ABSTRACT

We have isolated carcinoembryonic antigen (CEA)-related antigens from meconium and compared them with those in adult feces. Two CEA-related antigens were detected in meconium [nonspecific cross-reacting antigen 2 (NCA-2) and meconium nonspecific cross-reacting antigen] while four CEA-related antigens were found in adult feces [normal fecal antigen 1, normal fecal antigen 2 (NFA-2), normal fecal cross-reacting antigen, and fecal nonspecific cross-reacting antigen, respectively).

By conventional anti-CEA antisera, NCA-2 in meconium, NFA-2 in adult feces, and CEA in tumor tissues were indistinguishable from each other, but they could be distinguished by specific antibody preparations against a determinant unique to CEA (CEA-distinctive determinant) or to NFA-2 (NFA-2-distinctive determinant). Neither the CEA-distinctive determinant nor the NFA-2-distinctive determinant was detected on the NCA-2 molecule. No antigenic determinants unique to NCA-2 have been detected with the anti-NCA-2 antisera which we have prepared thus far.

The molecular weight of purified NCA-2 was estimated to be 150,000 to 170,000 as compared to 160,000 to 170,000 for NFA-2 and 170,000 to 180,000 for CEA. NCA-2 had amino acid and carbohydrate compositions similar to those of CEA and NFA-2.

All NFA-2 preparations and about one-half of the CEA preparations were sensitive to Pronase E digestion, which released two antigen fragments from these molecules, but NCA-2 preparations were resistant to such digestion.

INTRODUCTION

Although the clinical significance of CEA is now widely accepted, its cancer specificity has become obscure because of the discovery of several CEA-related antigens in nonmalignant tissues and secretions. Recently, these results have been reviewed in detail (1, 10, 15, 29, 32) and summarized comprehensively in a previous work of ours (13). It was reported by Matsuoka et al. (20) in 1973 that there existed a potent antigen cross-reactive with CEA in normal adult feces; this was subsequently designated NFA (21). NFA seemed to be very important among the CEA-related antigens reported thus far since most conventional anti-CEA antisera could not distinguish it from CEA in tumor tissues and the reactivity of these antisera with CEA could be completely absorbed out by addition of NFA (22). In 1973, Burtin et al. (2) also reported a cross-reactive antigen in meconium and in feces which seemed to be similar to NFA, and they named it NCA-2 since another cross-reactive antigen (NCA) had already been found in normal tissues such as lung or spleen (33). Subsequent reports revealed that similar antigens were detected in gastric juice (34), in bile (31), in normal colon washings (6, 30), and in normal colon mucosa (7).

The NFA and NCA-2 have been considered to be very closely related or almost identical to each other, but no precise comparisons of antigenic structure or of physicochemical properties have been carried out thus far. It should be decided, first of all, whether or not NFA is identical to NCA-2. Secondly, the antigenic relation of these antigens to CEA or NCA should be clarified further because NCA-2 has been reported to be closely related to NCA (2), whereas NFA has been reported to show less similarity to NCA (21). Thirdly, if NCA-2 from meconium and NFA from adult feces can be considered to be the representative of CEA-related antigens in fetal tissues and in adult tissues, respectively, comparison of chemical structure of these antigens with that of CEA in malignant tissues would provide interesting information about the molecular differentiation of the CEA-related glycoprotein family.

In the present study, we purified the CEA-related antigens in meconium and found 2 molecular species of CEA-related antigens, tentatively designated herein as NCA-2 and meconium NCA. The antigens in meconium were compared immunologically and physicochemically with the antigens in adult feces and with CEA in tumor tissues.

MATERIALS AND METHODS

Reference CEA and NCA Preparations. Preparations of CEA were isolated from liver metastases of colon carcinomas as described previously (20). The purity of one of the CEA preparations is demonstrated by IEP (Fig. 1) and SDS-PAGE (Fig. 2). The First British Standard (73/601-2/22J) for CEA was obtained from the National Institute for Biological Standards and Control, London, England, and was used in RIA as a standard CEA. NCA was prepared from the crude perchloric acid extract of pooled normal lungs obtained at autopsy as described previously (13).

