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CARCINOEMBRYONIC ANTIGEN (CEA) AND TWO CROSSREACTING ANTIGENS OF 165 KD AND 105 KD ISOLATED FROM MECONIUM EXHIBIT IDENTICAL AMINO TERMINAL SEQUENCES

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Two antigens of 165 kD and 105 kD crossreacting with the carcinoembryonic antigen (CEA) were isolated from human meconium by perchloric acid extraction, differential immunoaffinity chromatography, and size exclusion HPLC. The sequences of the 30 amino terminal amino acids of both antigens are identical with the corresponding sequence of CEA.

Carcinoembryonic antigen (CEA) and related antigens represent a family of glycoproteins which differ from each other with respect to structural and immunochemical properties (1). Major antigens crossreacting with CEA are the nonspecific crossreacting antigens with molecular weights of 55,000 (NCA-55) and 95,000 (NCA-95) (2-4). Both antigens are present in perchloric acid extracts of normal lung tissue (4) and, to variable amounts, in various human carcinomas (5). Amino terminal sequences (6-8) for CEA and nonspecific crossreacting antigens isolated from colonic cancer tissue and from spleen showed that the crossreacting antigens differ from CEA at position 21. Recently, the amino terminal sequences of CEA and two NCAs from colonic cancer were extended to 35 residues and further substitutions at positions 27-29 were found (9).

ABBREVIATIONS: CEA, carcinoembryonic antigen; HPLC, high performance liquid chromatography; MA-100, meconium antigen of 100 kD; MAb, monoclonal antibody; NCA, non-specific crossreacting antigen; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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Here we present amino terminal sequence data for the first 30 residues of two CEA-related antigens isolated from meconium. One antigen, with a molecular weight of approximately 165,000, is presumably identical with NCA-2 (10-14). The second antigen, previously identified by Western blots (13), has a molecular weight of approximately 100,000. A meconium antigen of somewhat lower molecular weight was described by Matsuoka et al. (12). In contrast to the nonspecific crossreacting antigens, the amino terminal sequences of both meconium antigens match that of CEA. This is the first report that CEA and crossreacting antigens have identical amino acid residues at positions 21 and 27-29.

MATERIALS AND METHODS

Extraction procedure. Meconium was collected from apparently healthy newborn children and kept frozen until processing which was performed at 4° C. The meconium was homogenized in saline (0.5 ml/g). To the homogenized mixture, an identical volume of 1.6 M perchloric acid was added dropwise while stirring. After 30 min., the mixture was centrifuged at 3000 x g for 30 min. The supernatant was neutralized immediately by the addition of a sodium hydroxide solution and dialyzed against water. The non-dialyzable residue was concentrated over a PM 10 membrane (Amicon, Witten, F.R.G) and was lyophilized.

Immunoaffinity chromatography. For the differential immunoaffinity chromatography of CEA related meconium antigens, two monoclonal anti-CEA antibodies were used (13, 15, 16). MAb T84.1 binds to a high and low molecular weight antigen, whereas MAb CEA.11 binds to the high molecular weight antigen only. The IgG fractions of the monoclonal antibodies were isolated from ascitic fluids and coupled to CNBr activated Sepharose 4B (Pharmacia, Freiburg, F.R.G.) as described (13). The lyophilized powder was dissolved in PBS to a final concentration of 100 g/L, and 0.5 ml of this solution was added to a gel volume of 4 ml. The samples were incubated with the immobilized IgG fractions for 1-2 hr at RT. Subsequently, the columns were washed by the addition of PBS, PBS containing 1% Triton-X-100, and again PBS. The bound fractions were eluted by a 0.2 M glycine-HCl buffer, pH 2.2, dialyzed against water, and lyophilized.

