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Protein Epitopes in Carcinoembryonic Antigen

Report of the ISOBM TD8 Workshop

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Key Words
Carcinoembryonic antigen · Epitopes · Monoclonal antibody

Abstract
To characterize antigenic sites in carcinoembryonic antigen (CEA) further and to investigate whether there are differences between colon tumor CEA and meconium CEA (NCA-2) that can be detected by anti-CEA monoclonal antibodies (MAb), 19 new anti-CEA MAb were analyzed with respect to specificity, epitope reactivity and affinity. Their reactivities were compared with 10 anti-CEA MAb with known CEA-domain binding specificity that have previously been classified into five nonoverlapping epitope groups, GOLD 1–5. Cross-inhibition assays with antigen-coated microtiter plates and immunoradiometric assays were performed in almost all combinations of MAbs, using conventionally purified CEA (domain structure: N-A1B1-A2B2-A3B3-C) from liver metastasis of colorectal carcinomas, recombinant CEA, meconium CEA (NCA-2), truncated forms of CEA and NCA (CEACAM6) as the antigens. The affinity of the MAbs for CEA was also determined. The new MAbs were generally of high affinity and suitable for immunoassays. Three new MAbs were assigned to GOLD epitope group 5 (N-domain binding), 3 MAbs to group 4 (A1B1 domain), 1 to group 3 (A3B3 domain), 3 to group 2 (A2B2 domain) and 3 to group 1 (also the A3B3 domain). Three MAbs formed a separate group related to group 4, they were classified as GOLD 4′ (A1B1 domain binding). The remaining 3 MAbs appear to represent new subspecificities with some relationship to GOLD groups 1, 2 or 4, respectively. Five MAbs, all belonging to epitope group 1 and 3, reacted strongly with tumor CEA but only weakly or not at all with meconium CEA, demonstrating that the two products of the CEA gene differ from each other, probably due to different posttranslational modifications.

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Introduction

The human carcinoembryonic antigen (CEA) is one of the most widely used serum tumor markers. Its main clinical utility is in monitoring colorectal carcinoma after surgical resection. CEA was discovered by Gold and Freedman in 1965 [1], cloned and sequenced by several research groups in 1987 [2–5], and the first tumor marker to be subjected to epitope mapping under the auspices of the International Society for Oncodevelopmental Biology and Medicine (ISOBM) [6]. CEA is a heavily glycosylated (50% carbohydrate) protein with a molecular mass of 150,000 [7]. It consists of 1 N-terminal immunoglobulin (Ig)V-like domain (N) and 6 Ig-constant type 2 (IgC2)-like domains (referred to as A and B). CEA is linked via a glycosyl phosphatidyl inositol anchor (referred to as C) to the apical surface of epithelial cells. Thus the domain formula for CEA is: [N-A1B1-A2B2-A3B3-C]. It is a monomer in solution and has an extended zigzag structure [7]. Soluble CEA in serum from cancer patients appears to be identical or closely similar to CEA cleaved from tumor cells by bacterial phosphatidyl inositol-specific phospholipase C [8]. CEA is a member of the CEA gene subfamily which, in turn, belongs to the immunoglobulin gene superfamily [9, 10]. The CEA subfamily contains 7 expressed members [10], the genes of which are located on chromosome 19q13:2 [11]. These are CEA, NCA (CEACAM6), BGP (CEACAM1), CGM1 (CEACAM3), CGM2 (CEACAM7), CGM6 (CEACAM8) and CGM7 (CEACAM4). The names in parentheses refer to the new nomenclature agreed in 1999 [12]. Due to the high degree of sequence homology between the molecules in the CEA subfamily, significant immunological cross-reactivity between members has been found. Thus a number of MAbs against CEA will cross-react, in particular, with NCA [13] and BGP [14, 15].

In the first CEA workshop, epitope mapping of CEA was performed, assigning a total of 43 anti-CEA MAbs into one of five essentially noninteracting epitope groups (GOLD 1–5), with each group containing between 5 and 15 MAbs [6]. The epitopes recognized by the MAbs belonging to GOLD 1–5 were peptide in nature. In a later study using recombinant CEA fragments, most results were confirmed, although for some of the antibodies different binding sites were suggested [16]. In that study it was also possible to identify in which domain of the CEA molecule GOLD group 1–5 epitopes were localized.

In this second CEA workshop we have extended the epitope-mapping endeavor to 19 new antibodies, together with 10 anti-CEA MAbs from the former workshop as reference antibodies, representing the GOLD epitope groups 1–5. Moreover, different CEA preparations, including recombinant CEA and fragments thereof, were used. In addition, we have studied the reactivity with meconium CEA (also termed NCA-2 or meconium antigen) [17, 18]. Meconium CEA is encoded by the CEA gene [19] and should therefore have the same primary peptide sequence as tumor CEA. However, secondary-processing events may be different. In fact, the two antigens display subtle differences in their glycosylation patterns [20, 21]. Thus NCA-2 appears to be more glycosylated than tumor CEA (27 versus 25 sugar chains/mole), lacks high mannose-type carbohydrate chains (0 versus 8 %), contains complex-type sugar chains with [Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-] outer chains, and has different proportions of some of the other complex-type sugar chains [21].

Materials and Methods

CEA and CEA-Like Antigens

Three CEA preparations were used: one preparation [referred to as 'CEA(Oslo)')] was purified by the Oslo group from liver metastases of a patient with colorectal carcinoma, using perchloric acid extraction followed by ion exchange chromatography and gel filtration [22]. The second CEA preparation was a gift from Dr. John E. Shively, Duarte, and is referred to as the 'CEA(Duarte)' preparation. It was pure by several physico-chemical criteria and was also used in the first CEA workshop [6]. The third preparation was a recombinant CEA produced in Chinese hamster ovary cells and recovered from the supernatant of the transfected cells grown in vitro [16]. The cell lines were kindly provided by Dr. Miyazaki, Suntory Biomedical Research Ltd, Osaka, Japan, who also provided cell lines producing three truncated forms of recombinant CEA: [N-A1B1], [N-halfA1-A3B3] and N [16]. Meconium CEA, also known as non-specific crossreacting antigen 2 (NCA-2), was purified from a pool of meconium from 8 infants born at term, using perchloric acid extraction followed by immunoaffinity chromatography with the MAb 12-140-2 [23]. Purified NCA (CEACAM6) was a gift from the late Prof. Pierre Burtin, Villejuif. It was purified from normal lung tissue by perchloric acid extraction and gel filtration [13]. All preparations were calibrated against a routine inhouse immunofluorometric assay (IFMA) for CEA, employing the mouse MAbs 12-140-10 and 12-140-1 [24].

MAbs

Fourteen new MAbs were received through the ISOBM TD-8 CEA Workshop as coded (ISOBM-298–ISOBM-311) aliquots at the beginning of the study. Five antibodies (ISOBM-342–ISOBM-346) were received at a later stage. Ten antibodies from the former workshop were also supplied as reference antibodies (two antibodies from each GOLD group) and were coded (ISOBM-288–ISOBM-297). Three of the reference antibodies (ISOBM-290, ISOBM-293 and ISOBM-295) were available only in smaller quantities, limiting their use in some applications. Three of the new antibodies (ISOBM-305, ISOBM-306 and ISOBM-307) were rat monoclonal IgG-antibodies;