Purification of CEA-related Antigens from Normal Adult Feces. The CEA-related antigens in normal adult feces were purified as described previously (13). Briefly, the antigens in feces were extracted with 0.6 M perchloric acid and then adsorbed to and eluted from goat anti-CEA adsorbent. The eluate contained 4 CEA-related antigens,
Comparison of CEA-related Antigens

Fig. 1. IEP of the crude and purified antigen preparations. Antigens applied to the wells were: tumor crude extract (T. Crude Ext.); purified CEA (CEA); fecal crude extract (F. Crude Ext.); meconium crude extract (M. Crude Ext.); purified NCA-2 (NCA-2). Antisera applied to the troughs were: rabbit anti-tumor crude extract antiserum (ä-T. Crude Ext.); rabbit anti-fecal crude extract antiserum (ä-F. Crude Ext.); rabbit anti-meconium crude extract (ä-M. Crude Ext.).

Fig. 2. SDS-PAGE (7.5% polyacrylamide, 0.6 x 10.0 cm) electrophoresis of the purified antigen preparations. a, CEA; b, NFA-2; c, NCA-2. C1 and C2, control gels. C1 contained membrane polypeptides prepared from human RBC marker proteins. Band 1 (Spectrin I), M, 240,000; Band 2 (Spectrin II), M, 220,000; Band 3, M, 90,000. C2 contained bovine serum albumin (BSA), M, 68,000; human IgG (H-chain), M, 50,000; human IgG (L-chain), M, 23,500; lysozyme (hen egg white), M, 14,300. All gels were stained with Coomassie Brilliant Blue. The same results were obtained by using 10% polyacrylamide gels.

Feecal NCA and 3 other molecular species (NFA-1, NFA-2, and NFCA) which had been inclusively called NFA (13, 18, 19, 21). The fecal NCA was isolated by specific goat anti-NCA adsorbent. Three other molecules were isolated by gel filtrations on columns of Sepharose 6B, Sephadex G-200, and Sephadex G-100 (superfine) and by chromatography on a column of DEAE-cellulose. The purity of these NFA-2 preparations is demonstrated by IEP (Fig. 1) and SDS-PAGE (Fig. 2).

Extraction of CEA-related Antigens from Meconium. Meconium was collected from more than 20 neonates of various blood types and frozen at -20° until use. The antigens in meconium were extracted with 0.6 M perchloric acid as described for NFA (13) and purified as described in "Results."

Antisera. Three different types of anti-CEA preparations (R-ä, Go-ä, and AAP-1) were used in this study. Preparation and specificity of these anti-CEAs have been described previously (22).

ä-NCA was prepared in a goat as described previously (13). ä-NFA-2s were raised in rabbits as described previously (13) and were reactive with both NFA-2 and CEA. By absorption with CEA, ä-NFA-2 was rendered specific for NFA-2-distinctive determinant and designated ä-NFA-2-Dis.

AAP-1 and ä-NFA-2-Dis were absorbed repeatedly with human erythrocytes from 3 adults, having antigens of A, Le+, Le++, M, and N; B, Le* and N; as well as H, Le++, and M. Antisera against human erythrocyte antigens (A, B, Le*, Le++, M, and N) were purchased from Ortho Pharmaceuticals, Raritan, N. J.

ä-NCA-2s were raised in rabbits which were immunized twice at 5-week intervals, each time with 200 µg of purified NCA-2 in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). These antisera were used without further absorption or after complete absorption with CEA.

Antisera against crude extracts of tumors, feces, or meconium were raised in rabbits and used for IEP.

Antigen and Antibody Adsorbents. Purified CEA, NCA, or NFA-2 was coupled with CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) (10 mg/g dry gel) according to the manufacturer's instructions.

Goat anti-CEA antibody, goat anti-NCA antibody, or rabbit anti-NFA-2-Dis antibody specifically purified by proper antigen adsorbent was conjugated to CNBr-activated Sepharose 4B (10 mg/g dry gel) and used for specific purification or separation of NFA, NCA, and NCA-2, respectively.

Gel Filtrations. Gel filtrations were performed with 0.05 M sodium phosphate-0.15 M NaCl (pH 5.2).

Proteolytic Enzyme Treatment. Proteolytic digestion of purified antigens by Pronase E (Kaken Kagaku Co., Tokyo, Japan) was performed as described previously (21). The digested sample was concentrated to the starting volume by pervaporation.

