

## An epitope on carcinoembryonic antigen defined by the clinically relevant antibody PR1A3

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**ABSTRACT** The monoclonal antibody PR1A3 has been used successfully for *in vivo* imaging of colorectal cancers, and several properties associated with this antibody, including minimal reactions of the antibody with circulating antigen in patients' sera, differentiate it from anti-carcinoembryonic antigen (CEA) antibodies used in similar studies. However, the antigen bound by PR1A3 was identified as CEA by analysis of somatic cell hybrids and by antigen expression from yeast artificial chromosomes, cosmids, and cDNA clones. The molecular weight, presence of a glycosyl-phosphatidylinositol anchor, elevation of surface expression by  $\gamma$ -interferon, and N-terminal amino acid sequence all confirmed the antigen identification as CEA. A series of biliary glycoprotein-CEA hybrid proteins was produced which demonstrated that the epitope bound by the antibody was at the site of membrane attachment and involved parts of the glycosyl-phosphatidylinositol anchor and the B3 domain of CEA to form a conformational epitope. Access to this epitope, although possible when the antigen was on the cell surface, appeared to be blocked when CEA was released from the cell. The nature and location of the epitope on CEA are proposed to be responsible for the unique properties of the antibody.

PR1A3 is a monoclonal antibody which has been successfully used for *in vivo* imaging and staging of colorectal tumors. It was produced from mice immunized with normal human colon epithelium and was selected for further study because PR1A3 bound to upper crypt columnar cells of the normal colon, although weak reactivity was also seen with the stomach, ileum, esophagus, tracheal, and breast epithelium (1). However, although this monoclonal antibody was produced by immunization with normal tissue and reacted accordingly, when  $^{111}\text{In}$ -labeled, and later  $^{99\text{m}}\text{Tc}$ -labeled antibody was used in colorectal cancer patients for tumor imaging, it bound specifically to the tumors and binding to normal tissues was not detected. Extensive *in vivo* imaging studies have shown that the antibody binds to both moderately and poorly differentiated carcinomas and to all stages of colorectal carcinoma (2), with an overall accuracy for sensitivity and specificity of 95% in a study involving 130 patients and 199 images (3). The apparent tumor specificity is thought to be a reflection of the restricted ability of the antibody to gain access to luminal spaces and hence the normal mucosa, whereas the disrupted tissue architecture associated with tumor growth allows access of the antibody to its target antigen. The *in vivo* and *in vitro* properties of PR1A3 differentiated it from other antibodies used for colorectal tumor imaging, especially anti-carcinoembryonic antigen (CEA) antibodies (4, 5). These differences included the observations that 59 of 60 tumors studied bound PR1A3 (1), in contrast to a clinical study where only 70% of colorectal tumors were

imaged by an anti-CEA antibody (6). Anti-CEA antibodies have, in some patients, labeled lymph nodes in the absence of tumor, probably because of trapped circulating CEA (7), whereas this has not been observed with PR1A3 (8). Additionally, anti-CEA antibodies readily complex with circulating antigen in colorectal cancer patients (9), whereas this study demonstrates that PR1A3 bound little or no circulating antigen in the sera of patients.

The *in vivo* imaging properties of PR1A3 make this antibody an excellent candidate for use as a therapeutic targeting agent but also pose questions about the identity of the antigen and nature of the epitope that is recognized. The study described here demonstrates that the antigen bound by PR1A3 is indeed CEA but that the epitope is uniquely associated with the site or region of membrane attachment.

### MATERIALS AND METHODS

**Cell Lines.** The following cell lines were used: MKN45, a human stomach cancer line (10); SW1417, SW837, SW620, HCT15, and LS174T, human colon carcinoma cell lines (11–14); CMT93, a mouse rectal carcinoma line (15); and COS-7, a simian virus 40-transformed monkey fibroblast line (16).

**CEA Assays.** ELISAs used a  $\beta$ -galactosidase/anti- $\beta$ -galactosidase detection system (17). Circulating serum CEA was measured by capture on plates coated with a 1:100 dilution of a rabbit anti-human CEA antibody (Dako A115). Assays of direct antibody binding to CEA used plates coated with CEA (2.5 ng per well) purified from liver metastases of adenocarcinoma (Scripps Laboratories).