Table 1. Summary

TD-No.		Antibody	Isotype	Species	Source	Provider	GOLD group	Reactivity with NCA
ISOBM-288	Ref	F6	IgG1	mouse	CIS bio international	L. Bellanger	1	
ISOBM-289	Ref	G15	IgG1	mouse	CIS bio international	L. Bellanger	2	
ISOBM-290	Ref	II-17	IgG1	mouse	Umeå University	S. Hammarström	3	
ISOBM-291	Ref	G12	IgG1	mouse	CIS bio international	L. Bellanger	4	
ISOBM-292	Ref	G13	IgG1	mouse	CIS bio international	L. Bellanger	5	
ISOBM-293	Ref	T84.66	IgG1	mouse	?	J.E. Shively/C. Wagner	1	
ISOBM-294	Ref	12-140-2	IgG2b	mouse	Norwegian Radium Hospital	O.P. Børmer	2	
ISOBM-295	Ref	CE 27	IgG1	mouse	?	J.P. Mach/F. Buchegger	3	
ISOBM-296	Ref	12-140-10	IgG1	mouse	Norwegian Radium Hospital	O.P. Børmer	4	
ISOBM-297	Ref	12-140-1	IgG1	mouse	Norwegian Radium Hospital	O.P. Børmer	5	
ISOBM-298	TD-ab	12-140-6	IgG1	mouse	Norwegian Radium Hospital	O.P. Børmer	4′	+
ISOBM-299	TD-ab	12-140-8	IgG1	mouse	Norwegian Radium Hospital	O.P. Børmer	4	+
ISOBM-300	TD-ab	12-664	IgG2b	mouse	Norwegian Radium Hospital	O.P. Børmer	5	–
ISOBM-301	TD-ab	DPC CEA 1	IgG1	mouse	DPC	P. Sibley	2	–
ISOBM-302	TD-ab	DPC CEA 2	IgG1	mouse	DPC	P. Sibley	4	+
ISOBM-303	TD-ab	IM 10/6.2	IgG1	mouse	SAPU	J.L. Young	5	–
ISOBM-304	TD-ab	IM 10/13.1	IgG1	mouse	SAPU	J.L. Young	4′	+
ISOBM-305	TD-ab	GFR44		rat	CIS bio international	L. Bellanger	3	–
ISOBM-306	TD-ab	GFR46		rat	CIS bio international	L. Bellanger	4″	–
ISOBM-307	TD-ab	GFR48		rat	CIS bio international	L. Bellanger	1	–
ISOBM-308	TD-ab	G25	IgG1	mouse	CIS bio international	L. Bellanger	1	–
ISOBM-309	TD-ab	G208	IgG1	mouse	CIS bio international	L. Bellanger	1[a]	+
ISOBM-310	TD-ab	CEA Ab-3	IgG2a	mouse	NeoMarkers	A.K. Tandon	5	–
ISOBM-311	TD-ab	CEA Ab-4	IgG1	mouse	NeoMarkers	A.K. Tandon	2	–
ISOBM-342	TD-ab	1C3	IgG1	mouse	Xema-Medica Co. Ltd.	Y. Lebedin	4	+
ISOBM-343	TD-ab	1C6	IgG1	mouse	Xema-Medica Co. Ltd.	Y. Lebedin	1	–
ISOBM-344	TD-ab	1C10	IgG1	mouse	Xema-Medica Co. Ltd.	Y. Lebedin	2[a]	–
ISOBM-345	TD-ab	1C11	IgG1	mouse	Xema-Medica Co. Ltd.	Y. Lebedin	4′	+
ISOBM-346	TD-ab	1C13	IgG1	mouse	Xema-Medica Co. Ltd.	Y. Lebedin	2	–

[a] This antibody was not inhibited by any other antibody. The number is the closest epitope group.

all others were mouse monoclonal IgG antibodies. All antibodies were provided in phosphate-buffered saline (PBS) with 1 g/l NaN_3 and were of high purity as judged by SDS-PAGE. Table 1 lists the donors and sources of the MAbs.

Coating of Microtiter Plates

Antibodies were preincubated in a buffer pH 2.5 containing 0.1 mol/l glycine (Sigma, St Louis, Mo., USA) for 10 min under continuous stirring, and then neutralized by an excess of 0.2 mol/l sodium dihydrogen phosphate buffer pH 4.3 to a final concentration of 5 mg/l. Aliquots of 200 µl (1 µg antibody) were added to Maxi Breakapart microtiter wells (Nunc, Copenhagen, Denmark). Wells were incubated in a humidified chamber at 37 °C for 20 h, washed twice with a buffer pH 7.8 [0.05 mol/l Tris 7–9 (Sigma), 0.15 mol/l NaCl, 1 g/l Germall II (ISP Sutton Laboratories, Chatham, UK) and 0.5 g/l Tween 20 (Serva, Heidelberg, Germany)], and blocked with 300 µl of 0.05 mol/l Tris-HCl [pH 7.0, containing 10 g/l BSA (Sigma A 4503), 60 g/l D-Sorbitol (Sigma) and 0.5 g/l NaN_3] for 20 h at room temperature in a humidified chamber. Plates were then aspirated and kept dry until use.

CEA and CEA-like antigens were suspended in a 0.2 mol/l carbonate buffer pH 9.3 to a molar concentration equivalent to 2.5 mg/l CEA. Aliquots of 100 µl (0.25 µg antigen) were added to Maxi Breakapart microtiter wells and incubated in a humidified chamber at 37 °C for 20 h. Wells were then aspirated and blocked using 200 µl PBS with BSA 10 g/l and 0.5 g/l NaN_3 added, incubated for 2 h, washed three times with PBS with 0.5 g/l NaN_3 and finally stored dry until use.

Radioiodination of Proteins

Antibodies and antigens were radio-iodinated with equimolar amounts of ^{125}I with the IODO-GEN Iodination Reagent (Pierce, Rockford, Ill., USA), using the indirect method described by the producer.

Table 2. Dissociation constants for the interaction between anti-CEA mAb and tumor CEA or NCA-2

Antibody name	Tumor CEA (Oslo) K_d in nmol/l		NCA-2 K_d in nmol/l	
	graphic estimation	Scatchard plot	graphic estimation	Scatchard plot
ISOBM-298	1	0.46	2	4.4
ISOBM-299	2	1.2	2	5.6
ISOBM-300	0.09	0.092	0.15	0.12
ISOBM-301	1	1.1	1	0.85
ISOBM-302	1	1.2	1	4.1
ISOBM-303	<0.06	0.06	<0.06	0.022
ISOBM-304	0.2	0.2	0.25	0.57
ISOBM-305	<0.06	0.028	low binding	
ISOBM-306	1	0.83	1.5	0.7
ISOBM-307	0.3	0.23	low binding	
ISOBM-308	0.7	0.3	low binding	
ISOBM-309	0.15	0.13	0.1	0.093
ISOBM-310	0.07	0.068	0.07	0.048
ISOBM-311	0.3	0.33	0.15	0.4
ISOBM-342	0.6	0.55	0.6	0.5
ISOBM-343	2.5		low binding	
ISOBM-344	5		0.1	0.095
ISOBM-345	1.5	1.7	1.5	2.5
ISOBM-346	>8		>12	
T84.66	<0.06	0.019	low binding	
12-140-10	0.3	0.29	0.25	0.47

Cross-Inhibition Assays

Antigen-coated wells were allowed to react overnight with 3 μg/well of inhibiting antibody in 100 μl of an assay buffer consisting of 0.05 mol/l PBS pH 7.4 with 10 g/l BSA added. To each well, 3 ng of radio-iodinated competing antibody was then added in 50 μl assay buffer, and wells were incubated for 2 h under continuous shaking, washed three times with PBS with 0.5 g/l NaN_3, and bound radioactivity was counted. Percent inhibition was calculated as 100 – 100 × [(bound – blank)/(total – blank)].

Immunoradiometric Assays

Antibody pairs in all combinations were tested for their reactivity with the following antigens: CEA(Oslo), recombinant CEA and meconium CEA (NCA-2). Antigens were suspended in an assay buffer pH 7.5 consisting of 0.05 mol/l of Tris 7–9, 0.10 mol/l NaCl, 10 g/l BSA, 0.5 g/l Tween 20 and 0.1 g/l merthiolate to a final antigen concentration with an activity corresponding to 80 μg/l of CEA. One hundred microliters of this solution was added to each antibody-coated well, then incubated under shaking for 2 h and then washed three times with the pH 7.8 washing buffer. Radio-iodinated tracer antibodies were diluted to 100 μg/l in assay buffer, and 125 μl (approx. 60,000 cpm) was added to each well and incubated for 2 h. Wells were then washed six times with washing buffer and bound radioactivity was counted. The immunoradiometric assay combinations were analyzed in duplicates, and the mean CV for duplicates was 5.2%.

Reactivity with NCA was measured by adding 125 μl of radio-iodinated NCA diluted in assay buffer to 100 μg/l (approx. 60,000 cpm/well) to each antibody-coated well, incubating for 2 h and then washing six times with the pH 7.8 washing buffer before counting. Radio-iodinated CEA and meconium CEA were used as positive controls in the NCA assay.