RIA. A sandwich-type solid-phase RIA system (28) for measuring the antigenic reactivity of CEA and related antigens was performed as described previously (14).

Immunological and Physicochemical Analyses. Antigenic properties of purified antigens were analyzed by microscale immunodiffusion tests as described previously (21). Physicochemical analyses such as IEP or SDS-PAGE and the determination of amino acid or carbohydrate composition were performed as reported in a previous paper (13).

RESULTS

Purification of CEA-related Antigens from Meconium. After treatment with perchloric acid to precipitate the protein, the antigens with CEA activity in meconium were adsorbed to and eluted from goat anti-CEA adsorbent. The eluate (NCA-2-rich eluate) was applied to a Sepharose 6B column. The elution profile is shown in Chart 1 in comparison with that of NFA-rich eluate obtained from normal adult feces (13). Antigens reactive
with anti-CEA antisera were detected in Fractions II and IV.

The concentrated Fraction II was chromatographed on another Sepharose 6B column and then on a Sephadex G-200 column. A CEA-related antigen was eluted from the column as a single peak. This high-molecular-weight antigen in meconium was designated NCA-2 according to the work of Burtin et al. (2). An antigen with NCA activity was detected in Fraction IV and further purified by specific goat anti-NCA adsorbent and designated meconium NCA. This was the only CEA-related antigen in Fraction IV from meconium, although 3 CEA-related antigens (NFA-1, NFCA, and fecal NCA) had been identified in a similar fraction of NFA-rich eluate from adult feces previously (13). The meconium NCA was antigenically indistinguishable from NCA purified from normal lungs or from adult feces.

Immunological Analyses. The results of immunodiffusion analyses for CEA, NCA-2, NFA-2, NCA, and NFA-2 with several antisera possessing different specificities are summarized in Table 1, and some relevant immunodiffusion patterns are demonstrated in Figs. 3 and 4. The findings were as follows: (a) no antigenic differences were detected by conventional anti-CEA (R-8 or Go-8) among CEA, NCA-2, and NFA-2; (b) CEA or NFA-2 each possessed a unique antigenic determinant referred to as CEA-distinctive determinant and NFA-2-distinctive determinant, respectively (13); and (c) NCA-2 does not seem to have any unique determinant, or if a unique determinant exists in NCA-2, it must be extremely weak. The reaction unique to CEA or NFA-2 was not affected by intensive absorption of the respective antisera with human erythrocytes.

Under some experimental conditions, NCA-2 preparations used in this study developed a faint precipitate line with â-NFA-2-Dis. This suggests that the NCA-2 preparations contain some NFA-2-like molecules or, alternatively, that the NCA-2 molecules themselves have a very weak cross-reactivity with the NFA-2-distinctive determinant. In order to clarify this point, 4.9 mg of NCA-2 were applied to an adsorbent column of Sepharose 4B to which 7 mg of anti-NFA-2-Dis were bound. About 3.9 mg of NCA-2 (79.6%) were not adsorbed to the adsorbent. When 2 mg of NFA-2 or CEA were applied on this column, 96% of NFA-2 was adsorbed, whereas most of the CEA molecules (about 99%) were not adsorbed to the column. The NCA-2 freed from the NFA-2-like molecules no longer reacted with anti-NFA-2-Dis (Fig. 4c) and gave a single precipitate line in IEP (Fig. 1) and a single diffuse band in SDS-PAGE (Fig. 2). The antigen which was adsorbed to the column of anti-NFA-2-Dis and eluted with acid was indistinguishable from NFA-2 purified from adult feces when tested with R-8, Go-8, or â-NFA-2. About 10 mg of NCA-2 (freed of NFA-2) were obtained from 300 g of meconium mass used as the starting material.