**$\gamma$ -Interferon Stimulation.** Five colorectal cell lines—SW1417, SW620, SW837, LS174T, and HCT15—were treated in culture with human  $\gamma$ -interferon (Boehringer Mannheim) at 1000 units/ml. After 24, 48, and 72 hr in culture, cells were tested for PR1A3 reactivity. CEA expression was also measured by using a CEA-specific monoclonal antibody from Sera-Lab (Crawley Down, Sussex, U.K.) and PR3B10. Antigen was quantitated by the solid-phase  $\beta$ -galactosidase/anti- $\beta$ -galactosidase ELISA (17).

**Phospholipase Treatment.** Phospholipase release of antigen from cells was achieved by treating 0.1 ml of cell suspensions ( $10^8$  cells/ml) with 1 unit of phosphatidylinositol-specific phospholipase C (Sigma) for 60 min at 30°C (18). Cells were removed by centrifugation and the supernatant was used for further analysis. SDS/PAGE and immunoblotting were performed on reduced and nonreduced samples containing 10  $\mu\text{g}$  protein which had been boiled with or without 50 mM 2-mercaptoethanol.

**Mapping Using Somatic Cell Hybrids.** Two sets of somatic cell hybrids were made. The mouse epithelial colorectal

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Abbreviations: BGP, biliary glycoprotein; CEA, carcinoembryonic antigen; GPI, glycosyl-phosphatidylinositol; NCA, nonspecific crossreacting antigen; YAC, yeast artificial chromosome.

carcinoma cell line CMT93, which is resistant to 6-thioguanine, was crossed with both the human stomach line MKN45 and the human colorectal carcinoma cell line SW1417 by polyethylene glycol cell fusion (19). CMT93 was chosen to allow epithelial-specific CEA expression, and MKN45 because it reacted strongly with PR1A3. PR1A3 was known to react with both human cell lines but not with the mouse line. Hybrids were selected in medium with 100  $\mu$ M hypoxanthine, 10  $\mu$ M methotrexate, 16  $\mu$ M thymidine, and 1  $\mu$ M ouabain and were tested for PR1A3 and PR3B10 reactivity by indirect immunofluorescence assay. Analysis for human chromosome content was carried out by probing unlabeled PCR-amplified inter-*Alu* hybrid sequences with  $^{32}$ P-labeled PCR-amplified human chromosome-specific inter-*Alu* sequences (20).

**Yeast Artificial Chromosome (YAC), Cosmid, and cDNA Transfectants.** A 180-kb YAC (ICRFy900C0244) containing most of the CEA gene cluster (CGM2-CEA-NCA-CGM1; NCA, nonspecific crossreacting antigen; CGM, CEA gene family member) in the region q13.1-q13.3 on the long arm of chromosome 19 was modified to include the neomycin-resistance gene (*neo*) by homologous recombination of the plasmid vector pRAN4 with the right-hand vector arm of pYAC4 (21). The resultant CEA/*neo* YAC was transferred to CHO cells by yeast spheroplast fusion (22). CEA-expressing cells were isolated by fluorescence-activated cell sorting (FACS) and were monitored by indirect immunofluorescence using PR3B10.

A full-length CEA cosmid clone (23) was cotransfected with pSVneo2 into the mouse colorectal carcinoma cell line CMT93 by lipofection. G418-resistant colonies that expressed CEA were cloned by FACS using monoclonal antibody PR3B10. Expression of CEA as a 180-kDa species was confirmed by SDS/PAGE and immunoblotting with phosphatidylinositol-specific phospholipase C-released material probed with PR3B10.

A CEA cDNA in the dexamethazone-inducible vector pMAMneo (Clontech) was introduced into CHO cells by electroporation. A NCA cDNA clone (24) was transfected by electroporation into the human colorectal cell line SW620, in which CEA and NCA are not significantly expressed. CEA transcripts of 4.2 kb and 3.8 kb and a NCA transcript of 1.5 kb were demonstrated in these cells by Northern blotting. Cell surface expression was confirmed by SDS/PAGE and Western immunoblotting and indirect immunofluorescence using PR3B10 and a polyclonal anti-CEA serum (Dako A115).

