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Detection of a circulating gastrointestinal cancer antigen in sera of patients with gastrointestinal malignancies by a double determinant immunoassay with monoclonal antibodies against human blood group determinants

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SUMMARY

Monoclonal antibodies (MoAbs) produced against determinants A and B of the human ABO blood group system and against the Le^a and Le^b determinants of the Lewis (Le) blood group system detected these determinants on molecules released by cultured cells of human colorectal, gastric and/or pancreatic carcinoma (Ca) but not by a variety of other cells maintained in culture. Circulating Le antigen could be demonstrated in sera of patients by inhibiting the binding of MoAbs to a target preparation. A double determinant radioimmunoassay (DDIA) was then developed to detect the association of blood group determinants with a previously defined gastrointestinal cancer antigen (GICA). The DDIA with the anti-blood group and anti-GICA antibody was in some cases more sensitive in detecting GICA in sera than using the anti-GICA MoAb alone. Of 55 sera from patients with primary and early recurrent colorectal carcinoma (CRC), 10 (18%) were scored positive in the DDIA using only anti-GICA MoAb. When MoAb binding to a determinant on Le^b and on H, type I, was used as first antibody in DDIA followed by anti-GICA MoAb 11 additional sera were reactive, increasing the percentage of positive sera to 38. Using the same combinations of MoAbs, the sensitivity of detection of GICA was only slightly improved from 63 to 66% in sera of patients with advanced CRC. The number of false positive sera from patients with non-malignant gastrointestinal diseases or from healthy donors remained at low levels when anti-blood group determinant antibodies were used together with anti-GICA MoAb. The results indicate that DDIAs with MoAbs against different blood group determinants and tumour associated antigens can improve the detection of circulating antigens in patients with early stage cancer.

Keywords monoclonal antibodies gastrointestinal tumours serum antigens

INTRODUCTION

Several monoclonal antibodies (MoAbs) produced by hybridomas obtained from mice immunized with human tumours are directed against carbohydrate sequences occurring in glycolipids or glycoproteins. Two of these antibodies define a monosialoganglioside (Magnani *et al.*, 1981) and

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bind specifically to gastrointestinal tumours (Atkinson *et al.*, 1982; Herlyn *et al.*, 1982; Koprowski *et al.*, 1979). These antibodies have been successfully used to detect antigen circulating in sera of patients with gastrointestinal tumors (Herlyn *et al.*, 1982; Koprowski *et al.*, 1981; Steplewski *et al.*, 1983). Other antibodies against glycolipids containing the Le^b determinant of the human Lewis (Le) and the B determinant of the human ABO blood group system have been characterized (Hansson *et al.*, 1983).

The present report describes the production and characterization of binding specificities of several MoAbs against Le^a, Le^b, the common Le structure (Le^a and Le^b), and against blood group determinants A and B. These antibodies were then used to improve a double determinant radioimmunoassay (DDIA) for the detection of a circulating gastrointestinal cancer antigen (GICA) in sera of patients with gastrointestinal malignancies.

MATERIALS AND METHODS

Cells and cell preparations. Cell lines SW620, SW707 and SW1222 were established from colorectal carcinoma (CRC) lesions of patients with blood group A; SW403, SW948 and SW1116, from CRC patients with blood group O; and SW48 from a patient with blood group AB (Leibovitz *et al.*, 1976). Gastric carcinoma (Ca) cell line Kato III and pancreatic Ca cell line Capan 2 were established from malignant lesions of blood group B patients (J. Fogh, personal communication). Other cell lines used in this study are described elsewhere (Herlyn *et al.*, 1979). The Le type of those patients from whom the cell lines were derived was unknown.

Cells were grown in L-15 medium supplemented with 10% fetal bovine serum or with insulin (5 μg/ml), and transferrin (5 μg/ml) as described (Steplewski *et al.*, 1981). Supplemented serum free medium (SSFM) was removed after 5 days, centrifuged, filtered and used as a source of CRC antigen. Teratocarcinoma cell line SSFM was kindly provided by Dr P. Andrews, The Wistar Institute.