The antigenic reactivity of NCA-2 with a goat anti-CEA anti-

![Chart 1. Sepharose 6B chromatography of NCA-2-rich eluate. Pooled NCA-2-rich eluate (5.8 ml) was applied on the column (2.5 x 141.0 cm). The effluent was collected in 4.0-ml portions and combined as indicated. Elution profile of NCA-2-rich eluate (- - -) is depicted as compared with that of NFA-rich eluate (•••). ■, fractions containing CEA-related antigens in NCA-2-rich eluate; □, those in NFA-2-rich eluate. Elution positions of blue dextran, CEA, and NCA from the same column are shown as references.](chart1)

![Chart 2. Immunodiffusion analyses for CEA, NCA, and NFA-2. Antibodies against CEA, NCA, and NFA-2 were reacted with相应抗原, and the results are shown in Table 1.](chart2)

### Table 1

Antigenic reactivities of CEA and CEA-related antigens upon immunodiffusion analyses

<table>
<thead>
<tr>
<th>Antigens</th>
<th>CEA</th>
<th>NCA-2</th>
<th>NFA-2</th>
<th>NCA</th>
<th>NFA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Absorbed with CEA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>(+)</td>
</tr>
<tr>
<td>Absorbed with NFA-2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Specific for CEA-distinct (AAP-1)</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>â-NFA-2</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Without absorption</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Absorbed with CEA (â-NFA-2-Dis)</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>â-NCA-2</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Without absorption</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Absorbed with CEA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Approximate molecular wt

| 180,000 | 160,000 | 170,000 | 80,000 | 20,000 |

Synonyms or similar antigens

<table>
<thead>
<tr>
<th>NCW (6)</th>
<th>NGP (16)</th>
<th>CEX (5)</th>
<th>CEA-No (7)</th>
<th>TEX (12)</th>
<th>CCA-III (24)</th>
</tr>
</thead>
</table>

*Initial reporters for each antigen listed are: Gold and Freedman (8, 9) for CEA; Burtin et al. (2) for NCA-2; Matsuoka et al. (20, 21) for NFA-2; von Kleist et al. (33) for NCA; Kuroki et al. (13) for NFA-1.

†, reactions indistinguishable from each other; (+), reactions partially identical to that of CEA with spur formation;

x, distinctive reactions. These reactions were not affected by intensive absorptions of antisera with human erythrocytes.

*Absorption with NCA-2 revealed the same results.

*Three different preparations of â-NCA-2 gave the same results.
serum was compared with those of NFA-2 and several CEA preparations including a WHO standard CEA in a solid-phase RIA. As can be seen in Chart 2, the dose-response curves obtained with NCA-2 and NFA-2 were similar to those of CEA. Some differences in weight were observed among CEA preparations, and the differences between CEA and NCA-2 or NFA-2 were in the same range as interpreparation differences of CEA. In this system, NCA showed a negligible reactivity (10,000 ng NCA corresponded to 3 ng CEA).

Susceptibility to Pronase Digestion. We have reported previously that 2 antigenic fragments were released either from the CEA (21) or NFA-2 (13) by proteolytic digestion with Pronase E and that one of the fragments was antigenically identical to NFA-1 and the other was similar to NFCA. It was found afterward that CEA preparations from various individuals could be divided into 2 groups: about one-half of the CEA preparations were susceptible to Pronase E digestion and released 2 antigenic fragments mentioned above; the rest were resistant to digestion under the same experimental conditions. Representative examples are demonstrated in Fig. 5. It is noteworthy that, in contrast to NFA-2, the antigenic reactivity of NCA-2 was not affected by digestion with Pronase E (Fig. 5a), although a slight increase in ninhydrin reaction of incubation mixture was observed.

Physicochemical and Chemical Analyses. Electrophoretically, CEA, NFA-2, and NCA-2 revealed β-region mobility as shown in Fig. 1. All antigens migrated as a single diffuse band in the similar position on SDS-PAGE (Fig. 2). Apparent molecular weights of 3 antigens determined by SDS-PAGE were 150,000 to 170,000 for NCA-2, 160,000 to 170,000 for NFA-2, and 170,000 to 180,000 for CEA. All antigens were stainable with both Coomassie Brilliant Blue and periodic acid-Schiff reagent.

The carbohydrate composition of NCA-2 is shown in Table 2 in comparison with that of CEA or NFA-2. All samples contained the same types of monosaccharides in similar weight percentage proportions, although the total carbohydrate content of CEA was slightly higher than that of the other 2. Table 3 shows the amino acid compositions of CEA, NFA-2, and NCA-2. Essentially, similar compositions were observed in these 3 antigens.

