresis for 2 to 3 hr. Gels were stained with Coomassie Brilliant Blue or periodic acid-Schiff reagent.

**Immunodiffusion and Immunoelectrophoresis.** Double immunodiffusion in 1.0% agarose was routinely performed on doubling dilutions of antigen solutions. The plates were incubated at room temperature and observed for 3 days before staining with 0.1% amido black. The Cornling agarose film system (Corning, Palo Alto, Calif.) was used for immunoelectrophoresis.

**Digestion with Pronase E.** Digestion of CEA and MA with Pronase E (Sigma; type XIV) was carried out with labeled antigens containing 0.1% carrier human serum albumin according to Matsuoka et al. (18), with an enzyme:carrier substrate ratio of 1:25. Following incubation at 37°C for 48 hr, the digest was heated to 65°C for 10 min and analyzed in RIA as well as by Sephacryl S-300 chromatography in 6.0 M guanidine HCl.

**Reference Standards.** Reference CEA (lot-88B), NCA, goat anti-NCA antiserum (No.-80), and immobilized second antibody were generously supplied by Dr. Hans Hansen and colleagues, Roche Research Center, Nutley, N. J.

**RESULTS**

Identification of NCA-unrelated, CEA-cross-reactive Antigens in Meconium. When NCA-neutralized anti-CEA antiserum was tested in double immunodiffusion against individual meconium samples, about an equal number of specimens gave one or 2 precipitin bands that usually formed very close to each other. In most specimens in which 2 bands were present, both formed a reaction of partial identity with CEA (Fig. 1), while in less than 10% of unextracted or PCA-extracted specimens, the second band gave a reaction of identity with CEA. The 2 CEA cross-reactive precipitin bands did not coalesce with NCA which is also present in meconium (Fig. 1). These gel diffusion patterns demonstrate the presence of 2 additional antibody specificities in the NCA-neutralized antiserum that recognize 2 common determinants on CEA unrelated to the CEA:NCA-shared epitope.

In order to determine whether the NCA-unrelated, CEA-cross-reactive antibodies in the CEA antiserum contributed to the measurement of CEA in meconium by RIA, the NCA-neutralized antiserum was further adsorbed with meconium samples that did not give a reaction of identity with CEA in double
CEA-related Antigen Purification

Fig. 1. Double immunodiffusion of NCA-neutralized goat anti-CEA antiserum (center well), goat specific anti-CEA antiserum (Well 5), and goat specific anti-NCA antiserum (Well 3) against CEA, 0.25 mg/ml (Well 6), raw meconium sample resuspended 1:4 (w/v) in PBS (Wells 1 and 4), and NCA, 0.2 mg/ml (Well 2). The band formed between the NCA-neutralized goat anti-CEA antiserum and the goat specific anti-CEA antiserum is due to a slight excess of meconium antigens in the latter that were used for absorption.

diffusion. This meconium-absorbed antiserum retained precipitating activity against CEA (Fig. 1), and its maximum binding of labeled CEA decreased about 10% as compared to that of unadsorbed antiserum. However, over 80% of the antibody activity initially present in the NCA-neutralized antiserum was removed by absorption with meconium. A comparison was made of CEA levels in meconium by RIA using Roche kit antibody, NCA-neutralized antiserum, or specific anti-CEA antiserum with CEA as both the labeled tracer and inhibitor. All 3 antisera were adjusted in concentration to yield the same sensitivity to inhibition by CEA. Analysis of 12 meconium samples revealed that the CEA content as measured with the Roche and NCA-neutralized antisera was 3- to 10-fold greater (p < 0.001, Student’s t test) than that obtained with the specific anti-CEA antiserum (Table 1).

Purification of MA from Meconium. At various steps during the purification of MA, antigenic activity was monitored by RIA using Roche Kit antibody, assumed to measure mainly NCA-unrelated, CEA-cross-reactive substances in meconium, and by the specific anti-CEA antiserum, in order to follow CEA activity. These antigenic activities, as well as specific NCA levels at various stages in the isolation procedure, are shown in Table 2. Extraction of meconium at pH 8.0 into 40% ethanol yielded about 75% of the initial MA activity in the ethanolic supernatant. Although a large precipitate formed with ethanol fractionation, the supernatant remained viscous and highly pigmented. Chromatography of the ethanolic extract on DEAE-cellulose removed the majority of the pigment but resulted in a subfractionation of the MA activity throughout the entire chromatogram (Chart 1). For brevity, the chromatographic profile beyond the 0.1 M NaCl step is not shown since only a few percentages of the applied MA activity were recovered in each doubling of the salt concentration up to 2.0 M. Most of the optical activity eluting from the column appeared in the application buffer fraction which also contained 90% of the applied