Affinity Measurements

Dissociation constants (K_d) for antibodies and antigens were estimated from the concentration of free antibody (in mol/l) needed to achieve half-maximal binding of the radio-iodinated antigen. Dissociation constants were also estimated from Scatchard plots. Increasing concentrations of antibodies (from 10 μg/l to 10 mg/l in 500 μl PBS with BSA 10g/l) were added to tubes containing 2 ng of radiolabeled antigen in 100 μl PBS/BSA and incubated overnight at 20°C. Polyclonal sheep anti-mouse antibodies coupled to paramagnetic polymer particles (Dynabeads M280; Dynal, Oslo, Norway), previously shown to have similar binding capacities for mouse and rat antibodies, were used in excess to separate bound and free antigen, followed by washing and counting of bound radioactivity.

Results

Antibody Affinity

K_d for the interaction between the 19 new anti-CEA MAbs and tumor CEA or meconium CEA were determined as described in Materials and Methods. The K_d values were determined in two ways: (a) by a graphic estimation of the concentration of free antibodies at 50% of the maximum binding of the antigen, and (b) by Scatchard plots. There was good agreement between the two methods of calculation. For the 3 antibodies with low affinities (K_d values above 2 nmol/l), the estimated values are only approximate; higher concentrations of antibodies would have been needed in order to obtain an accurate measurement. The K_d values for the interaction between CEA and the 19 MAbs ranged from 0.02 to 8 nmol/l (table 2). Fifteen of the new MAbs reacted with meconium CEA, with affinities ranging from 0.02 to 12 nmol/l, while 4 new MAbs and reference MAb T84.66 displayed weak binding only (table 2).

Immunoreactivity of each MAb was also calculated from the affinity determinations. The immunoreactive fraction was expressed as the percent of bound activity [100 × (counts after washing/counts added)] for each MAb relative to the activity for the reference MAb 12-140-1 (fig. 1). It can be interpreted as the percentage of available binding sites on tumor CEA or meconium CEA for each antibody. It is interesting to note that 5 MAbs (ISOBM-305, ISOBM-307, ISOBM-308, ISOBM-343 and T84.66) bind very poorly to meconium CEA (table 2; fig. 1) while displaying strong binding to CEA.

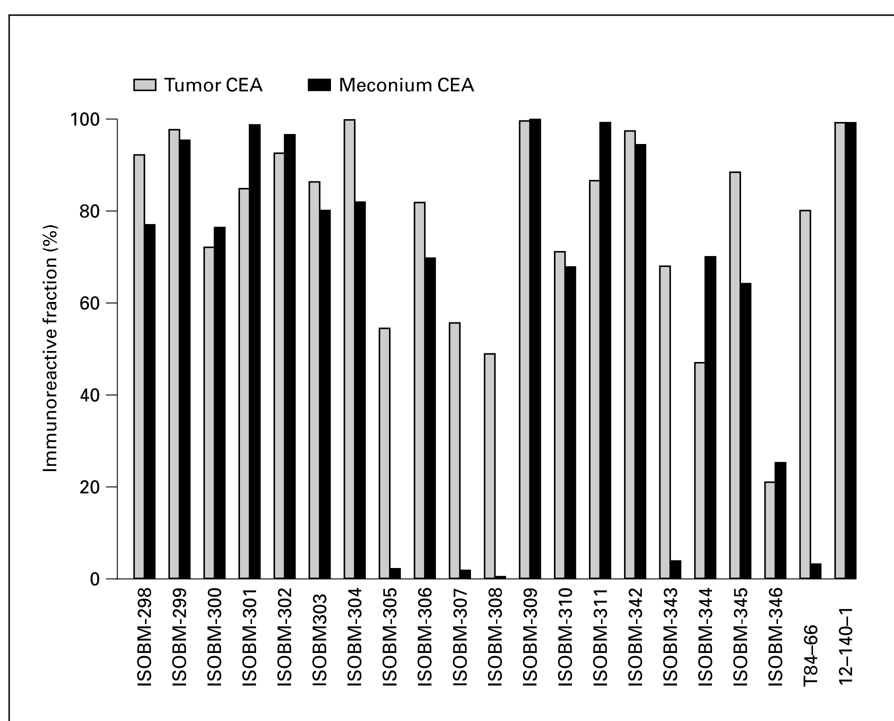

Fig. 1. Antibody binding of tumor and meconium CEA. The immunoreactive fraction of each antigen preparation was calculated as the percent of bound activity for each antibody relative to the activity for the reference antibody 12-140-1.

Cross-Inhibition Assays

The ability of an excess amount of unlabeled anti-CEA MAb to compete with labeled anti-CEA MAb for binding to tumor CEA (two different preparations) and meconium CEA-coated microtiter plates was studied for almost all combinations. The results are shown as percent inhibition (table 3–5). MAbs with similar inhibition patterns are grouped together, and the boxes in the tables denote antibodies showing complete inhibition within the group. The same groups of cross-inhibitory MAbs were seen irrespective of which of the 3 antigens were used.

Immunoradiometric Assays

Immunoradiometric assays were performed with 7 different antigens, tumor CEA(Oslo), recombinant CEA, meconium CEA, recombinant N-A1B1, recombinant N-halfA1-A3B3, recombinant N, and recombinant NCA. If the particular antigen under investigation has a binding site for the solid-phase antibody, the antigen will bind to solid phase. Bound antigen can then be detected if it also contains an accessible binding site for the tracer antibody. If, on the other hand, two antibodies react with the same binding site or with two closely situated binding sites, no signal will be seen. Recombinant N and recombinant NCA yielded only weak signals of doubtful significance in these immunoradiometric assays. These results are therefore not shown. The results for the other 5 antigens are shown in table 6a–c and table 7a, b. Two MAbs had to be excluded from the study because they either did not bind any antigen when used on the solid phase (MAb ISOBM-292), or they did not work on the solid phase or as a tracer (MAb ISOBM-346). Analyses of the results in table 6a–c show that MAbs expected to belong to different epitope groups on the basis of the cross-inhibition studies generally could form an immunoradiometric assay. It can also be seen that some combinations of MAbs gave particularly efficient assays, i.e., with a large percentage of the labeled MAb bound to the solid phase. This was at least partly due to these MAbs having a high binding affinity for tumor CEA. Tables 7a, b demonstrate that only MAbs tentatively classified as belonging to GOLD epitope groups 4, 4′ and 5 gave immunoradiometric assays if the truncated CEA form N-A1B1 was used as the antigen. Similarly, immunoradiometric assays could only be created if combinations of MAbs tentatively classified as belonging to epitope group 1, 3 and 5 were used when the truncated CEA form N-halfA1-A3B3 was used as the antigen.