DISCUSSION

Since NFA (20) and NCA-2 (2) were reported in 1973, it has been a long-pending question whether or not these 2 antigens are identical. As far as the reported immunological reactivity is concerned, both antigens appeared to be quite similar to each other. No precise comparison, however, has been done, mainly because no purified preparation of NFA was available until recently. As reported previously (13), 4 CEA-related antigens have been identified in normal adult feces and 3 of them (NFA-1, NFA-2, and fecal NCA) have been isolated in pure form. Thus, now it has become possible to compare these CEA-related antigens in adult feces with NCA-2 in meconium.

Burtin et al. (2) reported that NCA-2 was detected in meconium and in stools, but immunological and physicochemical characterization of NCA-2 has been performed only on antigen isolated from meconium (3, 4). In the present study, therefore, we designated the antigen in meconium as NCA-2 and compared it with NFA in normal adult feces. It was found that only 2 molecular species of CEA-related antigens (NCA and NCA-2) existed in meconium in contrast to the 4 molecular species (NFA-1, NFA-2, NFCA, and NCA) in adult feces. Since an
identical antigen (NCA) was found both in meconium and in adult feces, this antigen can be excluded from the comparison in this study. Lack of antigens in meconium corresponding to NFA-1 and NFCA of feces is one of the noteworthy findings in the present work.

The origin and production mechanism of fecal antigens are still unclear, but it seems most probable that NFA-1 and NFCA are produced from NFA-2 through degradation by bacterial or host enzymes in the alimentary canal. Aseptic conditions and probable lack of proteolytic enzymes in the fetal digestive tract may result in the absence of antigens in meconium corresponding to NFA-1 and NFCA.

Since NCA-2 and NFA-2 seem to be counterparts of CEA in malignant tissues in meconium and in adult feces, respectively, immunological and physicochemical features of these 3 antigens were compared. The results concerning the molecular weight and content of carbohydrates and amino acids indicate that NCA-2, NFA-2, and CEA are indistinguishable from each other with respect to these properties.

Antigentially, as far as the reactivity with conventional anti-CEA antisera is concerned, NCA-2, NFA-2, and CEA revealed a reaction of identity in immunodiffusion (Table 1; Fig. 3). NCA-2 or NFA-2 could not be distinguished from CEA by a solid-phase RIA either (Chart 2). With regard to this point, all of commercially available CEA assay systems thus far tested have given results similar to our RIA (data not shown). Since all CEA assays tested were negligibly reactive with purified NCA preparations, these results indicate that NCA-2 is more closely related to CEA than to NCA, although it has been observed by others (3, 17) that NCA-2 is close to NCA and these 2 antigens cross-react with CEA to a similar extent.

In spite of their similarities, NCA-2, NFA-2, and CEA were antigenically distinguishable from each other by means of specific antibody preparations such as AAP-1 and anti-NFA-2-Dis. The results indicate that CEA and NFA-2 each possess a unique determinant (CEA-distinctive determinant and NFA-2-distinctive determinant, respectively). These distinctive determinants seem to be unrelated to main blood group antigens, because no alteration was observed upon the specificity of AAP-1 or of 8-NFA-2-Dis after intensive absorption of antisera with packed human erythrocytes (Table 1). Furthermore, with very rare exception, most of CEA preparations derived from colorectal cancer patients with various blood types were equally reactive with antibody preparations against CEA-distinctive determinant, as reported previously (18, 19). No final conclusion on the cancer specificity of the CEA-distinctive determinant can be made yet, but it is certain that antibody preparations directed against a CEA-distinctive determinant, such as AAP-1 or A-1 in a previous paper (22), possessed narrower and consequently more cancer-specific reactivities. The possibility remains, however, that antibody reactivity against the CEA-distinctive determinant might be absorbed by an unknown antigen from a nonmalignant tissue.

