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Studies of the Association of the A, B and Lewis Blood Group Antigens with Carcinoembryonic Antigen (CEA)

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Summary. Carcinoembryonic antigen (CEA) was purified from primary tumour or from hepatic metastases obtained from ten cases of carcinoma of the colon. In nine cases the blood group antigens A, B, Le^a or Le^b were detected in CEA preparations by the binding of ^{125}I-labelled CEA by blood group antibodies. The extent of binding appeared to preclude simple contamination of CEA preparations by blood group glycoprotein. In all cases the blood group antigens detected were consistent with the patients' known blood groups. Blood group I and i activities were not detected.

It is concluded that the determinants of A, B and Lewis antigens and of CEA share the same glycoprotein carrier molecules.

INTRODUCTION

Carcinoembryonic antigen (CEA) was described by Gold and Freedman (1965a, b) as a tumour-specific antigen present in adenocarcinomas of the colon and rectum and in digestive organs of the foetus but absent from normal adult colonic mucosa. CEA may also be detected in the plasma of patients with malignant large bowel tumours by means of a radioimmunoassay (Thomson, Krupey, Freedman and Gold, 1969). The specificity of the assay for the diagnosis of carcinoma of the large bowel has not been confirmed by further studies. CEA has been detected in the plasma of cases of carcinoma of other organs and, in lower concentrations, of some non-malignant diseases (Lo Gerfo, Krupey and Hansen, 1971; Moore, Kupchik, Marcon and Zamchek, 1971). It has proved possible to extract immunologically identical CEA from non-digestive carcinomas and from non-cancerous colon mucosa although the yield of CEA from these tissues has been considerably less than from colonic cancer. (Martin and Martin, 1970; Burtin, Martin, Sabine and von Kleist, 1972; Pusztaszeri and Mach, 1973; Sizaret and Martin, 1973).

Carcinoembryonic antigen has been characterized as a glycoprotein soluble in 1 M perchloric acid, having a molecular weight of about 200,000 Daltons and comprising some 40–75 per cent carbohydrate (Krupey, Gold and Freedman, 1968; Terry, Henkart, Coligan and Todd, 1972). Evidence has been provided that the antigenic determinants reside in the carbohydrate moiety (Banjo, Gold, Freedman and Krupey, 1972).

At least two additional antigenic determinants have been described in purified preparations of CEA glycoprotein. One is a determinant common to CEA glycoprotein and to a glycoprotein extracted from several normal tissues (Mach and Pusztaszeri, 1972;

von Kleist, Chavanel and Burtin, 1972). The other determinant is the blood group antigen A which has been described as a contaminant of purified CEA preparations by Lo Gerfo, Herter and Bennett (1972) but as an antigenic determinant sharing the same glycoprotein carrier molecule as the CEA determinants by Turner, Olivares, Harwell and Kleinmann (1972) and by Gold, Freedman and Gold (1972). Simmons and Perlmann (1973) have also detected incompletely synthesized blood group antigens in partially purified CEA preparations.

Several blood group antigens, including those of the ABH, Lewis and Ii systems, are known to occur as high molecular weight glycoproteins in the tissues and secretions. The antigenic determinants reside in the terminal sugars of the oligosaccharide side chains and it is characteristic that blood group active glycoproteins may bear several different antigenic determinants on the same molecules (Watkins, 1972).

The purpose of this investigation was, therefore, to determine which of these blood group antigens could be detected in purified CEA preparations and to determine if the blood group antigens were integral parts of the CEA molecule.

MATERIALS AND METHODS

Carcinoembryonic antigen

Twelve CEA preparations were extracted from primary tumour or from hepatic metastases of large bowel carcinoma obtained from ten different patients. One CEA preparation was provided by Dr P. Gold (CEA-Montreal), four were provided by Dr F. Martin, Dijon (CEA-III, -IV, -V and -VI) and eight other CEA preparations were purified in our laboratory. The methods of purification and of subsequent iodination have been previously described (Thomson *et al.*, 1969; Martin and Martin, 1970; Krupey, Wilson, Freedman and Gold, 1972; Pusztaszeri and Mach, 1973).

Lewis^a glycoprotein

Le^a glycoprotein of ovarian cyst origin was obtained from Professor W. M. Watkins.

Antisera

Anti-CEA was raised in goats and absorbed as previously described (Mach and Pusztaszeri, 1972; Pusztaszeri, Mach and Dysli, 1972).

Blood group antisera. Anti-A, anti-B, anti-Le^a and anti-Le^b were obtained from healthy human donors who had been recently stimulated by pregnancy or by the intramuscular injection of blood group glycoprotein. Anti-Le^b was further concentrated by euglobulin precipitation with 0·002 M PO_4, pH 6·9, and by chromatography on Sephadex G-200. Anti-I was obtained from a patient with Cold Haemagglutinin Disease and two examples of anti-i were obtained from patients with infectious mononucleosis. The blood groups of the donors, the titres of the antibodies and the serological techniques employed are indicated in Table 1.

Absorption of blood group antisera by red cells. Aliquots of antisera were absorbed by incubation for 20 minutes at 20° with not less than one-fifth of their volume of washed packed red cells, the process being repeated five to ten times. In other experiments, Lewis antibodies were also absorbed by the addition of 3 volumes of serum from donors of known Lewis groups or by the addition of increasing concentrations of Le^a glycoprotein.