Patients' sera. Serum samples were obtained under informed consent from patients at the American Oncologic Hospital, Philadelphia, Pennsylvania and the Hurley Medical Center, Flint, Michigan, USA. Sixty-six serum samples from normal individuals 22–67 years of age were obtained from personnel at The Wistar Institute. Other normal sera were from patients without evidence of disease, though occult malignant disease cannot be excluded. Most of these sera were from patients 45–65 years old. Other sera were from patients with inflammatory gastrointestinal diseases or with benign or malignant neoplasms of the gastrointestinal tract. The ABO blood group or the Le type of the serum donors was unknown. Negative and positive serum control samples for the carbohydrate antigen 19-9™ were obtained from Centocor, Malvern, Pennsylvania, USA.

MoAbs. Production and characterization of MoAbs 1116 NS-10 (10), 1116 NS-19-9 (19-9), and 1116-NS-52a (52a) against CRC cell line SW1116 have been described (Koprowski *et al.*, 1979). The antigen detected by MoAb 10 was identified as Le^b (Brockhaus *et al.*, 1981); however, recently it was found that this antibody also reacts with the H, type I antigen (M. Blaszczyk, personal communication). The CRC antigen bound by MoAbs 19-9 and 52a was characterized from tumour tissue as a monosialoganglioside (Magnani *et al.*, 1981), whereas the same carbohydrate determinant detected by MoAb 19-9 in serum of CRC patients appears to be linked with mucin.

Of the additional MoAbs, which are described in detail elsewhere (Blaszczyk *et al.*, 1983), MoAb CO-51.4 is directed against Le^a; CO-51.2 and CO-51.3, against a common determinant of Le^a and Le^b; 33/25/1/17, against human blood group A antigen; $E_2$83-52, against the type 1 and 2 chains of human blood group B antigen (Hansson *et al.*, 1983); and MoAbs CO-56-22 and GA-29-1 define a sugar sequence of lactofucopentaose III (LNF III) (Brockhaus *et al.*, 1982; Hansson *et al.*, 1983). In addition to MoAb 10 (Brockhaus *et al.*, 1981), MoAbs CO-29.3, CO-29.4, CO-30.1, CO-43.1 and CO-43.2, which all bind to Le^b antigen, were also used (in Table 1). Purification of MoAbs from ascitic fluid of mice bearing the hybridoma tumour and radiolabelling of purified MoAbs by the Iodogen method have been described elsewhere (Herlyn *et al.*, 1983).

Binding assays. Indirect radioimmunoassay was performed to detect binding of MoAbs to live cells (Herlyn *et al.*, 1979) or SSFM (Herlyn *et al.*, 1982). The inhibition assay for the detection of

circulating antigen in patients' sera has been described in detail elsewhere (Herlyn *et al.*, 1982; Sears *et al.*, 1982). The percentage of inhibition of binding of MoAb to SSFM was calculated from the mean ct/min of triplicate wells as:

$$\%I = 100 - \left[\frac{(\text{test ct/min} - \text{control ct/min}}{(\text{maximum ct/min} - \text{control ct/min})}\right] \times 100.$$

where test ct/min represents cpm after incubation with inhibitor and maximum ct/min is determined after incubation of antibody with buffer. In controls, αCRC MoAb is replaced by supernatants from myeloma P3 × 63Ag8.

In DDIA, $\frac{1}{4}$ inch polystyrene beads (Precision Plastic Ball Co., Chicago, Illinois, USA) were first incubated with 1:1,000 diluted ascitic fluid of hybridoma tumour bearing mice, except for MoAb CO-51.3 which was used as tissue culture supernatant, diluted 1:90 in 0·2 M sodium borate, pH 8.2. The MoAbs used in this first step are referred to as 'catcher' antibodies. The beads were incubated overnight at 4°C, washed three times with phosphate-buffered saline (PBS) and incubated in PBS containing 2% bovine serum albumin (BSA) and 0·08% NaN_3 for 1 h at room temperature. The beads were then washed three times and incubated for 3 h at 4°C in assay buffer (PBS plus 2% BSA and 0·08% NaN_3). The beads were then incubated overnight at 4°C with 200 μl of the source of antigen (Serum, SSFM), then washed, and 200 μl of 19–9 MoAb labelled with ^{125}I in assay buffer was added before an additional overnight incubation at 4°C. This is referred to as 'tracer' antibody. The beads were then washed and counted for radioactivity bound.