Size exclusion HPLC. Size exclusion HPLC was performed over a TSK G 3000 SW column (LKB, Grafelfing, F.R.G.) in a 0.1 M phosphate buffer, pH 6.8, with 1 M NaCl; the flow rate was 0.5 ml/min. Positive fractions were identified by a solid phase enzyme immunoassay using the immobilized IgG fraction of a polyclonal anti-CEA antiserum (Dakopatts, Hamburg, F.R.G) and biotin-labeled MAb T84.1 as second antibody (17). The pooled fractions were dialyzed against water and lyophilized.

SDS-PAGE and Western Blots. SDS-PAGE and Western blots were performed as described (13, 17). Proteins were stained by AuroDye (Janssen Pharmaceutica, Beerse, Belgium) according to the instruction of the manufacturer.

Amino terminal sequence analysis. The lyophilized antigens were reconstituted with 1% acetic acid, and insoluble material was removed by centrifugation in a high-speed microfuge. Aliquots of each antigen were subjected to automated

Edman degradation on a gas-phase microsequencer built at the City of Hope as previously described for CEA and NCA (9). Phenylthiohydantoin amino acid derivatives were separated and identified by reverse-phase HPLC using a Beckman HPLC system coupled on-line to the microsequencer. The remainder of each antigen was chromatographed on a 4.6 x 30 mm Brownlee RP300 reverse-phase HPLC column. The proteins were eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. Both antigens eluted as single peaks at approximately 50% acetronitrile, with a small amount of contaminating material eluting earlier in the gradient in each case. Aliquots of each antigen were subjected to amino acid analysis using a Beckman 6300 amino acid analyzer. The amino acid compositions for the two antigens are similar to those reported for CEA and NCA (9). Each antigen was reanalyzed on the gas-phase microsequencer, with the results being identical to the first analysis.

RESULTS

The perchloric acid extract from meconium was passed over an immunoabsorbent containing MAb T84.1. This antibody of high affinity and broad specificity binds to all CEA related antigens described so far. The eluted fraction was further purified over a TSK G 3000 SW column (Fig 1a). Western blots performed with MAb T84.1 revealed that pool I contained a 165 kD antigen and pool III contained an antigen in the range of 100-120 kD. Pool II contained both antigens. Pool II from Fig. 1a was passed over immobilized MAb CEA.11. The bound and eluted fraction was further characterized by size exclusion chromatography. The immunoreactive material eluted as a homogeneous, slightly asymmetrical peak (Fig. 1b). The indicated fractions were pooled and added to pool I from Fig. 1a, dialyzed against water, and lyophilized. This material will be designated nonspecific crossreacting antigen 2 (NCA-2). The chromatogram of the fraction not bound by MAb CEA.11 is shown in Fig. 1c. The immunoreactive material elutes as two partly resolved peaks. The peak fraction of the first immunoreactive peak elutes significantly later than the peak fraction of the antigen bound to MAb CEA.11 (see Fig. 1b). The pooled fractions indicated in Fig. 1c were rechromatographed. As shown in Fig. 1d, the immunoreactive material elutes as a single symmetrical peak. The pooled material indicated in Fig. 1c was combined with pool III from Fig. 1a, dialyzed against water and lyophilized. This antigen will be designated meconium antigen 100 (MA-100).

The two antigen preparations were subjected to SDS-PAGE, transferred to nitrocellulose, and stained by the use of labeled monoclonal antibodies and