Table 3. Cross-inhibition study with anti-CEA MAbs and CEA (Oslo) as the antigen

Inhibiting MAb		Epitope Group	Labeled MAb ISOBM-303	ISOBM-310	ISOBM-300	ISOBM-342	ISOBM-299	ISOBM-302	ISOBM-306	ISOBM-298	ISOBM-304	ISOBM-345	ISOBM-346	ISOBM-301	ISOBM-311	ISOBM-344	ISOBM-309	ISOBM-343	ISOBM-307	ISOBM-308	ISOBM-305
ISOBM-303	IM 10/6.2	GOLD 5	99	100	100	-17	1	1	1	-16	2	70	45	6	11	39	1	-12	8	4	10
ISOBM-310	CEA Ab-3	GOLD 5	96	100	100	-36	0	-7	2	-2	-2	6	33	1	18	20	-4	-80	6	4	9
ISOBM-300	12-664	GOLD 5	97	100	100	-16	8	-4	2	7	12	24	44	6	5	37	5	-47	5	8	16
ISOBM-297	12-140-1	GOLD 5	97	99	99	-17	0	-5	6	8	8	22	45	-10	8	31	6	-76	-1	9	13
ISOBM-292	G13 Bu-103	GOLD 5	85	98	96	-44	5	-4	10	9	11	12	17	-2	-5	19	10	-116	5	13	17
ISOBM-342	1C3	GOLD 4	-32	-37	-32	98	98	99	28	28	23	26	56	27	-33	5	4	-5	-4	-55	-140
ISOBM-291	G12 Bu-102	GOLD 4	-54	-34	-61	98	99	100	89	-19	7	52	60	53	26	17	6	-118	23	3	13
ISOBM-296	12-140-10	GOLD 4	-41	-33	-49	100	97	99	66	-5	12	59	72	46	22	23	7	-68	9	4	15
ISOBM-299	12-140-8	GOLD 4	-36	-37	-46	98	99	98	67	-9	19	71	79	63	35	25	7	-92	17	4	6
ISOBM-302	DPC CEA 2	GOLD 4	-29	-25	-39	98	98	99	69	-1	14	58	80	55	18	32	7	-73	17	3	16
ISOBM-306	GFR 46	GOLD 4"	-39	-38	-50	-22	32	44	100	-14	6	35	18	7	1	14	-5	-116	0	-3	2
ISOBM-298	12-140-6	GOLD 4'	-38	-32	-46	32	38	47	22	100	98	96	50	-1	12	35	1	-48	-3	0	7
ISOBM-304	IM 10/13.1	GOLD 4'	82	96	88	48	42	50	16	100	100	103	88	27	13	72	-3	36	5	1	12
ISOBM-345	1C11	GOLD 4'	9	-78	-1	29	27	31	86	86	90	87	8	-13	4	12	4	8	13	-77	-4
ISOBM-346	1C13	GOLD 2	7	-15	-4	13	13	12	3	3	2	1	93	71	45	9	2	21	36	-7	-12
ISOBM-294	12-140-2	GOLD 2	-5	-6	-14	-23	19	7	7	0	4	15	100	99	90	30	-4	-123	9	4	7
ISOBM-289	G15 Bu-105	GOLD 2	-8	-2	-3	-51	7	4	5	-4	4	25	97	94	99	47	0	-130	24	8	17
ISOBM-301	DPC CEA 1	GOLD 2	-13	-8	-12	-11	11	9	3	-3	0	27	94	99	92	25	-2	-61	10	-2	18
ISOBM-311	CEA Ab-4	GOLD 2	0	-24	-1	-57	17	10	10	4	12	24	112	96	99	63	9	-38	20	13	24
ISOBM-344	1C10	GOLD 2	-16	-8	11	2	-10	3	0	0	-6	-3	33	-1	60	96	6	70	-46	-24	44
ISOBM-309	G208	GOLD 1	-29	-13	-27	-31	19	26	1	-12	-3	25	59	68	93	66	100	-85	100	99	10
ISOBM-343	1C6	GOLD 1	-24	-2	0	0	-10	-5	-2	-2	-7	-34	-19	-6	-23	14	10	101	71	-93	-104
ISOBM-293	T84.66	GOLD 1	-3	2	-7	-42	8	-1	5	-5	5	18	34	14	19	29	-1	89	91	25	-2
ISOBM-307	GFR48	GOLD 1	-24	-5	-20	-51	-1	-2	-3	-19	-11	15	22	23	8	29	-4	70	100	96	0
ISOBM-308	G25	GOLD 1	-27	-2	-30	-52	-3	-1	-4	-32	-11	12	23	31	2	27	-6	-121	97	100	4
ISOBM-288	F6 Bu-101	GOLD 1	9	0	-4	-46	5	2	5	-4	1	5	26	4	0	19	-1	-132	96	99	84
ISOBM-305	GFR44	GOLD 3	-15	-5	-14	-8	3	1	-1	-12	-6	15	24	67	1	33	-5	-43	9	2	100
ISOBM-295	CE27	GOLD 3	-11	0	-16	NT	0	-2	1	-9	-3	NT	NT	57	6	NT	-3	NT	6	1	86
ISOBM-290	II-17	GOLD 3	-8	-3	3	NT	4	-1	5	-1	9	NT	NT	62	16	NT	2	NT	25	7	-4

Results are shown as percent inhibition.
Boxed values = GOLD groups; dotted inlays = GOLD subgroups; NT = not tested.

Table 4. Cross-inhibition study with anti-CEA MAbs and CEA (Duarte) has the antigen

Inhibiting MAb		Epitope Group	Labeled MAb ISOBM-303	ISOBM-310	ISOBM-300	ISOBM-299	ISOBM-302	ISOBM-306	ISOBM-298	ISOBM-304	ISOBM-301	ISOBM-311	ISOBM-309	ISOBM-307	ISOBM-308	ISOBM-305
ISOBM-303	IM 10/6.2	GOLD 5	98	100	99	21	30	37	19	33	29	9	5	21	-1	9
ISOBM-310	CEA Ab-3		98	100	100	7	15	13	3	-1	16	15	5	15	-6	-2
ISOBM-300	12-664		98	100	100	11	17	16	0	3	14	9	3	24	-1	-1
ISOBM-297	12-140-1		97	99	99	12	20	18	-1	7	23	7	2	26	-2	8
ISOBM-292	G13 Bu-103		95	99	99	11	18	14	-2	3	12	6	3	12	1	-1
ISOBM-291	G12 Bu-102	GOLD 4	6	13	5	99	101	94	9	18	57	22	22	34	5	16
ISOBM-296	12-140-10		14	7	9	98	100	84	9	17	53	18	21	37	2	13
ISOBM-299	12-140-8		15	20	19	98	100	83	28	38	59	29	18	30	2	6
ISOBM-302	DPC CEA 2		15	3	11	98	100	84	11	14	50	22	16	31	2	11
ISOBM-306	GFR46	GOLD 4"	6	-2	12	42	40	100	6	9	17	6	4	22	-2	9
ISOBM-298	12-140-6	GOLD 4'	21	17	23	40	49	62	100	99	34	17	0	20	-4	1
ISOBM-304	IM 10/13.1		96	98	98	42	47	61	100	100	26	6	4	25	-1	3
ISOBM-294	12-140-2	GOLD 2	17	12	15	27	28	13	1	1	98	89	5	41	7	-1
ISOBM-289	G15 Bu-105		-12	-1	10	8	12	4	3	2	94	99	9	26	13	21
ISOBM-301	DPC CEA 1		10	13	24	24	28	13	1	2	100	88	3	29	-1	20
ISOBM-311	CEA Ab-4		17	25	33	20	22	15	0	10	97	100	16	28	8	17
ISOBM-309	G208	GOLD 1	4	6	7	36	53	20	5	3	64	92	100	100	98	17
ISOBM-293	T84.66		7	10	18	7	13	13	-1	0	30	13	9	94	32	1
ISOBM-307	GFR48		7	6	7	15	13	18	1	-2	35	15	12	100	93	0
ISOBM-308	G25		10	9	9	5	13	13	-1	-2	29	11	6	99	100	13
ISOBM-288	F6 Bu-101		8	-6	-8	3	7	4	3	5	-4	3	6	97	99	86
ISOBM-305	GFR44	GOLD 3	13	9	22	25	19	14	-1	-1	70	4	4	20	9	100
ISOBM-295	CE27		7	12	15	13	19	15	2	2	69	17	5	28	2	85
ISOBM-290	II-17		9	0	3	-1	6	0	-3	-2	51	12	0	22	4	-20

Results are shown as percent inhibition.
Boxed values = GOLD groups; dotted inlays = GOLD subgroups.

