Europ. J. Cancer, Vol. 16, pp. 539–551.

0014-2964/80/0401-0539 $02.00/0

A Human Colon Tumour Antigen Associated with β_2-Microglobulin and Isolated from Solid Tumour, Serum and Urine, is Unrelated to Carcinoembryonic Antigen*

D. M. P. THOMSON,† DONNA N. TATARYN,‡ JOSIE C. WEATHERHEAD, PAULA FRIEDLANDER, JOYCE RAUCH, ROSEMARIE SCHWARTZ, PHIL GOLD and JOSEPH SHUSTER

Division of Clinical Immunology and Allergy of the Montreal General Hospital and ‡Department of Surgery, Montreal General Hospital Research Institute, McGill University, Montreal, Canada

Abstract—*The antigen-induced leukocyte adherence inhibition response is directed to an organ-type specific neoantigen. Cancers of the colorectum, pancreas and stomach each expressed a unique organ-specific neoantigen. The relationship of the colon tumour antigen to the previously described carcinoembryonic antigen (CEA) of GIT cancers was examined by a tube blocking LAI assay and CEA radioimmunoassay. The colon tumour antigen was papain-solubilized from colon cancer membranes. An affinity column of horse anti-human β_2-microglobulin bound the colon tumour antigen but did not bind CEA. In contrast, the colon tumour antigen failed to bind to an affinity column of antisera prepared to cell surface proteins that had failed to bind to the anti-human β_2-microglobulin affinity column. Both the colon tumour antigen and CEA existed in the serum of patients with metastatic cancer. The colon tumour antigen co-isolated with the HDL fraction of serum by polyanion precipitation which suggested that the colon tumour antigen was lipoprotein in composition. By contrast, CEA was recovered in the non-lipoprotein fraction of serum. A proportion of the colon tumour antigen and CEA in the serum were eliminated into the urinary protein by filtration in the kidney. The results of the present study indicate that the colon tumour antigen epitope and the CEA epitope exist on separate molecules.*

INTRODUCTION

THE EXISTENCE of tumour-specific transplantation antigens (TSTA's) in most animal tumour systems studied were established on the basis of the rejection of experimentally induced tumours inoculated into previously immunized syngeneic recipients [1–3]. Comparable evidence for the existence of TSTA's in human tumours is not available, since this type of experimental design is precluded by both ethical consideration and the lack of a syngeneic donor–host relationship. The principal body of evidence supporting the concept that human tumours express neoantigens has been derived from *in vitro* assays of cell-mediated and humoral antibody responses to tumour cells [4, 5].

A promising *in vitro* technique for the measurement of host anti-tumour immunity is based on the phenomenon of tumour antigen-induced inhibition of leukocyte adherence (LAI) to glass [6], where leukocytes from patients with cancer, when incubated *in vitro* with extracts of tumours arising in the same organ

Accepted 14 September 1979.

*Supported, in part, by grants from the Medical Research Council of Canada, The Cancer Research Society Inc. of Montreal and the The National Cancer Institute of Canada.

†To whom reprint requests should be addressed: Montreal General Hospital, 1650 Cedar Avenue, Montreal, Canada H3G 1A4.

and of the same histological type, lose their normal properties of adherence to glass. With the tube LAI assay [7–10], the reactivity of the patient's leukocytes can be tested with isolates of the tumour to determine which component of the tumour is responsible for inhibition of leukocyte adherence to glass [11–15]. The tube LAI assay has been used to monitor the isolation of human tumour antigens from the cell membranes of hepatoma, malignant melanoma, breast and bowel cancer [11, 14].

The present experiments were undertaken to answer the question as to whether the colon tumour antigen recognized by the LAI response was related to the carcinoembryonic antigen (CEA), a tumour associated antigen, present in all gastro-intestinal cancers [16, 17] and located on the cell surface of the tumour [18].

MATERIALS AND METHODS

Donors of leukocytes

Heparinized blood samples were drawn from patients with adenocarcinoma of either the colon, breast or malignant melanoma. Buffy coat peripheral blood leukocytes (PBL) were processed for the tube LAI as previously described [7].