For calculation of units of antigen detected by MoAb 19-9 in serum or SSFM, positive and negative standards were used from the carbohydrate 19-9 assay kit (Centocor) (del Villano *et al.*, 1983). The ct/min obtained with 1:8 diluted SSFM of pancreatic Ca cell line Capan 2 as antigen source were then calculated into units using the Centocor standards as references. Counts per minute obtained with the other catcher antibodies using the same dilution of SSFM of Capan 2 as antigen source were given the same number of units as MoAb 19-9.

RESULTS

Binding pattern of MoAbs

As shown in Table 1, in solid phase radioimmunoassay with SSFM of human cell lines of various origin, nine MoAbs with specificities for the Le blood group determinants reacted with the SSFM from six cell lines of colorectal, gastric and pancreatic Ca. No release of antigen into SSFM was seen with three CRC cell lines or with 28 cell lines of other origin. Double staining studies using fluorescein and rhodamine as indicators for binding of MoAb showed that Le^a and Le^b antigens are expressed on the surface of the same individual cells (results not shown). Study of the reactivity of SSFM of several cell lines with various MoAbs (Fig. 1) indicates the same binding pattern for anti-Le^a and Le^b MoAbs as for anti-GICA (19-9 and 52a) MoAbs, whereas LNF III is the only antigen found in SSFM of rectal Ca cell line SW707. In contrast, SSFM of pancreatic Ca cell line Capan 2, does not react with LNF III MoAb, but reacts significantly with the MoAbs against Le^a, Le^b and GICA.

Fig. 2 shows examples of binding of MoAbs against the major blood group antigens A and B to red blood cells of 12 donors. The blood cells had been depleted of lymphocytes and monocytes by Ficoll-Hypaque gradients. MoAb 33/25/1/17 bound to types 1 and 2 of the blood group A antigen (not shown). Only cells of gastrointestinal tumours expressed either A or B antigen on their surfaces and shed into SSFM. No A or B antigen was detected by MoAbs 33/25/1/17 or $E_2$83-52 on cells or in SSFM of the following cell lines: mammary Ca (Two cell lines tested), lung Ca (two), ovarian Ca (one), bladder Ca (five), teratocarcinoma (five), melanoma (six), sarcoma (one), glioma (two), chemodectoma (one), lymphoma (two) and fetal fibroblasts (three).

Inhibition assays and DDIA

To determine whether either Le^a or Le^b antigen could be detected in human sera, sera obtained from

Table 1. Cell lines that shed antigens reactive with monoclonal anti-Le MoAbs*

αLe bind to SSFM of cell lines:	αLe do not bind to SSFM of cell lines:
CRC cell lines	CRC (3)†
SW403	Mammary Ca (2)
SW948	Prostate Ca (2)
SW1116	Ovarian Ca (2)
SW1222	Bladder Ca (5)
Gastric Ca cell line	Cervix Ca (1)
Kato III	Teratocarcinoma (6)
Pancreatic Ca cell line	Melanoma (6)
Capan 2	Leukemia (2)
	Lymphoma (2)
	Sarcoma (2)
	Astrocytoma (1)
	Hypernephroma (1)

* αLe^a, MoAb CO-51.4; αLe^b, MoAb 10, CO-29.3, CO-29.4, Co-30.1, CO-43.1, and CO-43.2; αLe^a and αLe^B CO-51.2 and CO-51.3.

† Figures in parentheses indicate number of different cell lines used in assays.