Table 5. Cross-inhibition study with anti-CEA MAbs and meconium CEA as the antigen

Inhibiting MAb		Epitope Group	Labeled MAb ISOBM-303	ISOBM-310	ISOBM-300	ISOBM-342	ISOBM-299	ISOBM-302	ISOBM-306	ISOBM-298	ISOBM-304	ISOBM-345	ISOBM-346	ISOBM-301	ISOBM-311	ISOBM-344	ISOBM-309
ISOBM-303	IM 10/6.2	GOLD 5	100	100	101	7	-2	-4	19	14	21	83	15	-13	-2	12	-2
ISOBM-310	CEA Ab-3		97	99	100	-36	-10	-17	-7	-2	-8	6	33	-22	0	20	1
ISOBM-300	12-664		98	100	100	4	-7	-5	-2	0	0	29	20	-7	-1	8	-2
ISOBM-297	12-140-1		98	100	99	0	-2	0	7	7	9	18	31	-4	10	2	11
ISOBM-292	G13 Bu-103		94	99	99	-1	0	3	3	6	6	35	26	-5	4	2	0
ISOBM-342	1C3	GOLD 4	-40	-59	-31	99	99	99	88	14	24	27	41	29	-8	-6	-2
ISOBM-291	G12 Bu-102		-6	20	-7	99	99	99	96	16	23	45	61	29	11	2	4
ISOBM-296	12-140-10		-1	23	0	100	99	99	88	17	25	54	55	32	5	1	11
ISOBM-299	12-140-8		9	34	15	99	98	98	81	26	29	86	72	37	17	6	2
ISOBM-302	DPC CEA 2		2-1	2	11	100	99	86	88	9	14	56	68	40	7	2	-1
ISOBM-306	GFR46	GOLD 4"	-4	22	0	-22	16	22	98	0	7	35	18	-25	0	14	-3
ISOBM-298	12-140-6	GOLD 4'	8	33	8	28	29	36	64	100	98	99	30	-2	3	3	8
ISOBM-304	IM 10/13.1		94	98	97	75	24	29	57	100	99	114	97	-12	2	81	-3
ISOBM-345	1C11		-17	-27	0	16	21	23	51	92	93	94	-2	7	-1	-9	20
ISOBM-346	1C13	GOLD 2	-12	-10	2	28	32	29	-19	2	9	18	98	88	65	3	-1
ISOBM-294	12-140-2		2	29	6	18	27	22	3	5	6	34	100	96	93	15	0
ISOBM-289	G15 Bu-105		5	2	7	-18	5	5	-3	1	2	26	100	94	99	19	7
ISOBM-301	DPC CEA 1		-15	32	14	15	17	-57	0	2	2	25	99	99	89	-1	-3
ISOBM-311	CEA Ab-4		-9	40	28	-57	-12	-21	-25	-4	-8	24	112	91	100	63	3
ISOBM-344	1C10		-30	-16	5	1	-2	2	-6	-3	-6	-7	20	15	50	100	-1
ISOBM-309	G208	GOLD 1	0	24	8	-31	13	20	0	2	-7	25	59	-21	84	66	100

Results are shown as percent inhibition.

Boxed values = GOLD groups; dotted inlays = GOLD subgroups.

Table 6. a Immunoradiometric assays with different combinations of anti-CEA MAbs and CEA (Oslo) as the antigen
b Immunoradiometric assays with different combinations of anti-CEA MAbs and recombinant CEA as the antigen
c Immunoradiometric assays with different combinations of anti-CEA MAbs and meconium CEA as the antigen

	Tracer MAb		Epitope Group	Solid phase MAb																								
				ISOBM-303	ISOBM-310	ISOBM-300	ISOBM-297	ISOBM-342	ISOBM-291	ISOBM-296	ISOBM-299	ISOBM-302	ISOBM-306	ISOBM-298	ISOBM-304	ISOBM-345	ISOBM-294	ISOBM-289	ISOBM-301	ISOBM-311	ISOBM-344	ISOBM-309	ISOBM-293	ISOBM-343	ISOBM-307	ISOBM-308	ISOBM-288	ISOBM-305
a	ISOBM-303	IM 10/6.2		2	1	0	0	37	5	6	5	5	3	6	5	8	1	4	4	4	6	5	4	21	3	3	4	3
	ISOBM-310	CEA Ab-3	GOLD 5	0	0	1	1	23	8	9	8	7	9	8	7	5	2	7	5	5	4	9	8	13	5	5	5	5
	ISOBM-300	12-664		0	1	0	-1	37	10	12	11	10	7	11	10	10	4	9	7	6	6	11	9	22	7	7	8	7
	ISOBM-299	12-140-8	GOLD 4	8	3	1	6	0	2	1	1	1	4	5	3	3	1	5	3	5	4	6	7	20	5	6	6	5
	ISOBM-302	DPC CEA 2		7	3	1	5	0	2	1	2	1	2	4	3	1	1	5	3	4	2	6	6	11	6	5	5	5
	ISOBM-306	GFR46	GOLD 4"	6	3	1	6	3	1	1	1	1	0	2	1	2	2	5	5	5	5	7	5	21	4	3	4	5
	ISOBM-298	12-140-6	GOLD 4'	12	5	1	9	15	4	5	4	3	3	1	1	0	5	12	11	10	8	13	11	31	9	8	9	8
	ISOBM-304	IM 10/13.1		7	4	1	5	4	2	3	2	2	2	1	1	0	2	6	6	3	4	10	7	15	5	5	5	5
	ISOBM-301	DPC CEA 1	GOLD 2	5	3	0	3	1	4	4	4	4	3	5	5	1	0	0	0	1	1	5	5	3	5	4	4	4
	ISOBM-311	CEA Ab-4		7	2	1	7	5	7	8	6	6	7	7	6	3	0	0	0	1	1	1	7	4	5	5	5	6
	ISOBM-309	G208		14	8	2	14	51	16	17	17	14	17	16	17	12	4	7	11	6	5	1	1	28	0	1	0	11
	ISOBM-307	GFR48	GOLD 1	3	2	0	4	15	4	4	5	4	3	3	3	3	1	3	4	2	0	1	0	0	0	0	1	3
	ISOBM-308	G25		7	4	2	7	33	8	9	8	7	8	7	8	8	3	8	7	7	1	0	7	24	0	0	0	8
	ISOBM-305	GFR44		8	3	0	8	35	10	11	10	9	5	10	10	8	2	8	3	2	0	10	11	24	6	8	0	0
	ISOBM-295	CE27	GOLD 3	5	2	0	3	NT	4	4	3	5	3	5	4	NT	1	1	1	1	NT	3	4	NT	4	5	6	0
	ISOBM-290	II-17		6	2	0	3	8	2	4	1	3	4	5	3	3	0	0	0	0	0	1	3	10	3	4	4	5
b	ISOBM-303	IM 10/6.2		1	1	0	0	49	6	6	5	6	3	7	6	11	4	6	5	4	23	6	2	16	2	2	2	2
	ISOBM-310	CEA Ab-3	GOLD 5	0	0	0	0	27	10	10	10	9	10	10	9	8	7	9	8	8	10	9	3	9	3	2	2	2
	ISOBM-300	12-664		1	1	0	0	47	13	14	11	12	8	13	11	14	10	12	10	5	19	13	4	16	3	5	3	3
	ISOBM-299	12-140-8	GOLD 4	11	5	1	8	1	1	1	1	1	4	6	5	4	4	9	5	9	24	9	4	21	4	4	3	3
	ISOBM-302	DPC CEA 2		512		1	10	0	1	0	1	1	3	6	5	3	5	10	7	11	16	10	4	11	5	4	4	3
	ISOBM-306	GFR46	GOLD 4"	6	3	1	6	3	0	1	1	1	0	3	2	3	5	6	5	6	23	6	2	13	2	1	1	1
	ISOBM-298	12-140-6	GOLD 4'	12	8	1	11	23	3	4	3	2	4	0	1	1	10	11	10	11	32	12	3	20	4	3	3	3
	ISOBM-304	IM 10/13.1		6	2	0	5	9	1	2	2	2	2	0	0	0	5	6	5	4	16	9	2	11	2	2	2	2
	ISOBM-301	DPC CEA 1	GOLD 2	8	2	0	8	13	9	10	8	7	7	9	8	8	0	0	0	0	23	10	4	12	4	3	3	3
	ISOBM-311	CEA Ab-4		7	2	0	7	15	7	7	7	7	7	7	6	4	0	0	0	0	4	0	2	6	2	2	2	2
	ISOBM-309	G208		15	8	2	14	63	15	16	15	14	14	15	16	15	13	3	14	3	25	1	0	26	0	1	0	5
	ISOBM-307	GFR48	GOLD 1	2	1	0	2	15	2	2	2	2	2	2	2	2	2	2	2	2	1	1	0	0	0	0	0	1
	ISOBM-308	G25		3	1	0	4	26	4	4	3	3	3	3	3	4	3	4	4	3	3	0	3	19	0	0	0	3
	ISOBM-305	GFR44		5	2	1	5	31	5	5	5	5	4	5	5	5	5	5	4	5	3	5	5	20	3	3	0	0
	ISOBM-295	CE27	GOLD 3	3	2	0	2	NT	2	3	1	3	2	3	2	NT	2	1	0	1	NT	2	3	NT	3	2	3	0
	ISOBM-290	II-17		3	1	0	1	7	0	1	0	1	2	3	1	3	1	0	0	0	1	1	1	11	2	2	1	3
c	ISOBM-303	IM 10/6.2		1	1	0	-1	37	5	6	4	5	2	6	4	6	1	5	2	4	27	4	0	0	1	1	0	0
	ISOBM-310	CEA Ab-3	GOLD 5	0	0	0	1	23	7	8	7	6	7	7	5	4	2	8	2	5	13	8	0	0	0	1	0	0
	ISOBM-300	12-664		1	1	0	0	38	11	12	9	9	5	10	7	9	4	11	4	9	23	12	0	0	1	3	0	1
	ISOBM-299	12-140-8	GOLD 4	6	3	0	3	0	0	0	0	1	2	3	2	1	0	7	1	7	28	6	0	0	0	0	0	0
	ISOBM-302	DPC CEA 2		6	3	1	5	0	1	0	1	1	2	2	2	1	1	7	2	6	23	7	0	0	1	1	0	0
	ISOBM-306	GFR46	GOLD 4"	5	2	1	6	1	1	1	1	1	0	1	1	1	2	5	3	5	25	5	0	0	1	1	0	1
	ISOBM-298	12-140-6	GOLD 4'	10	4	1	8	9	2	3	2	2	1	1	0	0	4	10	6	9	36	9	0	1	1	1	1	1
	ISOBM-304	IM 10/13.1		5	3	1	4	2	1	1	1	2	1	1	0	0	1	5	2	5	15	7	0	0	1	1	0	0
	ISOBM-301	DPC CEA 1	GOLD 2	4	2	1	3	1	4	5	4	4	3	4	4	0	0	0	0	1	3	5	0	0	1	1	1	1
	ISOBM-311	CEA Ab-4		7	3	1	7	23	8	9	8	7	6	5	5	4	0	0	0	1	6	0	0	0	0	1	1	0
	ISOBM-309	G208		11	5	2	11	58	14	15	15	13	10	10	11	9	6	1	6	1	32	0	0	0	0	0	0	0
	ISOBM-307	GFR48	GOLD 1	1	1	1	1	1	0	1	0	1	1	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0
	ISOBM-308	G25		1	1	1	1	1	0	1	1	1	0	0	0	0	1	1	1	1	0	0	1	0	1	1	0	1
	ISOBM-305	GFR44		1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
	ISOBM-295	CE27	GOLD 3	1	0	0	0	NT	0	0	0	1	0	1	0	NT	0	0	0	0	NT	0	0	NT	0	1	0	0
	ISOBM-290	II-17		2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0