<table>
<thead>
<tr>
<th>Sample</th>
<th>Roche</th>
<th>NCA neutralized</th>
<th>Specific anti-CEA</th>
<th>NCA</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>140</td>
<td>180</td>
<td>15</td>
<td>94</td>
</tr>
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<tr>
<td>12</td>
<td>27</td>
<td>34</td>
<td>8</td>
<td>57</td>
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Mean ± S.E. 124.3 ± 19.8 148.3 ± 26.5 20.8 ± 6.3 82.2 ± 6.2

Unextracted meconium samples were initially resuspended 1:4 (w/v) in PBS and then diluted in 0.05 M borate buffer, pH 8.5, prior to assay.

Values given are specific levels of NCA measured in NCA RIA.

Table 2
Comparative distribution and recovery of NCA, CEA, and MA during MA purification from meconium

<table>
<thead>
<tr>
<th>Isolation step</th>
<th>NCA</th>
<th>CEA</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting meconium</td>
<td>6.5</td>
<td>0.6</td>
<td>14.5</td>
</tr>
<tr>
<td>Ethanic supernatant</td>
<td>3.6</td>
<td>0.3</td>
<td>11.0</td>
</tr>
<tr>
<td>DEAE, 0.1 M NaCl</td>
<td>0.2</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>S-300</td>
<td>0.02</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Affinity chromatography</td>
<td>Goat specific anti-CEA (unadsorbed fraction)</td>
<td>0.006</td>
<td>0.01</td>
</tr>
<tr>
<td>Monoclonal CEA antibody, NP-3 (adsorbed fraction)</td>
<td>0.005</td>
<td>0.004</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Determined in RIA using NCA specific assay for NCA and CEA RIA, using goat specific anti-CEA antiserum for CEA or Roche kit antibody for MA.
NCA activity. The 0.1 M NaCl fraction was chosen for further purification on the basis of its low NCA and protein activity but enriched MA content.

Following chromatography of the 0.1 M NaCl fraction over Sephacryl S-300, the appearance of the MA activity overlapped at a slightly smaller size the elution of the radiolabeled CEA marker (Chart 2). The MA appearing between 225 and 270 ml elution volume was pooled and subjected to 2 affinity chromatography steps. The first involved passage over an immunoabsorbent containing specific anti-CEA antibody to remove the small amount of CEA remaining associated with the MA. Although this procedure removed 95% of the CEA, 45% of the MA was lost and was not found in the adsorbed fraction (Table 2). For the second step, an immunoabsorbent containing a murine monoclonal CEA antibody, NP-3, cross-reactive with at least one group of NCA-unrelated, CEA-cross-reactive substances in meconium, was used to select and purify further the MA. Over 80% of the applied MA activity was retained by this immunoabsorbent. Overall recovery of MA activity starting with 50 g meconium was 4%, or 20% of that present in the 0.1 M NaCl:DEAE fraction.

**Immunological Analysis of Purified MA.** On the basis of neutralizing activity in RIA, the finally purified MA contained less than 1.0% CEA or NCA (Table 2). Double immunodiffusion of the MA against NCA-neutralized anti-CEA antiserum gave a single precipitin band that formed a reaction of partial identity with CEA (Fig. 2). The 2 bands appearing between unextracted meconium and the latter antisem both fused with the single line formed by purified MA. The appearance of only a single precipitin band with the purified MA suggests, to the extent that can be determined by double immunodiffusion, that it did not contain the second NCA-unrelated, CEA-related antigen. The purified MA failed to react in double diffusion with specific anti-CEA antiserum, although this antisem retained its ability to precipitate CEA. The MA also gave a single precipitin band against NCA-neutralized CEA antisem in immunoelectrophoresis (not shown), migrating as an α-globulin relative to serum proteins.