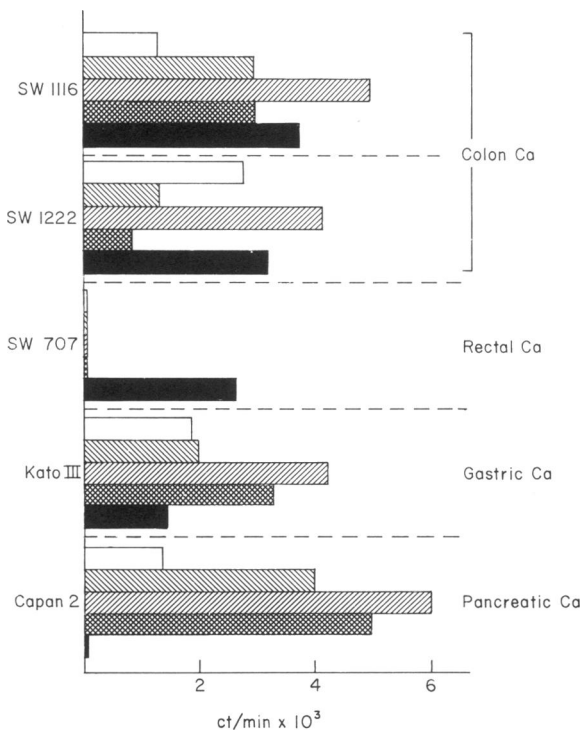

Fig. 1. Solid phase radioimmunoassay with undiluted SSFM of cell lines as targets. Binding of MoAbs was detected with ^{125}I-labelled rabbit IgG anti-mouse F(ab')$_2$ (40,000 ct/min/well). □ = αLe^a (C0 51.4); ▨ = αLe^b (CO 43.2); ▩ = αLe^{a+b} (CO 51.2); ▩ = GICA 19-9; ■ = αLNF III (CO 56-22).

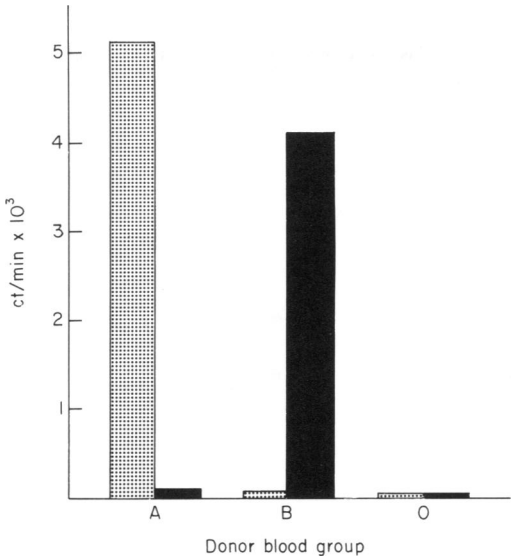

FiG. 2. Live cell radioimmunoassay with 50 μl packed red blood cells from donors with blood group A, B or O as targets. Binding of MoAb 33/25/1/17 (αA) (▦) or $E_2$83-52 (αB) (■) was detected with radiolabelled anti-mouse F(ab′)$_2$ (40,000 ct/min/well).

12 colorectal cancer patients and 22 healthy donors were tested in an inhibition assay for binding of MoAbs against Le^a and Le^b to SSFM of CRC cell line SW1116. The sera tested from healthy donors and patients with CRC contained an antigen detected by MoAb against Le^a and Le^b. These sera are apparently from Le positive and secreting individuals. As shown in Table 2, binding of MoAb 10, which defines Le^b and H type I, is inhibited by 38% of sera from normal volunteers and by 68% of sera from patients with CRC.

To test a combination of two MoAbs in detecting antigen(s) in sera of cancer patients, we used the DDIA. As shown in Fig. 3, the 19-9 MoAb as catcher followed by 19-9 as tracer antibody was more effective in detecting antigen present in SSFM of a pancreatic cancer cell line than the three other antibodies: 52a (αGICA), 10 (αLe^b and H) and CO-51.3 (αLe^a and Le^b). The specificity of DDIA was confirmed by negative results obtained with SSFM from a melanoma cell line. Similarly, MoAbs against blood groups A and B or against LNF III used as antigen catcher followed by 19-9 tracer detect antigen present in SSFM, but this combination was five- to 10-fold less effective than those illustrated in Fig. 3.

Table 2. Inhibition of binding of monoclonal anti-Le antibodies by circulating antigen in human sera

	Inhibition of binding of antibodies*	
Serum donors	CO-51.3 (αLe^a + Le^b)	10 α(Le^b + H_1)
CRC	12/12†	35/51
Healthy donors	10/10	11/29

* Target for the detection of binding of MoAb was SSFM of CRC cell line SW1116.