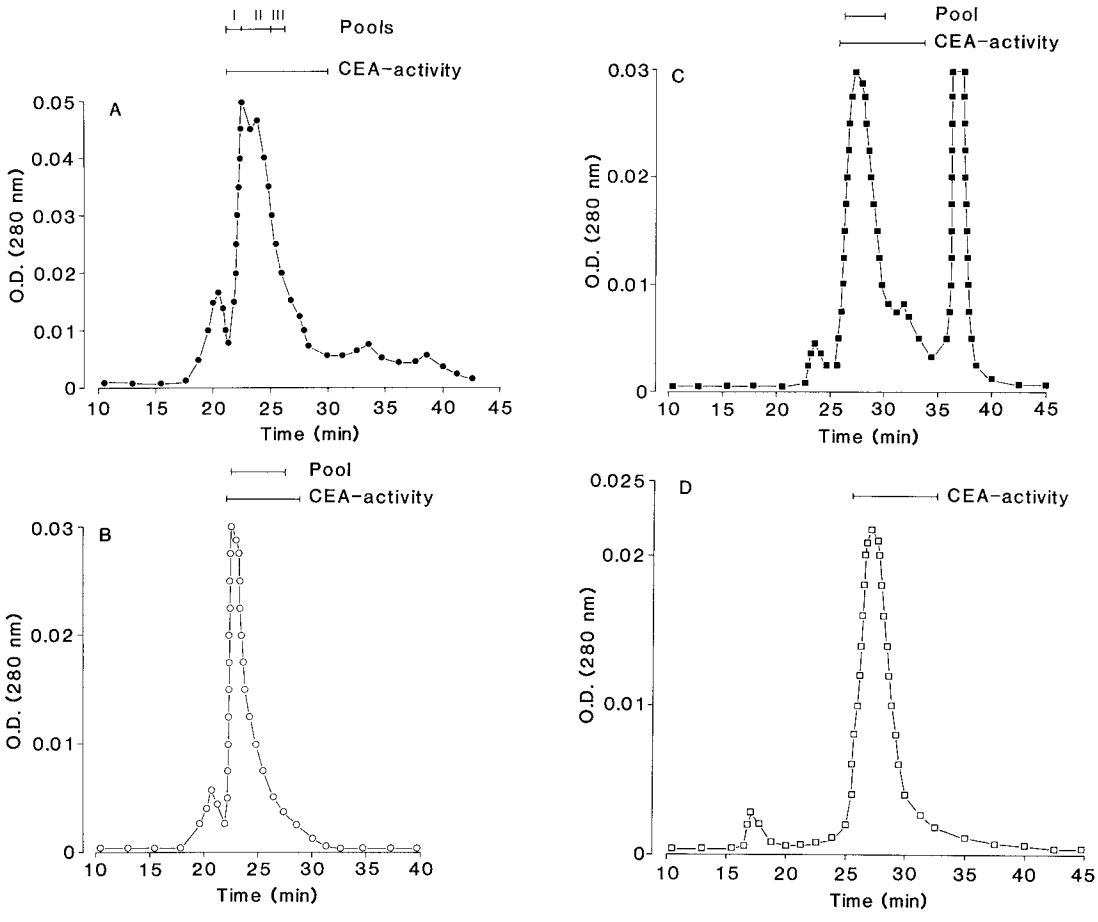
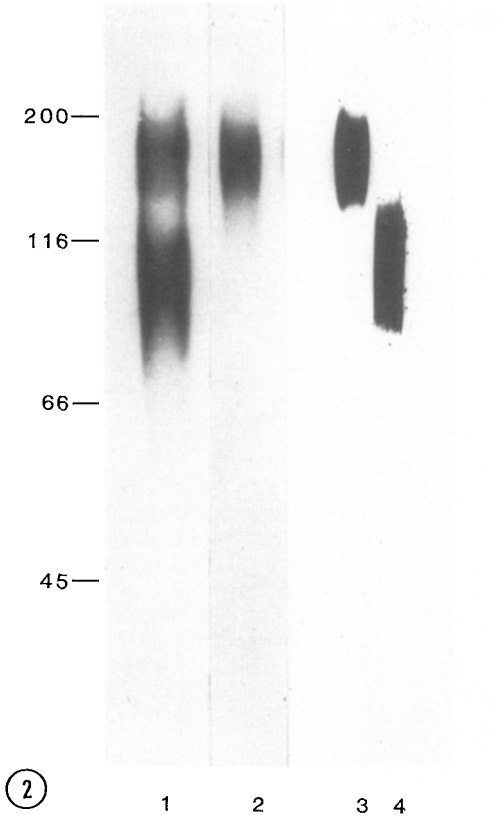