The antibody-binding characteristics of radiolabeled MA were evaluated in RIA. As depicted in Table 3, both the Roche kit antibody and the monoclonal CEA antibody, NP-3, bound similar amounts of MA, comparable of the binding of labeled CEA by these antibodies. Over 80% of the MA was immuno-reactive when tested shortly after labeling. Goat specific anti-CEA antisem was only marginally immunoreactive with the labeled MA, while goat anti-NCA antibody was unreactive. The former antisem and Roche kit antibody were similar in their reactivity with labeled CEA. Combining an excess of the specific anti-CEA and Roche kit antibodies together did not increase the maximum binding of CEA beyond that of the Roche kit antibody by itself (data not shown), demonstrating that the same major population of labeled CEA molecules was recognized by both antisera. Since inhibition assays and double diffusion did not reveal significant quantities of CEA in the MA, the low level of labeled MA binding to the goat specific anti-CEA antisem was most probably due to the presence of residual cross-reactive antibody in this antisem rather than reactivity with a labeled CEA contaminant.
Molecular Size of MA. Chromatography of the labeled MA over Sephacryl S-300 equilibrated in PBS gave a single, symmetrical peak eluting at only a slightly greater elution volume than that of CEA (Chart 3). This relationship in the elution patterns of MA and CEA was maintained following passage over Sephacryl S-300 equilibrated and eluted with 6.0 M guanidine-HCl. The overlap in size distribution between MA and CEA was also observed following SDS-PAGE electrophoresis of reduced samples on calibrated gels (Chart 4). Estimation of the molecular size for MA by interpolation from the log molecular size plot versus migration of standards gave a value of 185,000 as compared to 200,000 for CEA. Staining of reduced MA in SDS-PAGE gels with Coomassie Brilliant Blue or periodic acid-Schiff reagent showed a single diffuse band migrating in a similar position as CEA (not shown).

Binding of MA to Concanavalin A. The binding of radiolabeled MA to concanavalin A-Sepharose was compared to that of CEA and NCA. Over 90% of both CEA and NCA bound to this lectin, while less than 20% of the MA was reactive.

Pronase Digestion of CEA and MA. Digestion of CEA with Pronase E completely abolished its reactivity with goat specific anti-CEA antiserum and resulted in a 50% decrease in its binding to the NP-3 monoclonal antibody or Roche kit goat antibody. This loss or decrease in antibody-binding activity was not observed following similar treatment in the absence of enzyme. Chromatography of the enzyme digest on Sephacryl S-300 equilibrated in 6.0 M guanidine-HCl showed that the majority of the CEA was broken down into small fragments (Chart 5). By contrast, enzyme digestion of MA reduced its binding to the NP-3 and Roche kit antibodies by 20% and resulted in only a slight decrease in its molecular size (Chart 5).

DISCUSSION

Studies in our laboratory have used meconium as a source material for embryonic substances that may have developmental and chemical relationships to the CEA. In the present study, 2 antigens, in addition to NCA, were identified in meconium that cross-reacted with CEA derived from colon tumors. It was shown that these substances contributed significantly to the measurement of CEA when an antiserum containing cross-reactive antibodies was used in RIA. We immunologically defined CEA in this work as that molecular species recognized by an antiserum depleted of cross-reactive antibodies, and on this basis CEA was lowest in concentration with respect to the levels of related substances found in meconium. The low levels of CEA in meconium and, perhaps, adult feces explain the ease by which NCA-2 (2) and NFA-2 (14) were purified presumptively free of appreciable CEA contamination, since polyvalent anti-CEA antisera were used as an initial primary separation step for both antigens. The cross-reactive antibodies constituted the major portion of the CEA reactivity of our antiserum, and their presence in other conventionally prepared CEA antisera (2, 14, 17) supports the immunodominant nature of the determinants eliciting their production. Unlike the common CEA

Table 3
Comparative binding activities of radiolabeled NCA, CEA, and MA to goat and monoclonal antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>NCA</th>
<th>CEA</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat specific anti-CEA</td>
<td>0</td>
<td>62</td>
<td>8</td>
</tr>
<tr>
<td>Roche kit</td>
<td>63</td>
<td>69</td>
<td>63</td>
</tr>
<tr>
<td>Monoclonal CEA (NP-3)</td>
<td>0</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Goat anti-NCA</td>
<td>67</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

* Determined in antibody excess after incubation for 4 hr at 45°C. Solid-phase goat anti-mouse IgG or donkey anti-goat IgG was used to separate bound from free antigen.
epitope found on NCA (24), the shared determinants expressed on NCA-unrelated, CEA-related substances in meconium appear to be effective inhibitors in CEA RIA. Moreover, the competitive properties in RIA of the CEA-related substances formed the basis for an assay system to monitor them during purification.