† Number of sera inhibiting binding of MoAb to target by more than 30%/total number of sera tested.

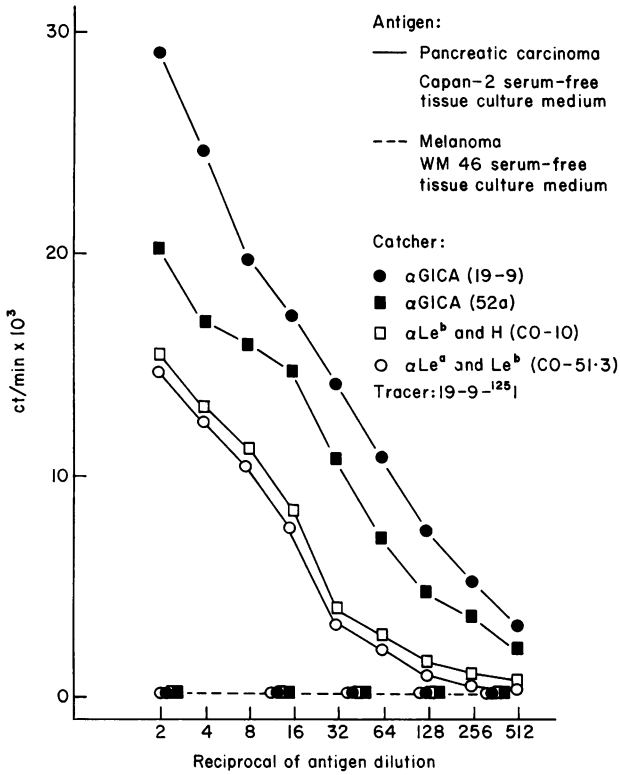

Fig. 3. DDIA using four different antibodies as catcher and one antibody (^{125}I-labelled 19-9, 100,000 ct/min) as tracer of antigen present in SSFM of cell lines.

Extending these studies to the detection of antigen in human sera, we used four antibodies, αGICA (19-9), αB ($E_2$83-52), αLe^a and Le^b (CO-51.3) and αLe^b+H, type I (10) as catchers and antibody 19-9 as tracer in DDIA. As shown in Table 3, antigen was detected by all four antibodies in the serum of one patient with CRC and in SSFM of Capan 2. The postitive 19-9 standards were useful for construction of standard curves for MoAb 19-9 only, not for the three other antibodies. For further experiments, we chose SSFM of Capan 2 as a positive standard since antigen(s) shed by this cell line reacted with all antibodies. We then calculated the ct/min of 1:8 diluted SSFM of Capan 2 for MoAb 19-9 into units, e.g., 407 units as shown in the example, from the positive standards. The ct/min obtained with the other three antibodies as catcher of antigen in SSFM of Capan 2 were given the same number of units, i.e., 407. We note that the sensitivity of DDIA was not increased by adsorption of more than one antibody as catcher to the same bead (not shown).

We then tested various MoAbs in DDIA as catcher antibodies to detect antigen in sera of cancer patients, patients with non-malignant gastrointestinal disease and healthy donors (Table 4). Elevated levels of antigen were found in 63% of sera from patients with advanced CRC, whereas only two out of 129 sera from healthy donors showed elevated antigen levels. When 41 units were arbitrarily chosen as 'cut-off' for positive and negative sera, the percentage of 'false positive' sera from patients with non-malignant gastrointestinal diseases was 2·5% for benign neoplasm, such as colonic and rectosigmoid polyps, and 3·8% for inflammatory diseases, such as colitis, duodenitis, and diverticulitis. Two of 17 patients (11·7%) with functional diseases such as spastic colon had elevated antigen levels in their sera. When anti-blood group B MoAb, $E_2$83-52, was used as catcher followed by 19-9 as tracer (Table 5) and three units were chosen arbitrarily as cut-off, three out of 12 (25%) sera from patients with advanced CRC had elevated antigen levels. The percentage of positive

Table 3. Detection of antigen in serum of CRC patient and healthy individual and in SSFM of a pancreatic CA cell line by DDIA with different MoAbs as catcher and 19-9 MoAb as tracer

Serum or SSFM, dilution	Catcher antibody*			
	19-9 (αGICA)	$E_2$83-52 (αB)	CO-51.3 ($\alpha Le^a + Le^b$)	10 ($\alpha Le^b + H$)
CRC patient, 1:4	12,256†	797	4,127	7,180
Healthy donor, 1:4	252	0	0	141
Positive 19-9 standard, 60 units for 19-9	2,647	0	0	114
Negative 19-9 standard, 4.1 units for 19-9	256	0	0	0
SSFM, Capan 2, 407 units for all antibodies	17,945	1,423	9,575	10,616

* ^{125}I-labelled MoAb 19-9 was used as tracer with an input of 100,000 ct/min/well.