**PR1A3 Antigen Purification.** PR1A3 antibody affinity chromatography was used to isolate antigen from phospholipase C-released material from MKN45 cells. After further purification by FPLC size exclusion on Superose 12 (Pharmacia) and SDS/PAGE, the stained protein band was covalently immobilized on the surface of poly(vinylidene difluoride) membrane by treatment with poly(allylamine) and 1,4-phenylenediisothiocyanate (25) and sequenced by solid-phase Edman degradation with a MilliGen 6600 solid-phase sequencer.

**Biliary Glycoprotein (BGP)-CEA Hybrids.** Fragments used in the construction of the chimeras were amplified from cDNA clones of CEA and BGP (36). Standard PCR amplification conditions were used (26). Fragments were joined by using 8 cycles (up to 18 cycles for difficult junctions) of 93°C for 45 sec, 37°C for 1 min, and 72°C for 2 min in the absence of outside primers, followed by addition of appropriate primers and a standard amplification program.

CHIM1 contained the BGP N-terminal domains amplified with primers 1 and 2 and the CEA C-terminal region amplified with primers 3 and 4. These fragments were joined by formation of PCR dimers between terminal overlaps, followed by rounds of amplification in the presence of the two outside primers, 1 and 4. CHIM2 contained BGP N-terminal

sequence (amplified with primers 1 and 5), a small fragment of CEA domain a3, all of the CEA domain b3 (amplified with primers 6 and 7), and the transmembrane fragment of BGP (amplified with primers 8 and 9). These fragments were joined as described above. CHIM3 introduced a stop codon 3' to the CEA b3 domain in CHIM2. Primers 1 and 10 were used to amplify this fragment from CHIM2 and to introduce the modifications. Primer sequences (5' to 3') were as follows: 1, CTCAAGCTTATGGGGCACCTC; 2, GAGGACATCCAG-CATGATGGG; 3, CCCATCATGCTGGATGTCTC; 4, GGTCTAGACTATATCAGAGCAAC; 5, GGATCGATG-CAGGTCAGGT; 6, CTCATCGATGAACCTGAGGCT; 7, GGCCCCAGGTGAGAGTCCAGATGCAGA; 8, TCTG-CATCTGGACTCTCACCTGGGGCC; 9, GGTCTAGAC-TATGAAGTTGGTTG; 10, GGTCTAGACTAAGATGCA-GAGAC.

## RESULTS

**Serum PR1A3 Detection.** Levels of CEA were studied in 107 serum samples, 84 from patients with various cancers and 23 from normal individuals. All tests were done in duplicate and most samples were repeated at least once. A positive reaction was recorded when the signal was more than twice the value of the negative control (phosphate-buffered saline). Results are shown in Table 1. With PR1A3, three positive reactions were recorded in the cancer-patient sera. In each case these sera were also positive with the other two anti-CEA antibodies. In contrast, when the CEA-specific monoclonal antibody from Sera-Lab was substituted for PR1A3, 22 sera were positive, 17 from cancer patients and 5 from normal individuals. With PR3B10, an antibody which reacts with CEA and other CEA family members, including NCA and BGP, 50 positive sera were identified, 36 from cancer patients and 15 from normal subjects. Many of the sera tested with PR3B10 and the Sera-Lab antibody gave results in excess of 1.0 and 0.6 fluorescence unit above background, respectively, whereas values with PR1A3 for the same sera were close to or indistinguishable from background, which was about 0.15 fluorescence unit.

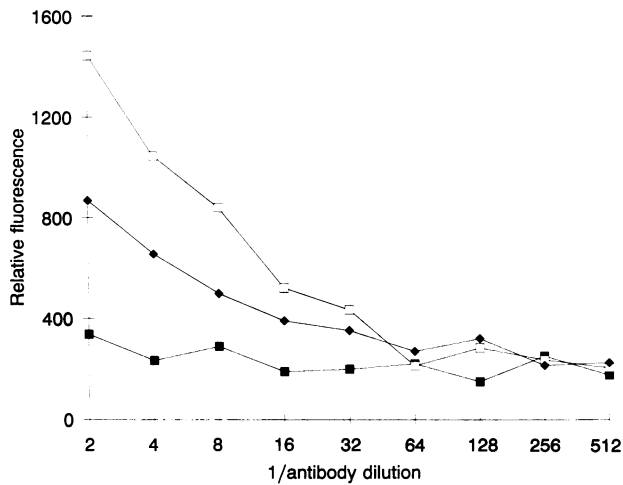
Additionally, the ability of PR1A3 to bind CEA was measured in an ELISA using plates coated with CEA purified from liver metastases of adenocarcinomas. The binding of PR1A3 to CEA in comparison to that of PR3B10 and the Sera-Lab antibody is shown in Fig. 1. No significant binding above background was given with PR1A3. The data shown are from plates coated with CEA at 2.5 ng per well; levels of  $\geq 25$  ng per well did not significantly affect this result.