Tumour extracts

Phosphate buffered saline extracts (PBS) pH 7.3, were prepared from tumour tissue metastatic to liver as described previously [7, 8, 15]. Protein concentration of the extracts and of all other samples were measured with bovine albumin as a standard [19].

Serum samples

Blood was drawn from patients with metastatic cancer whose leukocytes did not respond in the LAI assay to the sensitizing cancer extract and immediately stored at 4°C. After overnight retraction of the clot, the serum was separated and stored at −40°C.

Antigen-induced leukocyte adherence inhibition assay (tube LAI)

The tube LAI assay was performed as previously described by Grosser and Thomson [7, 8]. PBL were incubated in horizontally placed glass test tubes with the specific and non-specific tumour extracts at 37°C in a humidified atmosphere of 5% CO_2 in air. The tumour extracts were used at a concentration of ~100 μg protein/tube. After 2 hr, the tubes were placed upright and the contents at the bottom of the tube were gently agitated with a Pasteur pipet and a sample was placed on a haemocytometer for counting. In the latter half of the study the non-adherent cells were enumerated automatically by image analysis [10]. The results were expressed as a non-adherence index (NAI) [7]. $NAI = [(A-B)/B] \times 100$, where A equals the number of non-adherent cells in the presence of the specific antigen: and B equals the number of non-adherent cells in the presence of the non-specific antigen.

The difference in reactivity to the two tumour extracts is expressed by the NAI. The range of normal values is determined by the extent to which leukocytes from control subjects show differences in non-adherence to the two tumour extracts. More than 95% of subjects without the specific cancer had an LAI of <30. Hence, an NAI of $\geqq 30$ is positive and indicates the presence of anti-tumour immunity and a value of <30 is negative and indicates no anti-tumour immunity [8–10, 20, 21].

PBL were from patients with malignant melanoma, colon and breast cancer who reacted in the tube LAI assay against their respective cancer extracts. Leukocytes, a minimum of 1.3×10^7 in 0.5 ml of Medium 199, were preincubated with 0.5 ml of the experimental sample at 37°C in a 5% CO_2 atmosphere with frequent agitation of the plastic tubes. After 30 min, the cells were washed with 10 ml of Medium 199 to remove the experimental sample. The PBL were resuspended with Medium 199 to 1 ml and then 0.1 ml of the PBL suspension was plated in the glass test tubes with the specific and non-specific cancer extracts as decribed in the tube LAI assay. After completion of the assay, a sample of the nonadherent cells in each tube was counted and the NAI calculated [12].

A sample to be tested for tumour antigen activity was diluted to the appropriate protein concentration in Medium 199 containing 20% fetal calf serum (FCS) and 0.5 ml was preincubated with the PBL (1.3×10^7) in 0.5 ml of Medium 199. When the test sample specifically negated the LAI response of reactive leukocytes, tumour antigen activity was accepted as present. All samples tested in the blocking assay were unknown to the experimenter. When the various samples were tested, samples were included that might block and that should not block. All samples that bloc-

ked the LAI activity of leukocytes from patients with colon cancer were then tested on LAI reactive leukocytes from patients with an unrelated cancer to show that the blocking of LAI activity was immunologically specific [13].

Papain-solubilized tumour antigen from solid tumour

The membranes of colon cancer cells from metastatic deposits of solid tumour derived from liver metastasis were purified [11] and then digested with papain [11, 14]. The papain-soluble membrane material that passed through a column of DEAE Sephadex G-50 (0.38 M NaCl, 0.02 M Tris, pH 8.0) was chromatographed on a calibrated Sephadex G-150 column (5 × 87 cm) equilibrated with 0.01 M Tris, 0.3 M glycine, 0.2 M NaCl buffer, pH 8.0, containing 0.001 M disodium EDTA and the fractions were concentrated and dialyzed against PBS and tested for tumour antigen activity in the blocking LAI assay. The fraction that eluted from the Sephadex G-150 column in the molecular weight range of 70,000–150,000 (fraction 2) had maximal tumour antigen activity as determined by the blocking LAI assay [14]. The papain-soluble tumour antigen of colon cancer present in fraction 2 was purified further by horse anti-human β_2-microglobulin affinity chromatography. Tumour antigen from other cancers were isolated similarly.