An antigenic determinant unique to NCA-2 was not detected by our anti-NCA-2 sera, but the possibility remains that an antiserum distinguishing NCA-2 from the other antigens can be obtained by an intensive immunization with NCA-2. It has been reported by Burtin et al. (2) that NCA-2 possesses a unique determinant which was detected by an antiserum prepared against NCA-2. Therefore, by using their specific anti-NCA-2 antiserum, it should be possible to distinguish NCA-2 from NFA-2. Alternatively, however, the determinant claimed to be unique to NCA-2 by their a-NCA-2 may be the same as the NFA-2-distinctive determinant, since a small portion of molecules corresponding to NFA-2 was detected in our NCA-2 preparations and apparently the distinctive determinant of NFA-2 was a more potent immunogen than was NCA-2. In any case, it is clear that NCA-2, NFA-2, and CEA are distinguishable from each other. The difference in susceptibility to Pronase E digestion between NCA-2 and NFA-2 supports the idea that they are structurally different. The cause of the susceptibility difference is not clear yet. A difference in glycosylation of these antigens could be suspected to be a cause of this difference as well as the differences among various CEA preparations. The existence of antigenic and/or structural variations among CEA preparations from various tissues including fetal tissues has been suggested by several investigators (11, 23, 25), but precise comparisons in pure form of antigen preparations, especially between the antigens in meconium and those in normal adult feces, have not yet been reported.

The fact that NCA-2 did not possess either the CEA-distinctive determinant or the NFA-2-distinctive determinant leads us to a speculation that NCA-2 could be a prototype of CEA-glycoprotein family and that its place would be taken by NFA-2 in adult tissues and by CEA in tumor tissues. The fact that the NCA-2 preparations contained a small amount of molecules possessing the NFA-2-distinctive determinant may reflect a differentiation phase at the neonatal period during which most molecules have remained as prototype (NCA-2) but a small portion of molecules have already differentiated into the adult type (NFA-2). A concept of phase-specific variations in CEA molecules has been proposed by Rule and Golecki-Reilly (26, 27) on the basis of antigen distribution profiles of various tissue extracts upon amphoteric isoelectric focusing. No structural evidence, however, has been presented. The further structural analysis of CEA, NCA-2, and NFA-2 and a precise comparison of them may give us important information about molecular differentiation of this CEA-related glycoprotein family.

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Fig. 3. Immunodiffusion analyses of NCA-2, NFA-2, and CEA with conventional anti-CEA preparations. a, reactions with R-ä; b, reactions with Go-ä; c, antigenic relations of NCA-2 with NCA and NFA-1. NFA-2, purified NFA-2; CEA, purified CEA; NCA-2, purified NCA-2; NCA, purified NCA; NFA-1, purified NFA-2.

Fig. 4. Immunodiffusion analyses for detection of a determinant unique to each NCA-2 (a), CEA (b), and NFA-2 (c). No determinant unique to NCA-2 was detected with our α-NCA-2 antisera that we had prepared thus far, whereas a determinant unique to each CEA (CEA-distinctive determinant) and NFA-2 (NFA-2-distinctive determinant) was detected. α-NCA-2 nonabs, nonabsorbed rabbit anti-NCA-2 antiserum; AAP-1, specially prepared anti-CEA (see "Materials and Methods"); α-NFA-2 abs CEA, rabbit α-NFA-2 antiserum absorbed with CEA (referred to as α-NFA-2-Di in text). For descriptions of CEA, NCA-2, and NFA-2, see Fig. 3.

Fig. 5. Susceptibility to Pronase E digestion of purified preparations of CEA, NCA-2, and NFA-2. a, preparations resistant to Pronase E digestion; b, preparations susceptible to Pronase E digestion. CEA (T. Y.) na, native preparation of CEA (T. Y.); CEA (T. Y.) pro, CEA (M. Y.) pro, CEA (T. A.) pro, CEA (H. O.) pro. Pronase E-treated CEA preparations. Initials in parentheses, initials of the patients. NCA-2 pro, Pronase E-treated NCA-2; NCA-2 na, native preparation of NCA-2; NFA-2 pro, Pronase E-treated NFA-2; NFA-1, purified NFA-1; CEA (T. Y.), CEA (M. Y.), and NCA-2 were not affected by Pronase E digestion, whereas CEA (T. A.), CEA (H. O.), and NFA-2 were cleaved into 2 fragments corresponding to each NFA-1 and NFCA, although NCA was applied instead of NFCA in b.
Cancer Research


Immunochemical Differences among Carcinoembryonic Antigen in Tumor Tissues and Related Antigens in Meconium and Adult Feces

Yuji Matsuoka, Masahida Kuroki, Yoshiko Koga, et al.


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