† Counts per minute of duplicates with less than 5% s.d. minus ct/min of controls (SSFM of melanoma WM-46).

sera from patients with non-malignant gastrointestinal diseases and from normal donors varied between 2·5 and 8·6%.

Although MoAbs 10, reactive with Le^b and H and CO-51.3, reactive with Le^a and Le^b, reacted similarly in DDIA for detection of antigen in the Capan 2 SSFM (Fig. 3), they reacted differently in the detection of antigen in human sera. As shown in Table 6, anti-Le^a and Le^b MoAb, CO-51.3, binds antigen above a cut-off of three units in none of 92 sera from healthy donors and only in two

ıble 4. Detection of GICA in human sera in DDIA with 19-9 MoAb as catcher and tracer*

ısease of ːum donors‡	No. of sera binding with units†							Total No. of sera	Percentage of sera with elevated antigen levels (>41 units)
	0–6	7–12	13–24	25–41	42–96	97–192	>192		
ŘC:									
Advanced	4	3	2	2	6	12	1	30	63
Early recurrence	8	2	3	3	2	1	0	19	15
Primary, pre-therapy ncreatic or	5	14	12	1	6	1	0	39	17
stric Ca	1	0	0	3	4	3	1	12	66
ɔn-malignant gastro-estinal diseases:									
Benign neoplasm	12	10	10	4	1	0	0	39	2·5
Inflammatory	26	12	11	1	2	0	0	52	3·8
Functional (spastic colon)	11	2	0	2	2	0	0	17	11·7
ı disease	49	29	33	16	2	0	0	129	1·5

* 1:1,000 dilution of ascitic fluid of MoAb 19-9 was absorbed to polystyrene beads and used as antigen catcher and after ɛelling of purified antibody, as tracer (input 100,000 ct/min/well).

† Units were obtained from standard curve unit positive standard from Centocor carbohydrate 19-9^{TM} kit.

‡ Sera were diluted 1:4.

Table 5. Detection of antigen in human sera in DDIA with MoAb $E_2$83-52 as catcher of antigen and 19-9 MoAb as tracer*

Disease of serum donors‡	No. of sera binding with units†								Total No. of sera	Percentage of sera with elevated antigen levels (>3 units)
	0–3	4–6	7–12	12–24	45–48	49–96	97–192	>192		
CRC:										
Advanced	9	1	0	1	0	1	0	0	12	25
Early recurrence	3	1	0	0	0	0	1	0	5	40
Primary, pre-therapy	30	0	0	0	0	0	1	1	32	6·6
Pancreatic or gastric Ca	3	0	0	1	1	0	0	0	5	40
Non-malignant gastro-intestinal diseases:										
Benign neoplasm	21	0	0	1	0	0	0	1	23	8·6
Inflammatory	21	0	0	1	0	0	0	0	40	2·5
Functional	54	0	1	0	0	0	1	1	57	5·2
No disease	62	0	0	0	1	0	3	0	66	6·0

* 1:1,000 dilution of ascitic fluid of anti-blood group B MoAb $E_2$83-52 was absorbed to beads (catcher) and ^{125}I-MoAb 19-9 (100,000 ct/min) was used as antigen tracer.

† For calculation of units: No. of units of 1:8 diluted SSFM of pancreatic Ca Capan 2 with MoAb 19-9 as catcher and tracer was used as reference. Counts per minute obtained with MoAb $E_2$83-52 as antigen catcher and MoAb 19-9 as tracer were designated the same number of units. Control ct/min were subtracted (1:8 diluted SSFM of melanoma WM-46) before calculation of units.