Horse anti-human β_2-microglobulin

Pure β_2m was kindly provided by Dr. M. D. Poulik (William Beaumont Hospital, Royal Oak, MI). Antiserum to β_2-microglobulin was prepared by immunization of horses [11, 14]. This β_2m was used for radiolabelling with ^{125}I, and as the standard β_2m preparation in the establishment of the radioimmunoassay for β_2m by the double antibody technique as previously described [22]. The IgG derived from the antiserum to β_2m [23] was absorbed with normal human serum coupled to AH-Sepharose 4B to remove any potential reactivity to human immunoglobulin. The IgG was then coupled to AH-Sepharose 4B as described by Cambiaso [24].

The horse anti-β_2m antiserum did not react with normal human serum by double immunodiffusion. By radioimmunoelectrophoresis, the antiserum gave a single band with the low molecular weight fraction of serum or urine rich in β_2m. The antiserum for affinity chromatography was selected for its low titre to make possible more efficient elution from the affinity column [14]. By indirect membrane immunofluorescence [25], the anti-β_2m antisera was shown to stain the cell surface of lymphocytes and cancer cells and when β_2m on the lymphocytes was capped by the anti-β_2m antisera, all of the alloantigenic activity co-capped. When alloantigens were capped by anti-HLA alloantisera, β_2m was also co-capped, although some residual staining of the cell surface remained detectable. Specificity of the horse anti-human β_2m immunoadsorbent affinity column has recently been described in detail [11, 14, 26, 27].

Rabbit anti-human non-β_2-microglobulin

Papain-soluble breast cancer, normal liver and malignant melanoma material from Sephadex G-150 fraction 2 that had not bound to the horse anti-β_2m affinity column was used to immunize rabbits by three intramuscular injections over 10 days with 1 mg of material emulsified in complete Freund's adjuvant. After 4 weeks they were boosted by an intramuscular injection of 500 μg of the same preparation in incomplete Freund's adjuvant and bled 1 and 2 weeks later. The IgG derived from this anti-serum was coupled to AH-Sepharose 4B. Antiserum to normal human serum protein (NHS) was prepared as previously described [13] and was also coupled to AH-Sepharose 4B. The anti-human non-β_2m and anti-NHS antisera, linked to AH-Sepharose 4B, constituted the "anti-non-β_2m affinity column".

Affinity chromatography

Fraction 2 from the Sephadex G-150 column with tumour-antigen activity, assayed by blocking LAI, or similarly prepared control material was applied to the affinity column in PBS. Approximately 15 mg of material was applied and after the effluent containing the unbound fraction had been washed through, the column was washed with an additional 10 column volumes of PBS. Next, the affinity column was prewashed with 1.0 M NaCl, NaOH–glycine buffer, pH 9.0 to remove non-specifically absorbed proteins [28]. The bound material was then eluted with 3 M KSCN. All procedures were performed at 4°C. The bound and eluted fraction was immediately dialyzed against PBS at 4°C. After overnight dialysis, the bound and eluted fraction was centrifuged at 75,000 ***g*** for 1 hr and concentrated by ultrafiltration. The unbound fraction was treated in an identical manner. β_2m in the various fractions was measured by radioimmunoassay [22].

Co-isolation of serum tumour antigen with high-density lipoproteins (HDL)

HDL were precipitated and isolated from whole serum with the polyanions, sodium phosphotungstate and magnesium chloride as previously described [29, 30]. A supernatant fraction free of HDL and HDL fraction of serum were obtained. The fractions were returned to their original serum volumes to be assayed.

Isolation of urinary tumour antigen

Tumour antigen was isolated from urine by 80% saturated $NH_4(SO_4)_2$ precipitation and affinity chromatography with Blue Sepharose CL-6B, as previously described [30]. The isolate of urinary protein was dialyzed against PBS pH 7.3 and concentrated before being assayed by blocking LAI.

Sodium-dodecyl sulphate (SDS) polyacrylamide gel eletrophoresis (PAGE)

High resolution SDS slab gels (0.75 mm thick) were run by the discontinuous method of Laemmli [30] with the running gel having a continuous gradient of 5–20% polyacrylamide. The samples were radiolabelled with I^{125} by the chloramine T method or with the Bolton–Hunter reagent (New England Nuclear). The samples were prepared for electrophoresis as described by Laemmli [30]. The patterns of the radiolabelled materials on SDS–PAGE was determined by autoradiography.