**$\gamma$ -Interferon Upregulation.** Of the five colorectal cell lines examined with PR1A3 after treatment with  $\gamma$ -interferon, two showed increased expression compared with untreated cells.

Table 1. Detection of circulating CEA in sera from normal subjects and cancer patients

Patient status	No. of patients	No. positive in CEA assay		
		PR1A3	PR3B10	Sera-Lab
Breast tumor	23	1	9	5
Ovarian tumor	8	1	4	3
Osteosarcoma	5	0	4	0
Colorectal tumor	28	0	8	2
Lung tumor	20	1	10	7
Normal	23	0	15	5
(Total)	107	3	50	22

CEA was captured by a polyclonal anti-CEA antibody and the binding of PR1A3, PR3B10, or a CEA specific antibody from Sera-Lab to this captured CEA was measured. A reaction was considered positive when the fluorescent signal obtained was more than twice background.



**FIG. 1.** Binding of PR1A3 monoclonal antibody to purified CEA in an ELISA. Plates were coated with 2.5 ng of CEA per well, and the binding of PR1A3 (■), PR3B10 (□), and Sera-Lab antibody (◆) was measured over a series of doubling dilutions.

In one line, SW1417, expression was increased by 15%. In the other line, LS174T,  $\gamma$ -interferon induced a greater increase, 27%, of expression of PR1A3 antigen. In both cell lines, CEA as measured by the Sera-Lab monoclonal antibody was increased by similar amounts. In SW1417, PR3B10 expression was found to be very high before treatment and was not increased with  $\gamma$ -interferon. In LS174T, PR3B10 expression was doubled. The other three cell lines, HCT15, SW620, and SW837, were all very low or negative for PR1A3, the Sera-Lab antibody and PR3B10 reactivity, and  $\gamma$ -interferon had no stimulatory effect on any of these cells.

**Mapping Using Somatic Cell Hybrids.** To give a chromosome assignment to the PR1A3 antigen gene, somatic cell hybrids were made. In total, 62 hybrids were isolated. Table 2 contains results of analysis for human chromosome content and reactivity with PR1A3 and PR3B10 on 22 of them. As expected, PR3B10 reactivity showed concordance with the presence of chromosome 19. PR1A3 reactivity was identical,

thus showing the same concordance. Two hybrids, 1793.A3 and 1793.B3, did not react with either antibody although chromosome 19 was found to be present. Since the PCR chromosome analysis could detect a partial chromosome, a possible explanation for the absence of PR1A3 and PR3B10 staining in these hybrids could be the loss of the long arm or part of the long arm of chromosome 19; alternatively, the sensitivity of PCR could have led to detection of a minor population of hybrid cells carrying chromosome 19. In no case was PR1A3 binding found when chromosome 19 was not present, and no consistent presence of another chromosome could be associated with antibody binding.

**PR1A3 Expression in YAC, Cosmid, and cDNA Transfectants.** In CHO cells transfected with a YAC containing most of the CEA gene cluster, populations of PR1A3-positive cells were identified (Fig. 2A). It was not possible to identify the molecular weight of the expressed product by immunoblotting because the PR1A3-positive cell populations were too small. However, in a series of CEA cosmid CMT93 transfectant clones, PR1A3 antigen was found on the cells (Fig. 2B) and a 170- to 180-kDa product was detected by immunoblotting of material released by phospholipase C treatment. CEA cDNA CHO transfectant clones treated with 2  $\mu$ M dexamethazone for 16 hr reacted with PR1A3 in indirect immunofluorescence assays and ELISAs, and phospholipase C-released material was shown by immunoblotting to contain PR1A3 antigen. COS cells transfected with CEA cDNA were labeled by PR1A3 in indirect immunofluorescence assays (Fig. 2C). PR3B10 but not PR1A3 reacted with NCA cDNA-transfected cells in immunofluorescence and immunoblot assays (data not shown).