Our isolation procedure for one of the NCA-unrelated, CEA-related molecular species in meconium, tentatively designated MA for reasons described below, avoided application of an immunoseparation step at an early stage. This was due to our failure to find PCA solubilization alone (14), or in combination with alcohol (2), as a means to provide an extract with characteristics favorable for affinity chromatography. Among the different alcohol concentrations and pH ranges tested, 40% alcohol at pH 8.5 yielded the best solubilization of MA and maximum precipitation of irrelevant protein. Subsequent chromatography on DEAE-cellulose had the desirable feature of retaining most of the pigment while the bulk of irrelevant protein and NCA were not adsorbed. These advantages outweighed the subtraction of MA activity observed on the ion exchanger. In the final stages, the MA was specifically adsorbed to an immunoadsorbent containing a cross-reactive monoclonal antibody to CEA, NP-3 (23), analogous to the use of conventional cross-reactive antibodies for NCA-2 and NFA-2 purification (2, 14). However, the use of a monoclonal antibody has the advantage of selecting a molecular species homogeneous in the expression of the epitope recognized by the antibody, although differences may exist with respect to other determinants.

The relationship and/or identity of MA with NCA-2 (2, 3) and the NFA family of CEA-like antigens in adult feces, particularly NFA-2 (14, 17), is presently unclear. NCA-2 and NFA-2 are similar to CEA in molecular size as well as carbohydrate and amino acid composition. Immunologically, NCA-2 shares 2 epitopes with CEA, while 3 cross-reactive sites have been demonstrated on NFA-2. In the next paper (23), we have shown with monoclonal antibodies to CEA that MA, which represents one of the 2 NCA-unrelated, CEA-cross-reactive substances in meconium (Fig. 1), shares at least 3 epitopes with CEA. Inspection of the double immunodiffusion pattern depicted in Fig. 2 shows that the second CEA-related antigen in meconium is deficient in at least one cross-reactive site that is present on both CEA and MA. Recently, Matsuoka et al. (19) found that NCA-2 isolated from meconium did not cross-react with an antiserum specific for NFA-2, suggesting that these 2 antigens are in fact different. Furthermore, they reported that NCA-2, unlike NFA-2, and about one-half of their CEA preparations, was resistant to Pronase E proteolytic digestion. Since we found MA resistant to Pronase E digestion, this could indicate that our MA is the same as NCA-2. However, we are reluctant to equate the 2 antigens on this basis alone, since both Pronase E-resistant and -susceptible preparations of MA, NCA-2, and NFA-2 might be encountered, as already observed for CEA (19). It is noteworthy to mention that Matsuoka et al. (19) also identified NFA-2 in meconium, and thus the 2 CEA-related antigens we have described might ultimately be categorized as NCA-2 and NFA-2.

Even though it is not possible as yet to differentiate MA from other CEA-related antigens in meconium previously described, the specific identification and further characterization of this antigen are necessary to resolve its developmental relationship to CEA and its potential biological importance. MA has a similar molecular size as that of CEA but is antigenically different in that it lacks at least one determinant distinguished on CEA by the conventional antisera used in the present study, and this difference is also noted with monoclonal antibodies to CEA (23). Furthermore, MA has a much lower affinity for concanavalin A than either CEA or NCA. Burtin et al. (3) found NCA-2 to have a lower mannose content than CEA, but the concanavalin A-binding properties of both NCA-2 and NFA-2 have not been reported. The low affinity of MA for this lectin does provide another possible method, one not requiring specific immunoadsorbents, for the separation of MA from CEA and NCA. Nonetheless, the CEA cross-reactivity, molecular size, and concanavalin A-binding characteristics of MA suggest that it contributed in part to the various species of CEA isoantigens described in other studies of normal and tumor specimens that used polyvalent anti-CEA antiserum for CEA detection (9, 10, 26-28). Our observation that MA and the other NCA-unrelated, CEA-related antigen together appear at about a 6-fold-higher concentration than CEA in meconium may indicate that these antigens are earlier markers of differentiation than CEA. Therefore, a precise definition of the chemical structure and antigenic properties of MA, as well as the other CEA-related antigen in meconium, is required in order to determine their biological distinction from CEA and their potential role as tumor markers.

The studies reported herein were in part prompted by the anticipation that murine monoclonal antibodies to CEA, like conventional antisera, would react with epitopes on CEA that are shared among the family of CEA-like antigens. As shown in the accompanying study (23), the availability of purified MA has facilitated the delineation of epitope specificity of monoclonal antibodies to CEA and the distribution of these epitopes among CEA, MA, and NCA.

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Immunological Heterogeneity of Carcinoembryonic Antigen: Purification from Meconium of an Antigen Related to Carcinoembryonic Antigen

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