CEA radioimmunoassay

Isolates of papain-solubilized tumour cell membranes, serum and urine were examined for their content of CEA by a double antibody CEA radioimmunoassay.

RESULTS

Organ-type specific neoantigen recognized by LAI response

Leukocytes from patients with early colon cancer showed significantly more non-adherence in the tubes when incubated with an extract of colon cancer than extracts of normal colon mucosa, villous adenoma, pancreatic, stomach or lung cancer (Table 1). By contrast, leukocytes from the control subject were equally non-adherent with the various extracts. Leukocytes from the patient with stomach cancer showed increased non-adherence when incubated with the extract of stomach cancer but not with the other extracts (Table 1). Likewise, leukocytes from the patient with early pancreatic cancer showed increased non-adherence when incubated with the extract of pancreatic cancer in comparison to the other extracts (Table 1).

In addition, more than 200 subjects with disease unrelated to the colon were tested against the colon and lung cancer extract and less than 3% showed a positive LAI response to colon cancer [21]. In 49 patients with Dukes' stage A, B and C colon cancer, 100%, 75% and 61% were LAI^+ (LAI-positive) to the colon cancer antigen [21]. Leukocytes from patients with other GIT cancers did not react with the extract of colon cancer. Similarly, leukocytes from the LAI^+ positive patients with colon cancer did not react with extracts of pancreatic and stomach cancer [21]. Hence, the LAI response in patients sensitized to cancer of the GIT is directed to an organ-type specific neoantigen.

Isolation of papain-soluble colon tumour antigen by anti-human β_2m affinity chromatography

Papain-solubilized membranes of colon and breast cancer were chromatographed separately on Sephadex G-150 and the material pooled into 4 fractions as previously described [11, 14]. Maximal tumour antigen activity was found in fraction 2, the material that eluted in the molecular weight range of about 70,000–150,000.

The LAI reactivity of leukocytes from colon cancer patients was negated by preincubation with the papain-soluble colon cancer membranes from Sephadex G-150 fraction 2 (Table 2). The blocking of the LAI activity was specific since the same leukocytes retained their reactivity in the LAI assay to the extract of colon cancer when preincubated with material isolated in an identical manner from breast cancer membranes (Table 2). Conversely, LAI^+ leukocytes from patients with breast cancer were blocked when preincubated with the isolated papain-soluble breast cancer material but not with the identically prepared papain-soluble colon cancer material (Table 2).

Papain-soluble malignant melanoma, colon and breast cancer materials from fraction 2 of the Sephadex G-150 column were separately fractionated by horse anti-human β_2m affinity chromatography. Material with colon tumour antigen activity was bound by the anti-human

Table 1. Specificity of colon tumour antigen-induced leukocyte adherence inhibition in glass test tubes

Diagnosis of leukocyte donor	Mean number ± S.D. of non-adherent cells incubated with phosphate buffered saline extracts of:* Colon cancer	Villous‖ adenoma	Normal colon mucosa	Pancreatic cancer	Stomach cancer	Lung cancer	NAI†	*P*¶
Colon cancer	64±6	41±4	45±1	42±4	40±2	43±5	42‡	(<0.05)
Colon cancer	62±10	42±6	41±3	43±3	44±4	46±4	35‡	(<0.05)
Ovarian cancer	36±5	35±1	34±2	33±5	36±3	37±3	9§	(N.S.)
Pancreatic cancer	52±4	N.D.	N.D.	84±8	51±4	46±2	83	(<0.001)
Control subject	45±3	N.D.	N.D.	51±3	48±3	49±3	4	(N.S.)
Stomach cancer	35±10	N.D.	N.D.	41±6	88±10	43±7	104	(<0.005)

*Tube LAI assay is done in triplicate and the mean ± S.D. of a sample of the counts from each tube is shown.

†NAI—the non-adherence index is calculated as follows: $(A-B)/B \times 100$, where A are the non-adherent cells to the specific antigen, and B are the non-adherent cells to the non-specific antigen.