**Phospholipase C-Released Antigen.** Treatment of MKN45 cells with phosphatidylinositol-specific phospholipase C released antigen which on separation by SDS/PAGE and immunoblotting with PR1A3 revealed a broad band at  $\approx$ 180 kDa. A band of identical size and appearance was given by the CEA-reactive antibody PR3B10 (Fig. 3). Evidence for the influence of conformation on the epitope was obtained by varying the time of boiling the sample in nonreducing buffer prior to loading on gels. On immunoblotting the strength of labeling of the antigen increased with time of boiling, being maximal at 1 min, but faded on prolonged heating up to 5 min.

**Table 2.** Human chromosomes in the somatic cell hybrids and reaction of the hybrids with monoclonal antibodies PR1A3 and PR3B10

Hybrid	Human chromosomes present	PR1A3	PR3B10
1793.C4	1, 19, 21	+	+
1793.A3	5, 18	-	-
1793.B3	8, 19	-	-
1793.D3	6, 7, 8, 9, 10, 13, 15, 19, 21	+	-
1793.C5	2, 3, 4, 6, 8, 9, 11, 12, 14, 15, 19, 21, X	+	+
1793.B4	1, 3, 6, 11, 20, 21, X	-	-
1793.H1	1, 2, 3, 5, 9, 11, 12, 14, 15, 17, 19, 20, 22	+	+
4573.23.B1*	2, 3, 15, 19, 20	+	+
1793.D4	3, 15, 19	+	+
1793.G3	2, 3, 19	+	+
1793.F7	3, 5, 11, 19, 20, 22	+	+
4593.23.E2*	3, 6, 8, 13, 15, 20, X	-	-
1793.G4	2, 15, 19, X	+	+
1793.F3	5, 7, 9, 11, 17, 19	+	+
1793.H2	2, 5, 6, 9, 19	+	+
1793.H3	2, 5, 8, 18, 22	-	-
1745.17.H1	1, 2, 3, 7, 18, 19, 21	+	+
1745.23.B1	1, 19	+	+
1745.23.G3	2, 5, 8, 10, 13, 15, 19	+	+
1745.23.C3	1, 5, 8, 9, 19, X	+	+
1793.J1	1, 15, 19	+	+
4593.17.C4*	5, 9, 11, 13, 14, 19	+	+

\*MKN45-CMT93 hybrids; the others are SW1417-CMT93 hybrids.

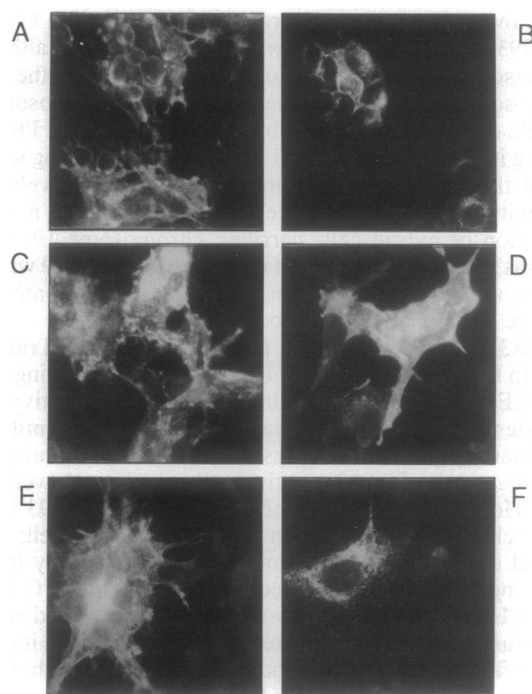


FIG. 2. Transfected cell lines assayed by indirect immunofluorescence. (A) YAC-transfected CHO cells with PR1A3. (B) Cosmid-transfected CMT93 cells with PR1A3. (C) COS-7 cells transfected with CEA cDNA, assayed with PR1A3. (D) COS-7 cells transfected with CHIM1 DNA, encoding BGP with the CEA b3 domain and the glycosyl-phosphatidylinositol (GPI) anchor, assayed with PR1A3. (E) COS-7 cells transfected with CHIM2 DNA, encoding BGP with CEA b3 and BGP transmembrane sequence, assayed with PR3B10. (F) COS-7 cells transfected with CHIM3 DNA, encoding BGP with CEA b3 alone, assayed with PR3B10.