Results are shown as percent bound radioactivity.
Boxed values = GOLD groups; dotted inlays = GOLD subgroups; NT = not tested.

Table 7. a Immunoradiometric assays with different combinations of anti-CEA MAbs and recombinant CEA fragment N-halfA1-A3B3 as the antigen
b Immunoradiometric assays with different combinations of anti-CEA MAbs and recombinant CEA fragment N-A1B1 as the antigen

	Tracer MAb		Epitope Group	Solid phase MAb																								
				ISOBM-303	ISOBM-310	ISOBM-300	ISOBM-297	ISOBM-342	ISOBM-291	ISOBM-296	ISOBM-299	ISOBM-302	ISOBM-306	ISOBM-298	ISOBM-304	ISOBM-345	ISOBM-294	ISOBM-289	ISOBM-301	ISOBM-311	ISOBM-344	ISOBM-309	ISOBM-293	ISOBM-343	ISOBM-307	ISOBM-308	ISOBM-288	ISOBM-305
a	ISOBM-303	IM 10/6.2	GOLD 5	1	1	0	0	6	1	0	1	1	0	0	0	0	0	0	0	1	0	5	1	0	4	3	4	4
	ISOBM-310	CEA Ab-3		0	0	0	0	4	1	1	3	1	0	0	0	0	0	0	0	1	0	7	2	0	5	6	6	6
	ISOBM-300	12-664		0	1	0	0	8	3	1	4	1	1	1	1	1	0	0	0	1	0	9	2	0	7	9	8	9
	ISOBM-299	12-140-8	GOLD 4	3	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	1	0	2	0	0	3	4	1	2
	ISOBM-302	DPC CEA 2		0 0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	ISOBM-306	GFR46	GOLD 4''	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	ISOBM-298	12-140-6	GOLD 4'	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-304	IM 10/13.1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-301	DPC CEA 1	GOLD 2	0 0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-311	CEA Ab-4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	ISOBM-309	G208	GOLD 1	9	3	1	10	8	3	1	6	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	8
	ISOBM-307	GFR48		2 5		1	5	4	2	1	3	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	5
	ISOBM-308	G25		9	5	1	9	10	3	1	5	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	9
	ISOBM-305	GFR44	GOLD 3	0 12	2		11	8	2	0	4	1	0	0	2	0	0	0	0	0	0	10	3	0	4	9	0	0
	ISOBM-295	CE27		7	3	1	6	NT	2	0	3	1	1	0	1	NT	0	0	0	1	NT	7	2	NT	6	6	8	1
	ISOBM-290	II-17		8	3	1	5	4	0	0	2	0	0	0	0	0	0	0	0	1	0	6	1	0	7	8	6	8
b	ISOBM-303	IM 10/6.2	GOLD 5	0	0	0	0	3	7	1	2	2	12	11	10	25	0	0	0	0	0	1	0	0	0	0	0	0
	ISOBM-310	CEA Ab-3		0	0	0	0	2	9	1	2	4	14	15	15	15	0	0	0	0	0	1	0	0	0	0	0	0
	ISOBM-300	12-664		0	1	0	0	4	13	1	3	5	19	17	18	27	0	0	0	0	0	1	0	0	0	0	0	0
	ISOBM-299	12-140-8	GOLD 4	5	1	1	5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-302	DPC CEA 2		2 9		2	7	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-306	GFR46	GOLD 4''	16	5	3	14	0	0	0	0	0	0	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-298	12-140-6	GOLD 4'	24	8	6	23	2	13	0	0	2	20	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	ISOBM-304	IM 10/13.1		15	6	4	13	0	6	0	1	2	12	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
	ISOBM-301	DPC CEA 1	GOLD 2	0 0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-311	CEA Ab-4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-309	G208	GOLD 1	1	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-307	GFR48		0 0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-308	G25		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-305	GFR44	GOLD 3	0 0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ISOBM-295	CE27		0	0	0	0	NT	0	0	0	0	0	0	0	NT	0	0	0	0	NT	0	0	NT	0	0	0	0
	ISOBM-290	II-17		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Results are shown as percent bound radioactivity.
Boxed values = GOLD groups; dotted inlays = GOLD subgroups; NT = not tested.

In order to compare conventionally purified tumor CEA with recombinant CEA directly, the values obtained in immunoradiometric assays with conventionally purified CEA were divided by the values obtained in assays with recombinant CEA for each pair of MAbs. The result is shown in table 8a. Table 8b shows the same type of comparison, now dividing the results from the immunoradiometric assays for meconium CEA by the results obtained with conventionally purified tumor CEA. In both tables, only assay combinations with more than 6 percent bound activity are shown. This limit was arbitrarily set to avoid assay combinations clearly not suitable for immunoassays. The preparations were calibrated using the combination of MAbs 12-140-10 and 12-140-1 set as 1.00 in both tables. A result outside the range 0.5–2.0 cannot be explained by assay variation alone. As can be seen from table 8a, most assay combinations gave values within this range. However, a few combinations gave values outside this range, possibly indicating that tumor CEA and recombinant CEA were slightly different. By contrast, a number of combinations of MAbs demonstrated that tumor CEA was clearly different from meconium CEA (table 8b). Surprisingly, three combinations of MAbs, all involving MAb ISOBM-344, gave immunoradiometric assays in which meconium CEA was more efficiently detected than tumor CEA.