‡Colon cancer is the specific antigen and the other extracts are the non-specific antigens. The NAI to the colon cancer extract is greater than 30 with any of the control extracts.

§The NAI to the colon extract is less than 30 with any of the control extracts.

¶*P*—Student's *t*-test was used to calculate the statistical significance of the difference in leukocyte non-adherence to the specific and nonspecific (lung) extract. N.S. where $P>0.05$.

‖The villous adenoma was from a patient whose leukocytes were LAI-negative.

β_2m affinity column and eluted with 3.0 M KSCN (Table 2). The papain-soluble melanoma and breast cancer material, also bound and eluted from the anti-human β_2m affinity column, did not alter the LAI reactivity of leukocytes from patients with colon cancer (Table 2). Conversely, the LAI^+ leukocytes from malignant melanoma or breast cancer patients were not blocked by the bound and eluted colon cancer material. However, the LAI^+ leukocytes from the malignant melanoma or breast cancer patient were blocked by the bound and eluted papain-soluble melanoma or breast cancer material, respectively, that were isolated in the same way from the anti-human β_2m affinity column (Table 2).

CEA was detected in the colon cancer material that did not bind to the affinity column of anti-human β_2m (Table 2), whereas β_2m was found in the fraction that bound and was eluted from the affinity column of anti-human β_2m. Affinity chromatography with anti-human β_2m achieved about a 25 fold purification of the colon cancer antigen. The fraction that blocked LAI activity contained β_2m, whereas the unbound fraction was free of β_2m but contained CEA.

Anti-non-β_2m affinity chromatography

Papain-soluble colon cancer material from fraction 2 of the Sephadex G-150 column was applied to the affinity column of rabbit antisera raised to cell surface proteins not linked to β_2m and to NHS, anti-human non-β_2m. The colon tumour antigen was recovered in the unbound fraction (Table 2). The bound and eluted fraction had no blocking activity (Table 2). The unbound fraction with the colon tumour antigen activity did not impair the LAI reactivity of leukocytes from patients with breast cancer (Table 2). CEA was present in both the unbound and bound and eluted fractions, whereas β_2m was detected in the unbound fraction.

SDS–PAGE

Figure 1 shows a high resolution SDS slab gel electrophoresis autoradiograph pattern of radiolabelled papain-soluble membrane material from the Sephadex G-150 fraction 2 that bound and was eluted from the affinity column of anti-human β_2-microglobulin. Polypeptide chains are visible at molecular weights of about 12,000, 25,000, 40,000, 50,000, and 60,000. The band at 50,000 mol. wt and a proportion of the band at 25,000 mol. wt, probably represented H and L chains of IgG that have bled from the affinity colum. Isolates with colon or breast tumour antigen activity have similar patterns on SDS gel electrophoresis (Fig. 1).

Figure 2 shows the pattern on SDS–PAGE of papain-soluble colon cancer material bound and eluted, and unbound from the affinity column of anti-non-β_2-microglobulin. The bound and eluted material shows multiple bands that are strikingly different compared

Table 2. Papain-solubilized human colon cancer tumour antigen from solid tumour isolated and separated from CEA by anti-β_2m affinity chromatography

Preincubation treatment of donor leukocytes	Total† protein conc. (mg/l)	CEA§ (ng)	β_2m¶ (ng)	Diagnosis of leukocyte donor	Leukocyte NAI* before incubation	NAI‡ after blocking
Sephadex G-150 fraction 2 of papain-soluble cancer membranes:						
Colon cancer	100	>25	10	Colon cancer	100	−7
	25					−10
	10					87
Breast cancer	100					80
Breast cancer	50			Breast cancer	48	2
Colon cancer	100					41
Anti-β_2m affinity fractions of:						
Colon cancer, unbound	100	>25	0	Colon cancer	100	74
bound	100	0	27			−3
	1.0					16
	0.5					109
Melanoma, bound	100	0	34			70
Breast cancer, bound	100	0	28			52
Colon cancer, bound	100	0	27	Malignant melanoma	53	47
Melanoma, bound	1.0	0	34			10
Colon cancer, bound	50			Breast cancer	33	84
Breast cancer, bound	1.0					8
Anti-non-β_2m affinity fractions of:						
Colon cancer, unbound	50	>25	40	Colon cancer A	81	10
bound	50	12	0			126
unbound	50			B	62	−3
bound	50					83
unbound	50			C	38	2
bound	50					102
unbound	50			D	42	−4
bound	50					58
unbound	50			Breast cancer	49	61

*NAI was calculated from the number of non-adherent leukocytes when incubated with extracts of colon, breast cancer or melanoma as specific antigen for patients with colon, breast cancer or melanoma, respectively; and the non-specific antigens were lung, breast cancer or melanoma for patients with colon, melanoma or breast cancer, respectively.