**PR1A3 Antigen Purification.** Purified antigen, immunoreactive with PR1A3 and PR3B10, was transferred from SDS/polyacrylamide gels to poly(vinylidene difluoride) membranes for amino acid sequencing. The N-terminal 16 residues of the antigen, Lys-Leu-Thr-Ile-Glu-Ser-Thr-Pro-Phe-Asn-Val-Ala-Glu-Gly-Lys-Glu, were identical to those of the N domain of mature CEA (27).

**Epitope Mapping.** A CEA-maltose-binding protein fusion expressed in *Escherichia coli* did not contain the PR1A3 epitope (data not shown), suggesting the involvement of posttranslational modification or conformation in the epitope. Since CEA is a member of the immunoglobulin super-

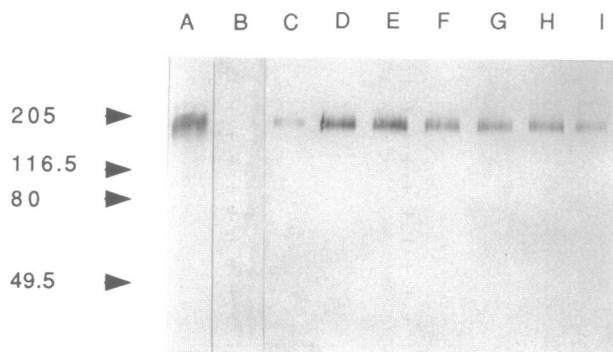


FIG. 3. Immunoblotting of phospholipase C-released material from MKN45 cells. Reduced samples were boiled for 4 min and analyzed by SDS/PAGE and immunoblotting with PR3B10 (lane A) or PR1A3 (lane B). Unreduced samples were boiled for 0, 0.5, 1, 2, 3, 4, or 5 min (lanes C–I, respectively) before SDS/PAGE and immunoblot analysis with PR1A3. Positions of molecular size (kDa) standards are indicated at left.

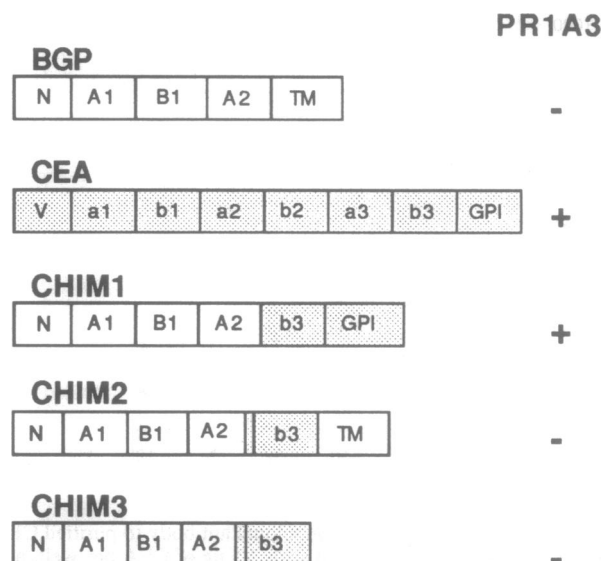


FIG. 4. Diagram of BGP-CEA chimeras and their reaction with PR1A3. Letters V, a, and b represent domains of CEA; N, A, and B are domains of BGP. GPI is the sequence of CEA replaced by a GPI anchor, and TM is the transmembrane domain of BGP.

family, to conserve domain structures for epitope analysis, it was fused to other members of the family and expressed in mammalian cells. Initial studies failed to detect the PR1A3 epitope when the N-terminal domains of CEA (V-a1-b1-a2) were fused to the Fc region of IgG (data not shown). Therefore, further studies concentrated on the C-terminal portion of CEA expressed as a fusion with BGP, which is a member of the CEA gene family (28) but does not carry the PR1A3 epitope. The BGP chimeric proteins whose outline structures are represented in Fig. 4 were expressed in COS cells and screened by indirect immunofluorescence using PR1A3 and, as a positive control, PR3B10, which binds to both CEA and BGP.