Reactivity with NCA

The results for conventionally purified NCA are shown in table 1. A cut-off limit of 5% bound activity was used to determine whether a MAb should be considered NCA-reactive or not.

Discussion

All antibodies included in the study bound conventionally purified tumor CEA, assumed to be an equivalent to CEA in serum from cancer patients. The affinities of the tested antibodies were generally high. Only ISOBM-346, with the lowest affinity, should be considered inferior for assay use. This antibody also showed a falsely low immunoreactive fraction (fig. 1), as not all of the antigen was bound at the highest antibody concentration. Differences between anti-CEA MAbs in immunoreactivity against tumor CEA have been seen before [6, 25–27], and they have implications in the selection of promising antibodies for immunoassays and other purposes, such as immunoscintigraphy [28].

There was a good agreement between the results from the cross-inhibition assays and the immunoradiometric assays (IRMA) for almost all MAbs. The cross-inhibition studies defined the epitope groups, and the epitope groups were confirmed by the IRMA studies. The IRMA studies using recombinant CEA fragments N-A1B1 and N-halfA1-A3B3 indicated roughly where the epitope groups were located on the CEA molecule. However, there was no total agreement, e.g. ISOBM-304, which, despite forming good IRMA combinations with GOLD group 5 antibodies, also inhibited those antibodies in the cross-inhibition studies. Experience from the previous workshop suggests steric effects as the most probable explanation. No pair of MAbs recognized recombinant CEA fragment N. This fragment is either too small to accommodate the 2 MAbs required for the formation of a sandwich assay, or all the N-domain reactive GOLD 5 MAbs studied here recognize the same epitope.

Epitope groups could readily be assigned for most MAbs. Thus ISOBM-343, ISOBM-307 and ISOBM-308 were found to belong to GOLD group 1; ISOBM-301, ISOBM-311 and ISOBM-346 to GOLD group 2; ISOBM-299, ISOBM-302 and ISOBM-342 to GOLD group 4, and ISOBM-300, ISOBM-303 and ISOBM-310 to GOLD group 5. Three new antibodies (ISOBM-298, ISOBM-304 and ISOBM-345) formed an epitope group of their own, from hereon referred to as 4′, to denote that they react with epitopes close to the epitopes recognized by GOLD group 4 MAbs in the A1B1 domain. We tentatively assigned MAb ISOBM-305 to GOLD group 3. However, it only inhibited one of the reference MAbs, CE27, and not the other, II-17. Furthermore, ISOBM-305 inhibited F6 Bu-101, one of the reference MAbs for GOLD group 1. However, both GOLD group 1 and 3 MAbs react with epitopes located in the C-terminal A3B3 domain of CEA, and there may be steric interference between some MAbs binding to the same general area. Three antibodies (ISOBM-306, ISOBM-309 and ISOBM-344) could not readily be classified into any group. However, the epitope of ISOBM-306 must be located in the A1B1 domain of CEA in the vicinity of the epitopes recognized by GOLD group 4 and 4′ MAbs. ISOBM-309 inhibits ISOBM-307 and ISOBM-308, 2 antibodies of GOLD group 1 MAbs that do not bind to meconium CEA or to NCA. However, ISOBM-309 binds both to meconium CEA and NCA. Moreover, an IRMA is formed with several GOLD group 5 MAbs when the N-halfA1-A3B3 fragment is used as the antigen, but not with the N-A1B1 fragment. We therefore consider ISOBM-309 to have an epitope located in the A3B3 domain of CEA close to where the tumor CEA-spe-

Table 8. a Relative activity for tumor CEA compared to recombinant CEA[1]
b Relative activity for meconium CEA compared to tumor CEA[2]

	Tracer Mab		Epitope Group	Solid phase MAb ISOBM-303	ISOBM-310	ISOBM-300	ISOBM-297	ISOBM-342	ISOBM-291	ISOBM-296	ISOBM-299	ISOBM-302	ISOBM-306	ISOBM-298	ISOBM-304	ISOBM-345	ISOBM-294	ISOBM-289	ISOBM-301	ISOBM-311	ISOBM-344	ISOBM-309	ISOBM-293	ISOBM-343	ISOBM-307	ISOBM-308	ISOBM-288	ISOBM-305
a	ISOBM-303	IM 10/6.2	GOLD 5					0.75		0.92				0.87		0.72					0.25			1.37				
	ISOBM-310	CEA Ab-3	GOLD 5					0.83	0.83	0.91	0.85	0.81	0.92	0.86	0.85			0.76				0.99	2.61	1.47				
	ISOBM-300	12-664	GOLD 5					0.80	0.81	0.90	0.93	0.85	0.88	0.86	0.89	0.67		0.72	0.67	1.17	0.34	0.89	2.58	1.39	2.35	1.57	2.32	2.07
	ISOBM-299	12-140-8	GOLD 4	0.77			0.76															0.74	1.80	0.96		1.56	1.66	
	ISOBM-302	DPC CEA 2	GOLD 4	0.57																		0.56	1.51	0.99	1.22			
	ISOBM-306	GFR46	GOLD 4"	0.95			0.89															1.05		1.62				
	ISOBM-298	12-140-6	GOLD 4'	1.00			0.85	0.65										1.06	1.05	0.90	0.27	1.09	3.45	1.57	2.45	2.57	3.59	2.90
	ISOBM-304	IM 10/13.1	GOLD 4'	1.15														1.01	1.19	0.80		1.18	3.30	1.30				
	ISOBM-301	DPC CEA 1	GOLD 2																									
	ISOBM-311	CEA Ab-4	GOLD 2	0.91			1.03		0.94	1.10	0.95	0.91	1.03	1.02	1.06								2.90					2.45
	ISOBM-309	G208	GOLD 1	0.99	1.09		0.96	0.81	1.04	1.11	1.10	1.00	1.18	1.08	1.07	0.81		2.33	0.81	1.76				1.06				2.25
	ISOBM-307	GFR48	GOLD 1					1.02																				2.06
	ISOBM-308	G25	GOLD 1	2.06			1.85	1.29	2.27	2.32	2.24	2.16	2.58	2.41	2.68	1.77		2.04	1.85	2.67			2.52	1.25				2.47
	ISOBM-305	GFR44	GOLD 3	1.76			1.58	1.15	1.88	2.26	2.09	1.89		2.10	2.10	1.52		1.66				1.98	2.19	1.17	2.11	2.51		
	ISOBM-295	CE27	GOLD 3																							1.99	1.77	
	ISOBM-290	II-17	GOLD 3	2.15				1.17																0.94				
b	ISOBM-303	IM 10/6.2	GOLD 5					1.01		0.98				1.05		0.71					4.49			0.02				
	ISOBM-310	CEA Ab-3	GOLD 5					1.02	0.87	0.89	0.87	0.82	0.80	0.82	0.74			1.18				0.86	0.05	0.02				
	ISOBM-300	12-664	GOLD 5					1.01	1.01	0.97	0.87	0.97	0.73	0.91	0.73	0.97		1.29	0.54	1.38	3.64	1.02	-0.02	0.02	0.13	0.39	0.05	0.20
	ISOBM-299	12-140-8	GOLD 4	0.69			0.56															0.88	-0.04	0.01		0.00	0.01	
	ISOBM-302	DPC CEA 2	GOLD 4	0.94																		1.13	0.00	0.02	0.11			
	ISOBM-306	GFR46	GOLD 4"	0.90			1.00															0.81		0.01				
	ISOBM-298	12-140-6	GOLD 4'	0.81			0.86	0.63										0.83	0.59	0.93	4.20	0.71	0.01	0.02	0.10	0.07	0.06	0.07
	ISOBM-304	IM 10/13.1	GOLD 4'	0.76														0.80	0.41	1.51		0.72	-0.04	0.00				
	ISOBM-301	DPC CEA 1	GOLD 2																									
	ISOBM-311	CEA Ab-4	GOLD 2	1.04			0.95		1.19	1.16	1.19	1.14	0.88	0.78	0.79								0.06					0.05
	ISOBM-309	G208	GOLD 1	0.76	0.66		0.81	1.14	0.87	0.86	0.86	0.87	0.60	0.60	0.65	0.72		0.13	0.57	0.17				0.01				0.03
	ISOBM-307	GFR48	GOLD 1					0.03																				0.11
	ISOBM-308	G25	GOLD 1	0.10			0.16	0.04	0.05	0.10	0.07	0.07	0.06	0.05	0.02	0.04		0.12	0.10	0.18			0.09	0.02				0.06
	ISOBM-305	GFR44	GOLD 3	0.09			0.00	0.03	0.00	0.01	0.03	0.07		0.05	0.03	0.01		-0.01				-0.02	-0.01	0.01	0.08	0.06		
	ISOBM-295	CE27	GOLD 3																							0.12	0.08	
	ISOBM-290	II-17	GOLD 3	0.33				0.04																0.02				

[1]The results from the IRMA combinations for tumor CEA have been divided by the results from the IRMA combinations for recombinant CEA.
[2]The results from the IRMA combinations for meconium CEA have been divided by the results from the IRMA combinations for tumor CEA.