†Total amount of protein of the isolate added to the preincubation tube, which has a final volume of 1 ml.

‡The leukocytes of the donor were tested against their appropriate specific and non-specific tumour extracts to determine if LAI reactivity had been negated. An NAI of $\geqq 30$ is positive and <30 is negative.

§Determined by CEA radioimmunoassay: ng of CEA/100 μg protein.

¶Determined by β_2m radioimmunoassay: ng of β_2m/10 μg protein.

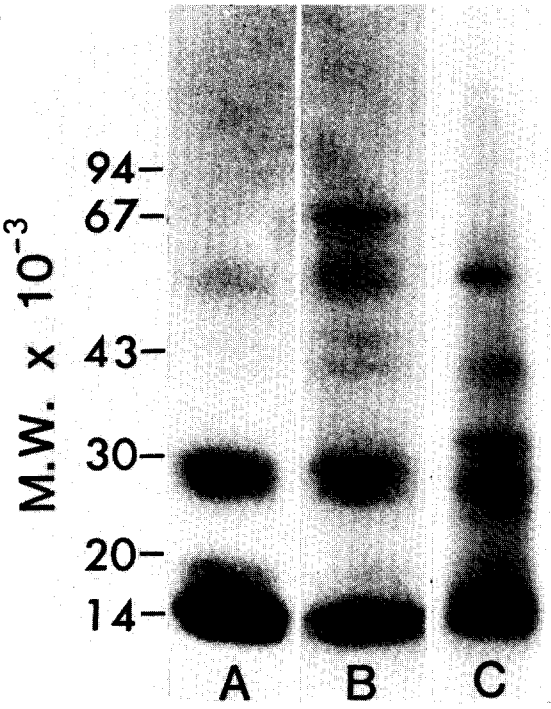

Fig. 1.

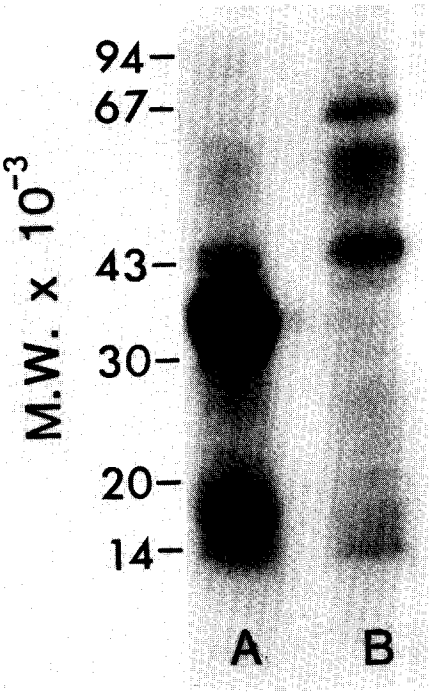

Fig. 2.

Fig. 1. *Autoradiogram of SDS gel electrophoresis of papain-solubilized colon and breast cancer membranes isolated from Sephadex G-*150 *fraction* 2, *bound and eluted from the affinity chromatography column of horse anti-human* β_2*-microglobulin. A, material with colon cancer antigen activity radiolabelled with chloramine T; B, another isolate with colon cancer antigen activity radiolabelled with the Bolton–Hunter Reagent; C, material with breast cancer antigen activity radiolabelled with chloramine T. The material shown in columns A, B and C had specific activity in the tube blocking LAI assay. Although not well visualized, colon cancer material shown in slot A had two bands visible at about* 40,000 mol wt *similar to the bands of the material in column B.*

Fig. 2. *Autoradiogram of SDS gel electrophoresis of papain-solubilized colon cancer membrane, unbound, and bound and eluted from the affinity column of anti-non-*β_2*-microglobulin. A, unbound material; B, bound and eluted material. The material in column A had specific colon tumour antigen activity, whereas material in column B had no tumour activity.*

to the material that was bound and eluted from the anti-β_2m column shown in Fig. 1.