Fig. 1. Size exclusion HPLC of CEA related meconium antigens isolated by differential immunoaffinity chromatography. CEA activity was determined by enzyme immunoassay. (A) extract bound to MAb T84.1; (B) pool II from Fig. 1a bound to MAb CEA.11; (C) pool II from Fig. 1a not bound by MAb CEA; (D) pooled fractions from Fig. 1c.

colloidal gold, respectively (Fig.2). In the fraction eluted from the T84.1 immunosorbent, MAb T84.1 binds 2 antigens (lane 1), whereas MAb CEA.11 binds the high molecular weight antigen only (lane 2). Both, the high molecular weight (lane 3) and low molecular weight (lane 4) meconium antigen preparations are pure when stained by colloidal gold. The molecular weight of NCA-2 corresponds to 165,000, the molecular weight of MA-100 to approximately 105,000. The Western blot of the purified antigens performed with MAb T84.1 exhibited a staining pattern comparable to the colloidal gold stain indicating the absence of contaminating antigens in the respective antigen fractions (not shown).



(2)

(3)

Antigen	Amino terminal sequence		
		10	20
CEA	KLTIESTPFNVAEGKEVLLLVHNLPQHLF		
NCA-2			
MA-100			
NCA-95	KLTIESTPFNVAEGKEVLLLAHNLPQNRI		
NCA-55			

Fig. 2 Western blots of purified CEA-related meconium antigens. Molecular weights of standard proteins (kD) are indicated at the left margin. Lane 1: fraction eluted from immobilized MAb T84.1 and stained by the use of the same antibody; lane 2: fraction eluted from immobilized MAb T84.1 and stained by the use of MAb CEA.11; lane 3: NCA-2 preparation stained by colloidal gold; lane 4: MA-100 preparation stained by colloidal gold.

Fig. 3. Amino terminal amino acid sequences of NCA-2 and MA-100 in comparison with the respective sequences published for CEA, NCA-95, and NCA-55 by Paxton et al. (9). The CEA, NCA-2, and MA-100 sequences are identical, differing from NCA-95 and NCA-55 at positions 21 and 27-29 (boxed).

Fig. 3 shows the amino terminal sequences for the meconium antigens in comparison with CEA and the crossreacting antigens NCA-95 and NCA-55, respectively. Both meconium antigens share their amino acid sequences with CEA. The NCA-specific amino acid substitutions at positions 21 and 27-29 are lacking.

DISCUSSION

Two antigens crossreacting with CEA were isolated from meconium, purified to homogeneity, and amino terminally sequenced. For both antigens, the

sequences match that of CEA and differ from the respective sequences of two nonspecific crossreacting antigens. It follows that the amino terminal sequence of CEA can no longer be regarded as CEA-specific as it may be shared by other antigens of the CEA family. The high molecular weight meconium antigen (NCA-2) has been shown to closely resemble CEA, both with respect to biochemical and immunochemical properties (10-12). Therefore, the identity of the amino terminal sequences of CEA and NCA-2 is not unexpected. As judged from electrophoretic mobility and antibody binding, the low molecular weight meconium antigen, MA-100, is much more similar to NCA-95 (4) than to CEA (13). For this reason, the low molecular weight meconium antigen was designated meconium NCA by Matsuoka et al. (12). The amino terminal sequence, however, clearly distinguishes MA-100 from NCA. Thus, MA-100 is a distinct new member of the family of CEA related antigens as suggested previously (13).