‡ Sera were diluted 1:4.

Table 6. Detection of antigen in human sera in DDIA with monoclonal anti-Le^a and Le^b MoAb CO-51.3 as catcher of antigen and 19-9 MoAb as tracer*

Disease of serum donors‡	No. of sera binding with units†							Total No. of sera	Percentage of sera with elevated antigen levels (>3 units)
	0–3	4–6	7–12	13–24	25–48	49–96	97–192		
CRC									
Advanced	13	1	0	2	1	1	1	19	31·5
Early recurrence	12	1	0	0	0	0	0	13	7·6
Primary, pre-therapy	31	3	1	0	1	0	0	36	3·8
Pancreatic or gastric Ca	9	1	0	1	1	1	1	14	28·5
Non-malignant gastrointestinal diseases:									
Benign neoplasm	60	0	2	0	0	0	0	62	3·2
Inflammatory	41	0	0	0	0	0	0	41	0
Functional	15	0	0	0	0	0	0	15	0
No disease	93	0	0	0	0	0	0	92	0

* 1:90 dilution of tissue culture supernatant of MoAb CO-51.3 was adsorbed to beads for catching of antigen which was detected with ^{125}I-labelled MoAb 19-9 (100,000 ct/min).

† For calculation of units: No. of units of 1:8 diluted SSFM of pancreatic Ca Capan 2 with MoAb 19-9 as catcher and tracer served as reference. Counts per minute obtained with MoAb CO-51.3 as catcher and MoAb 19-9 as tracer of antigen in SSFM of Capan 2 were designated the same number of units. Control ct/min (SSFM of melanoma WM-46) were subtracted before calculations.

‡ Sera were diluted 1:4.

sera from patients with benign neoplasm. On the other hand, six out of 19 patients with advanced CRC and five out of 14 patients with pancreatic or gastric Ca showed elevated antigen levels. In the cases of early recurrences or primary cancer, the antigen was detected in few sera. In contrast, as shown in Table 7, anti-Le^b and H, type I, antibody 10 as catcher detected elevated antigen levels in a larger number of sera. When the 'cut-off' was chosen at 24 units, one out of 107 sera from healthy donors was positive, whereas 56% of sera from patients with advanced CRC and 58% of sera with gastric or pancreatic Ca showed elevated levels of antigen. Although these results do not seem to differ substantially from those shown in Table 4, the fact that antigen was detected in 31% of early recurrences and in 26% of primary cancer cases made this combination more sensitive than the 19-9/19-9 combination. When the results of the two DDIAs with 19-9/19-9 and 10/19-9 are considered together, the frequency of the detection of early recurrences and primary cancer is higher (Table 8) than with either of the two assays separately. This is primarily due to the fact that antigen which could not be detected in sera of 11 patients in this category by 19-9/19-9 DDIA was detected by the 10/19-9 combination. However, as seen in Table 8, some sera were positive in 19-9/19-9 DDIA but negative in 10/19-9 DDIA.

DISCUSSION

We have shown in this paper that combinations of anti-blood group antibodies as solid phase antigen catcher and iodinated anti-GICA MoAb 19-9 as tracer improved the sensitivity of detecting elevated GICA levels in sera of patients with primary CRC and early recurrences of CRC. Using 19-9 MoAb as both catcher and tracer, only 17% of primary cancer patients and 15% of those with early recurrences showed the presence of CRC antigen in their sera. When the same panel of sera was assayed in DDIA using anti-Le^b MoAb as catcher and 19-9 MoAb as tracer, the percentages in

Table 7. Detection of antigen in human sera in DDIA with anti-Le^b and H type I MoAb 10 as catcher of antigen and 19-9 MoAb as tracer*

Disease of serum donors‡	No. of sera binding with units†								Total No. of sera	Percentage of sera with elevated antigen levels (> 24 units)
	0–3	4–6	7–12	13–24	25–48	49–96	97–192	> 192		
CRC										
Advanced	4	5	2	2	6	6	4	1	30	56
Early recurrence	4	4	1	2	4	1	0	0	16	31·2
Primary, pre-therapy	7	7	7	8	8	1	1	0	39	26
Pancreatic or gastric Ca	1	2	0	2	2	1	3	1	12	58
Non-malignant gastro-intestinal diseases:										
Benign neoplasm	20	10	10	13	3	0	0	0	56	5·3
Inflammatory	29	8	6	4	2	0	0	0	49	4·1
Functional	13	2	2	1	0	1	0	0	19	5·2
No disease	60	19	17	10	1	0	0	0	107	0·9

* 1 : 1,000 dilution of ascitic fluid of MoAb 10 was adsorbed to beads for catching of antigen which was traced with ^{125}I-labelled MoAb 19-9 (100,000 ct/min/well).