Separation of serum HDL and colon TSA from CEA

The serum from patients with metastatic colon cancer contained more than 25 ng of CEA/ml and when the serum was fractionated by polyanion precipitation of HDL, CEA was recovered in the supernatant fraction which contained no HDL. Whole serum from patients with metastatic colon cancer blocked the LAI response of reactive leukocytes from patients with limited colon cancer, whereas serum from patients with metastatic breast cancer did not block the LAI response of leukocytes from patients with colon cancer (Table 3). Conversely, the LAI activity of leukocytes from breast cancer patients was blocked by serum from patients with metastatic breast cancer but not metastatic colon cancer. After polyanion precipitation of HDL from serum, the supernatant fraction of serum from patients with metastatic colon cancer and breast cancer lacked blocking activity although this fraction contained CEA. The HDL fraction of serum, free of CEA, from patients with metastatic colon cancer negated the LAI response of leukocytes from patients with limited colon cancer. Nullification of LAI activity was not the result of a non-specific factor in the HDL fraction of serum since HDL prepared from serum of patients with metastatic breast cancer did not interfere with the LAI activity of leukocytes from colon cancer patients (Table 3). Moreover, when the HDL fraction of serum from patients with metastatic colon or breast cancer was preincubated with LAI$^+$ leukocytes from patients with limited breast cancer, only the HDL fraction of serum from patients with metastatic breast cancer nullified the LAI response.

Urinary colon TSA and CEA

A portion of the soluble human tumour antigen in the circulation is cleared by glomerular filtration [13]. Urinary protein isolated from patients with metastatic colon cancer specifically blocked LAI activity of reactive leukocytes from patients with colon cancer (Table 4). The blocking was mediated by the colon tumour antigen since similarly isolated urinary protein from patients with metastatic breast cancer had no effect on the LAI reactivity of leukocytes from colon cancer patients. LAI reactivity of leukocytes from breast cancer patients was blocked by the tumour antigen present in the isolated urinary protein from patients with metastatic breast cancer but not from metastatic colon cancer. The isolated urinary protein that contained the colon tumour antigen also contained CEA and β_2m.

DISCUSSION

The results of the present study indicate that the epitopes of the colon cancer antigen and CEA are on separate molecules. The putative colon cancer antigen detected by blocking LAI is an organ-type specific neoantigen. On the other hand, CEA is a tumour associated antigen derived from cancers arising in all digestive tract organs, and the recognition of CEA is dependent upon immune serum prepared in xenogeneic animals by immunization with isolates from colon cancer [16–18].

The papain-soluble colon tumour antigen bound specifically to an affinity column of anti-human-β_2-microglobulin, whereas CEA did not bind. The papain-soluble colon tumour antigen was purified about 25 fold by this procedure. High resolution SDS–PAGE of the isolated material with colon tumour antigen activity revealed bands, at about 12,000, 25,000, 40,000, 50,000 and 65,000 mol. wt. The bound and eluted fraction contained β_2m while the unbound fraction had no β_2m.

The papain-soluble colon tumour antigen did not bind to an affinity column of antisera (anti-non-β_2m affinity column) directed to cell surface proteins that had failed to bind to the anti-human-β_2m affinity column. With this affinity column, CEA was recovered in both the unbound and bound fractions. β_2m was recovered in the unbound fraction. Interestingly, the SDS-PAGE pattern of the unbound material resembled the material bound and eluted from the anti-human β_2m affinity column. The results of the present study show that the papain-soluble colon tumour antigen co-isolates with β_2m.