Among the five monoclonal anti-CEA antibodies with different epitope specificities used in this and our previous studies, NCA-2 binds 4 antibodies and MA-100 3 antibodies. As each of the 5 antibodies binds to the protein moiety of CEA (16), it is probable that the structural differences between CEA, NCA-2, and MA-100 reside in the protein moieties of the respective antigens. As concluded from restriction fragments of genomic DNA, it has been suggested that the CEA gene family may comprise 9-11 different genes (13). Thus, NCA-2 and MA-100 could represent distinct gene products. Alternatively, the structural differences between the antigens could be a result of alternative RNA splicing or post-translational modifications. Further protein sequencing and molecular cloning studies will ultimately answer these questions.

One important conclusion drawn from this study regards the identification of different genes of the CEA family. The identity of the deduced amino acid sequence of the amino terminus can no longer be taken as major argument to classify a gene as "CEA-specific" (19). Considering the extensive structural homologies between CEA and the high and low molecular weight NCAs (9) additional information on internal sequences of the different CEA-related

antigens are needed in order to unequivocally relate nucleotide sequences of cloned genes to the respective antigens.

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REFERENCES

1. Rogers, G.T. (1983) Biochim. Biophys. Acta 695, 227-249.
2. Mach, J.-P. and Pusztaszeri, G. (1972) Immunochemistry 9, 1031-1034.
3. Kleist, S. von, Chavanel, G. and Burtin, P. (1972) Proc. Natl. Acad. Sci. USA 69, 2492-2494.
4. Buchegger, F., Schreyer, M., Carrel, S. and Mach, J.-P. (1984) Int. J. Cancer 33, 643-649.
5. Geilgens, A., Schwonzen, M. and Wagener, C. (1987), submitted.
6. Terry, W.D., Henkart, P.A., Coligan, J.E., and Todd, C.W. (1972) J. Exp. Med. 136, 200-204.
7. Engvall, E., Shively, J.E., and Wrann, M. (1978) Proc. Natl. Acad. Sci. USA 75, 1670-1674.
8. Kessler, M.J., Shively, J.E., Pritchard, D.G. and Todd, C.W. (1978) Cancer Res. 38, 1041-1048.
9. Paxton, R.J., Mooser, G., Pande, H., Lee, T.D., and Shively, J.E. (1987) Proc. Natl. Acad. Sci. USA 84, 920-924.
10. Burtin, P., Chavanel, G. and Hirsch-Marie, H. (1973) J. Immunol. 111, 1926-1928.
11. Burtin, P., Roubertie, P., Chavanel, G., Sambine, M.C. and Hirsch-Marie, H. (1976) In Protides of the Biological Fluids, Proc. 24th Colloqu. (H. Peeters, ed.) pp. 447-449. Pergamon Press, Oxford, New York.
12. Matsuoka, Y., Kuroki, M., Koga, Y., Kuriyama, H., Mori, T. and Kosaki, G. (1982) Cancer Res 42, 2012-2018.
13. Neumaier, M., Fenger, U. and Wagener, C. (1985) J. Immunol 135, 3604-3609.
14. Koga, Y., Kuroki, M., Matsunaga, A., Shinoda, T., Takayasu, T. and Matsuoka, Y. (1985) Mol. Immunol. 22, 67-73.
15. Wagener, C., Clark, B., Rickard, K.J. and Shively, J.E. (1983) J. Immunol. 130, 2302-2307.
16. Wagener, C., Yang, Y.H.J., Crawford, F.G. and Shively J.E. (1983) J. Immunol. 130, 2308-2315.
17. Neumaier, M., Fenger, U. and Wagener, C. (1985) Mol. Immunol. 22, 1273-1277.
18. Thompson, J.A., Pande, H., Paxton, R.J., Shively, L., Padma, A., Simmer, R.L., Todd, C.W., Riggs, A.D. and Shively, J.E. (1987) Proc. Natl. Acad. Sci. USA 84, 2965-2969.
19. Oikawa, S., Nakazato, H. and Kosaki, G. (1987) Biochem. Biophys. Res. Comm. 142, 511-518.