† For calculation of units: No. of units of 1 : 8 diluted SSFM of pancreatic Ca Capan 2 with MoAb 19-9 as catcher and tracer served as reference point. Counts per minute obtained with MoAb 10 as antigen catcher and MoAb 19-9 as tracer of antigen in SSFM of Capan 2 were designated the same number of units. Control ct/min (SSFM of Melanoma CO-46) were subtracted before calculation.

‡ Sera were diluted 1 : 4.

Table 8. Increased sensitivity of detecting in DDIA GICA in human sera with αLe^b and H MoAb 10 as antigen catcher and MoAb 19-9 as tracer

Disease of serum donors	No. of sera evaluated*	No. of sera positive in 19-9/19-9†‡ DDIA (%)	No. of sera positive in 10/19-9 DDIA§ (%)	No. of sera negative in 19-9/19-9 DDIA but positive in 10/19-9 DDIA	No. of sera positive in 19-9/19-9 DDIA but negative in 10/19-9 DDIA	Percentage of positive sera in 19-9/19-9 and/or 10/19-9 DDIA
CRC:						
Advanced	30	19 (63)	17 (56)	1	2	67
Early recurrence	16	3 (19)	5 (31)	4	1	44
Primary, pre-therapy	39	7 (17)	10 (26)	7	4	36
Pancreatic or gastric Ca	12	8 (66)	7 (58)	1	1	75
Non-malignant gastrointestinal diseases:						
Benign neoplasm	39	1 (2)	3 (51)	2	0	7
Inflammatory	49	2 (4)	2 (4)	2	0	6
Functional	17	2 (11)	1 (5)	0	1	11
No disease	107	2 (2)	1 (0·7)	1	2	3

* Only sera tested at same time were compared.

† Antigen catcher/antigen tracer.

‡ Sera with more than 41 units were considered positive.

§ Sera with more than 24 units were considered positive.

the two groups of patients rose to 26 and 31%, respectively. This increase is mainly due to the difference in the chosen cut-off between positive and negative sera for each catcher antibody. The results using anti-Le^a and anti-Le^b MoAb as catcher (and 19-9 MoAb as tracer) showed little difference, if any, from the results obtained with 19-9 MoAb alone. If one excludes $Le^{(a-b-)}$ individuals, who represent 5–7% of the population and who cannot synthesize GICA because this antigen appears to derive from Le^a glycolipid through sialylation of its carbohydrate moiety (Koprowski *et al.*, 1982), the actual percentage of CRC positive sera among early stage patient's increased.

It is not surprising that the antigenic determinant recognized by anti-Le^b MoAb is detectable in serum of early stage CRC patients since Le^b antigen can be detected in the mucosa of a great majority of colon cancers even though corresponding normal tissue such as the distal colon mucosa does not express this antigen at all. Concentration of the Le^b antigen in tumour tissue may account for its 'spillover' into the bloodstream and thus, the subsequent improvement of the diagnostic procedure.

The epitope recognized on cancer cells by anti-Le^b MoAb probably differs from that recognized by the anti-Le^a and Le^b MoAb, and this may account for the negligible improvement of the assay using anti-Le^a and Le^b MoAb as catcher.

In contrast to the tumour cell antigen, the serum antigen of CRC cancer patients is not a glycolipid but rather a glycomucin. Also, the number of carbohydrate chains linked with the mucin molecule is quite large, and MoAbs directed against carbohydrate chains other than Le^b and GICA may further improve the assay; this may eventually lead to a procedure which will enable the diagnosis of gastrointestinal cancer at the earliest, perhaps even pre-clinical, stages of the disease.

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