Initially, the HLA large component and no other membrane components were reported to be bound to β_2m [31, 32]. More recent studies [33] indicate that β_2-microglobulin does bind certain membrane components that are the same in molecular size as the HLA large components but are different antigenically from the HLA large components. The

Table 3. Isolation by polyanion precipitation of colon tumour antigen and CEA from serum

Preincubation treatment of donor leukocytes	Donor diagnosis	Leukocyte NAI* before incubation	Total protein conc. (mg/l)	NAI* after blocking	CEA† (ng)	β_2m‡ (ng)
Whole serum:						
Metastatic breast cancer	Colon cancer A	59	18	53	4	
Metastatic colon cancer			18	−10	>25	
			4.0	6		
			1.8	−28		
			0.8	25		
			0.4	48		
High density lipoprotein fraction of:						
Metastatic colon cancer serum	Colon cancer B	41	0.1	13	0	37
Metastatic breast cancer serum			0.1	75	0	
Supernatant of:						
Metastatic colon cancer serum			18	81	>25	17
Metastatic breast cancer serum			18	46	2.5	
High density lipoprotein fraction of:						
Metastatic colon cancer serum	Malignant melanoma	53	0.1	43	>25	
Metastatic breast cancer serum			0.1	40	2.5	
Metastatic colon cancer serum	Breast cancer	40	0.1	51	>25	
Metastatic breast cancer serum			0.1	−3	2.5	

*The leukocytes from the patients with colon cancer were tested against extracts of colon cancer (specific antigen) and lung cancer (non-specific antigen). The leukocytes from the patients with breast cancer were tested against extracts of breast cancer (specific antigen) and melanoma (non-specific antigen) and the specific and non-specific antigens were reversed for the melanoma patients. The NAI is the difference in non-adherence to the two extracts and a value of 30 or ≧ is positive and <30 is negative.

†ng of CEA/100 μl of whole serum, supernatant and HLD; with the supernatant and HDL fractions equal to the original serum volumes.

‡ng of β_2m measured by radioimmunoassay and expressed per 100 μg of protein in the sample as estimated by the method of Lowry *et al.* [23].

Table 4. Isolation of colon tumour antigen and CEA from urinary protein

Preincubation treatment of donor leukocytes	Donor diagnosis	Leukocyte NAI* before incubation	Total protein conc. (mg/l)	NAI* after blocking	CEA† (ng)	β_2m‡ (ng)
Urinary proteins unbound from Blue-Sepharose CL-6B:						
Colon cancer A	Colon cancer	52	50	9	>25	8
B			50	−24		13
C			50	8		
Breast cancer A			50	77	<2.5	
B			50	62		
Colon cancer A	Breast cancer A	69	50	75	>25	8
B			50	83		13
Breast cancer A	B	47	50	4	<2.5	

*The leukocytes from the patients with colon cancer were tested against extracts of colon and lung cancer, the specific and non-specific antigens, respectively, whereas the leukocytes from patients with breast cancer were tested against the extracts of breast cancer and malignant melanoma, the specific and non-specific antigens, respectively. The NAI represents the difference in reactivity to the two tumour extracts and a value of ≥ 30 is positive and <30 is negative.
†ng of CEA/100 μl of the isolated and concentrated urinary proteins.
‡ng/100 μg of protein.

T/a antigen and Qa-2 antigens in the mouse are reported to be linked to β_2m. In addition, the male-specific antigen (H-Y) is associated with β_2m although the H-Y antigen is coded for by the Y chromosome [34]. Hence, β_2m may play a regulatory role in the expression of a series of membrane antigens during development and differentiation.

HLA antigens are integral membrane proteins and when HLA antigens are shed into the circulation they retain their lipoprotein composition and co-isolate with the HDL of serum [35]. Human organ-specific neoantigens are integral membrane proteins and also retain their lipoprotein structure when they are shed from the tumour cell surface into the circulation. The colon tumour antigen was precipitated from the serum along with HDL by polyanions and purified about 8 fold. CEA, in the serum of the patient with metastatic colon cancer, was not precipitated by the polyanions and was recovered in the supernatant fraction. Hence, when the colon tumour antigen and CEA epitopes are in the circulation, they are on separate molecules. Both colon tumour antigen and CEA are eliminated, in part, by filtration in the kidney. The procedures used for isolation of the urinary protein did not separate the colon tumour antigen and CEA molecules.

The putative organ-specific tumour antigens of human cancer appear to have many properties in common [11, 13, 14] which suggest that they arise from similar molecules that have some unexplained relationship to organ-definition.

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