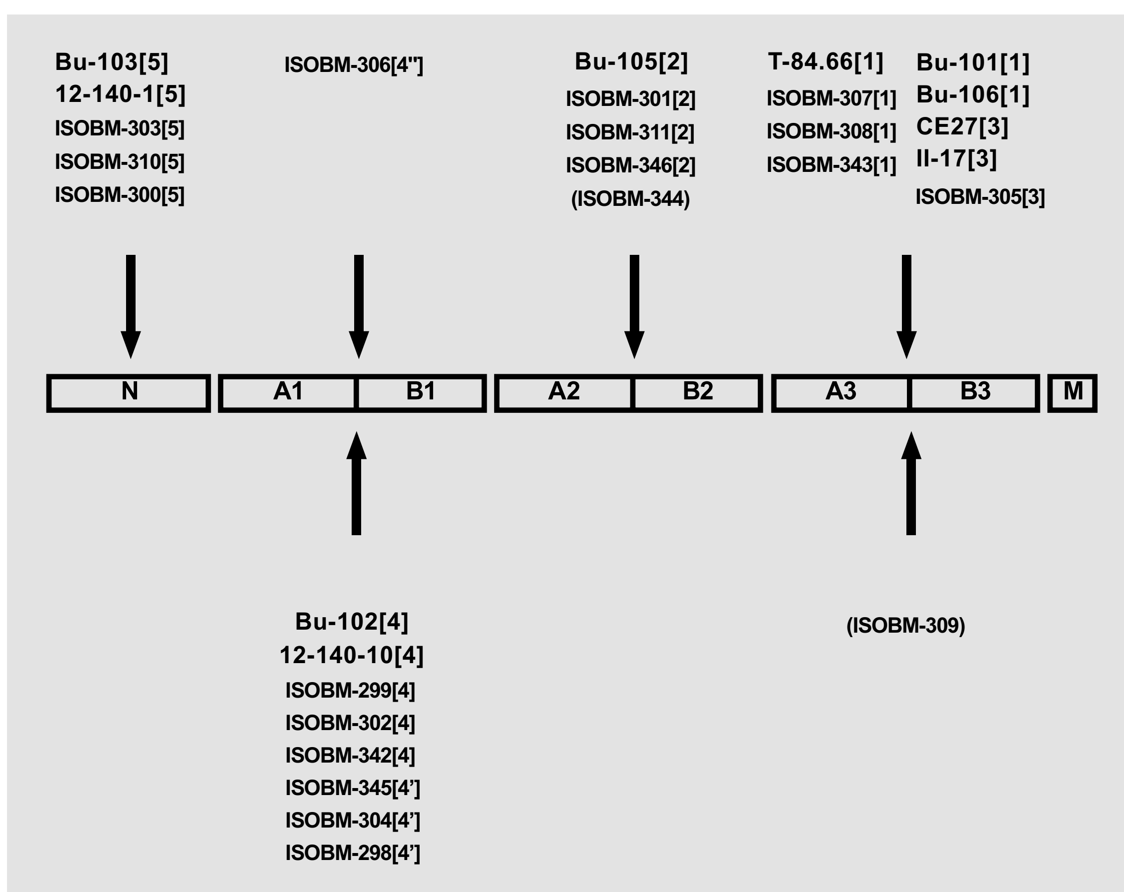

Fig. 2. Proposed binding sites for antibodies. Using the epitope classification of Murakami et al. [16], the proposed binding sites for the new CEA antibodies are drawn (arrows). Antibodies in bold font are reference antibodies previously investigated by Murakami et al. [16]. Figures in square brackets indicate GOLD groups.

cific group 1 epitopes are located. However, this epitope is also present in meconium CEA. The most reasonable GOLD group assignment for ISOBM-309 would then be group 1. ISOBM-344 differs from all other antibodies in the workshop by having a higher affinity for meconium CEA than for tumor CEA. This MAb inhibits GOLD group 2 MAbs. The GOLD group assignments are listed in table 1 and the proposed binding sites for the antibodies are illustrated in figure 2.

The presence of repetitive epitopes was suggested during the work of the former workshop [6, 25], although our studies do not confirm this. There could be several reasons for this disagreement. Firstly, some of the MAbs in the first workshop were of low affinity. For these antibodies, the differences in affinity between the main epitope and a similar but slightly different epitope could be very small. Secondly, some of the CEA preparations used in the former workshop may have been partly in complexes, which could mimic repetitive epitopes. Thirdly, true repetitive epitopes may exist for some antibodies from the former workshop, but not among the antibodies tested now.

We also studied antibody reactivity with NCA. The binding of NCA to cross-reactive MAbs was too low to allow estimating their affinity, which certainly is much lower than their affinity for CEA. However, in some patients, the serum NCA concentration can be very high [29, 30]. Therefore, when selecting antibodies for an immunometric CEA assay, at least one in the pair should be without NCA reactivity.

Recombinant CEA has been proposed as a possible and convenient source of CEA for calibrators in assays. In this study we compared recombinant CEA to two sources of conventionally purified CEA from hepatic metastases

of a colorectal tumor, CEA(Oslo) and CEA(Duarte). Both conventional sources have previously been used in CEA research and are well characterized. In addition, both preparations were used for the cross-inhibition studies without finding any differences between them. Unfortunately, there were insufficient amounts of antibodies to perform the IRMA studies with both preparations, so the comparison was done between recombinant CEA and the CEA(Oslo) preparation. The antibodies with epitopes close to the C-terminal end of the molecule clearly bound tumor CEA(Oslo) stronger than recombinant CEA. This is an important finding and warrants further studies, using several sources of both recombinant and conventionally purified CEA to reveal whether this is a general phenomenon, and which preparation would be the more appropriate calibrator.

It has been demonstrated that a CEA variant similar to meconium CEA is present in serum from several cancer patients, contributing to the well-known discrepancies between commercial assays [23]. Mapping antibody reactivity with this antigen is therefore of interest, even if the clinical relevance (if any) is still not known [31]. Five MAbs with A3B3 reactivity reacted poorly with meconium CEA, clearly demonstrating that the two forms of the CEA gene product are different. Two mutually not exclusive explanations seem possible: differences in glycosylation, with meconium CEA being more completely glycosylated than tumor CEA; and proteolytic cleavage of the meconium CEA peptide chain at the C-terminal. As indicated earlier, there is chemical evidence for glycosylation differences between NCA-2 and tumor CEA [19, 20].

The finding that MAb ISOBM-344 has a higher affinity for NCA-2 than for tumor CEA is interesting. Firstly, it indicates that assays specific for this CEA variant may be set up. Secondly, it points to an unrecognized problem in the standardization of CEA assays. We not only have to consider if meconium CEA in serum samples is measured, but also how efficiently the various commercial assays are measuring meconium CEA compared to tumor CEA. In this study, assay combinations using ISOBM-344 gave 4 times the signal for equimolar concentrations of meconium CEA compared to tumor CEA.

The ISOBM TD8 Workshop has performed epitope mapping and measured affinities for 19 new antibodies against CEA, expanding the knowledge of the CEA epitope structure. We hope that this knowledge will contribute to more sensitive and more specific CEA assays, to the benefit of patients.

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