The BGP-CEA hybrids were cloned into the *Hind*III and *Xba*I sites of pCDM8 and transfected into COS cells; expression was detected by immunofluorescence. CHIM1 was composed of the first 378 amino acids of BGP followed by residues 554–668 of CEA. Expression of this construct produced a chimeric protein attached to the cell surface through a GPI anchor. CHIM3 was a nominally secreted product and contained residues 1–314 of BGP and 490–643 of CEA but lacked the hydrophobic C terminus and, therefore, the GPI processing information. CHIM2 added the C-terminal residues of BGP to CHIM3 (residues 391–430) and resulted in the attachment of the molecule to the membrane by a transmembrane protein sequence and not through a GPI anchor. The chimeric proteins are described in Fig. 4 together with the results of epitope screening. The PR1A3 epitope was detected when the b3 domain and GPI anchor were present but was absent when the GPI anchor was replaced by the BGP transmembrane domain or when the hybrid was expressed, with no means of membrane attachment, as a potentially secreted product (Fig. 2). In this latter instance, although the product would be expected to be secreted, fluorescence was found within the cell but was of a punctate form.

## DISCUSSION

Tumor imaging by immunoscintigraphy has demonstrated that PR1A3 has distinct advantages in targeting to colorectal carcinomas. The high degree of specificity of this antibody (3), together with the apparent lack of reactivity with circu-

lating antigen and purified CEA, appeared to differentiate PR1A3 from anti-CEA antibodies. However, evidence from somatic cell hybrids and from YAC, cosmid, and cDNA expression, as well as a variety of molecular and biochemical properties, demonstrated that PR1A3 was specific for cell-bound CEA. The apparent inconsistency in these data could imply that there are unusual properties associated with the epitope recognized by the antibody. The absence of the epitope on bacterially expressed CEA and the sensitivity of the epitope to reduction and heating suggest that it is conformational and/or a posttranslational modification. Both linear and conformational determinants have been described for CEA (29, 30), although the ability to position conformational epitopes on the molecule has been limited by difficulties in expressing individual domains of CEA (24). CEA-specific conformational determinants can be defined by construction of hybrid proteins between CEA and other members of the CEA family. Analysis of BGP-CEA chimeras localized the epitope for PR1A3 to the extreme C terminus of the protein. The epitope was present when both the b3 domain and the GPI membrane attachment anchor were present. Removal of the GPI anchor and expression of a BGP-CEA hybrid as a potentially secreted protein also removed the PR1A3 epitope. This secreted product was detected by immunofluorescence assays of cells where the antigen appeared to be trapped within the cells, or on its way to secretion, and it may not have been fully processed. Therefore, hybrids attached to the cell surface by the BGP transmembrane domain and not by a GPI anchor were constructed. Replacement of the GPI anchor with a BGP transmembrane domain also removed the PR1A3 epitope. The structures of several GPI anchors are known (31), and although the glycosyl moiety of the GPI anchors from protozoa can be antigenic in some animals (32), the specificity of PR1A3 for CEA and absence of binding to NCA, a related member of the CEA family which also has a GPI anchor, would exclude the GPI anchor alone from being the epitope. Therefore in keeping with the conformational nature of the epitope, elements of the GPI anchor make up part but not all of the PR1A3 epitope, the additional elements of the epitope being provided by the b3 domain.

The close association of the epitope with the site of cell attachment goes some way to explaining the preference of the antibody for cell-bound antigen but does not explain the absence of antibody binding to circulating antigen. This absence of binding could result from the loss of part or all of the b3 domain and the glycosyl moiety of the GPI anchor when CEA is released into the serum. Partial degradation of this type has been proposed for CEA released from cells grown *in vitro* (33). Alternatively, release of CEA from the membrane and loss of the hydrophobic anchor may result in conformational changes which could affect the spatial arrangement of the different elements of the epitope. An additional possibility is that dimerization of CEA may give steric hindrance and prevent PR1A3 access to the epitope. CEA has been proposed to form head-to-tail homodimers during cell-cell binding (34), and evidence suggests that soluble CEA is a dimer (35). CEA on the cell surface is monomeric and the PR1A3 epitope is exposed, but formation of dimers in solution may sterically hinder access to the epitope.

In conclusion, PR1A3 binds to CEA close to the site of membrane attachment. The epitope involves both the b3 domain of CEA and part of the GPI anchor. The nature of the epitope probably gives the clinically advantageous properties associated with this antibody.

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