Elution of antisera. Antibodies were eluted from red cells into a medium of 3 per cent

bovine serum albumin in 1 per cent saline by the method of Landsteiner and Miller (1925).

Fractionation of antisera. Sera were fractionated by chromatography on Sephadex G-200 or on DEAE–cellulose (DE-52, Whatman) using a gradient from 0·02 M to 0·5 M Tris–HCl, pH 8·0.

Serological techniques. Standard techniques were used (Mollison, 1972).

Detection of blood group antigens on CEA

A modification of the Farr technique was used to investigate the ability of blood group antibodies to bind ^{125}I-labelled CEA. Antibody dilutions were made in a medium of 1 mg/ml bovine serum albumin in 0·15 M PO_4, pH 7·4. Iodinated CEA was diluted in the same buffer to give 10–30,000 cpm per 0·5 ml. Equal volumes (0·5 ml) of antibody dilution and of ^{125}I-labelled CEA dilution were mixed and incubated for 2 hours at 37°. One milligram of bovine gamma globulin (Merck) in 0·1 ml was then added and this was immediately followed by the addition of 1 ml of saturated ammonium sulphate. The mixture was incubated at 4° for 30 minutes and centrifuged at 4° for 30 minutes at 1200 *g*. The supernatant was removed and the precipitate kept for counting. The non-specific binding of each ^{125}I-labelled CEA preparation was determined by the addition of normal group A or AB Le(a-b+) serum in the same dilution as that of the antibody. The non-specific binding amounted to 8–20 per cent of the total radioactivity added. The cold reacting antibodies anti-I and anti-i were incubated with ^{125}I-labelled CEA for 18 hours at 4° prior to the addition of saturated ammonium sulphate.

RESULTS

BINDING OF ^{125}I-LABELLED CEA BY GOAT ANTI-CEA

Immediately upon iodination, the purity of the CEA preparations, as judged by the percentage of counts that was specifically bound by goat anti-CEA antiserum, ranged from 70–90 per cent for nine CEA preparations obtained from hepatic metastases and 51–60 per cent for three CEA preparations obtained from primary carcinomas.

BINDING OF ^{125}I-LABELLED CEA BY BLOOD GROUP ANTIBODIES

The percentage of ^{125}I-labelled CEA that could be bound by blood group antibody was estimated within two weeks of iodination and the percentage of ^{125}I-labelled CEA that could be bound by anti-CEA was determined simultaneously.

ANTI-Le^a

Extensive binding was obtained with only a single iodinated CEA preparation, CEA-38. Two anti-Le^a antibodies Tuf. and Far. bound a maximum of 63 per cent and 74 per cent respectively of the total radioactivity present (Fig. 1). Eighty-eight per cent of CEA-38 was simultaneously bound by anti-CEA antibody. The shape of the binding curve suggested that a higher percentage of CEA-38 would have been bound by a more potent anti-Le^a antibody. Chromatography of the anti-Le^a antisera on Sephadex G-200 revealed approximately equal binding activity in the IgG and IgM fractions.

The Le^a specificity of these reactions was confirmed by absorption experiments. Absorption of serum Tuf. by group O Le (a+b−) red cells partially inhibited the reaction

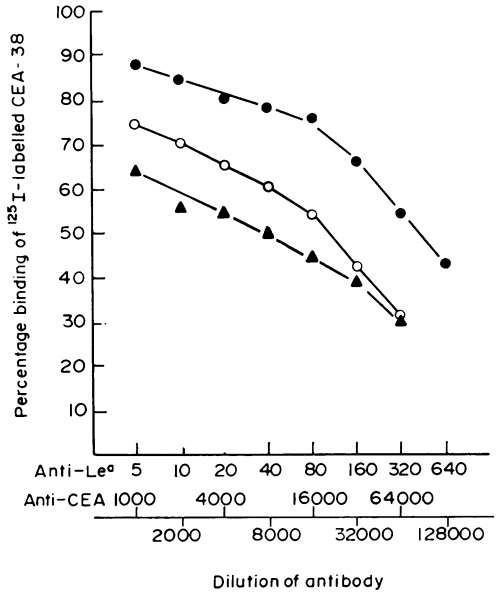

FIG. 1. The percentage binding of ^{125}I-labelled CEA-38 by dilutions of (●) anti-CEA and of two examples of anti-Le^a ((▲)Tuf. and (○)Far.).

but absorption by group 0 Le(a−b−) red cells had no effect (Fig. 2). Antibody activity to CEA-38 could be eluted from Le (a+b−) cells but not from Le(a−b−) cells. Partial inhibition of the activity of the two anti-Le^a antisera toward CEA-38 was also obtained by the addition of group Le(a+b−) serum. Sera from group Le(a−b+) and from Le (a−b−) donors had no inhibitory effect.

Complete inhibition of the reaction between anti-Le^a and ^{125}I-labelled CEA-38 was, however, obtained by the addition of Le^a glycoprotein. The inhibition curves obtained by the addition of increasing concentrations of unlabelled CEA-38 and of Le^a glycoprotein

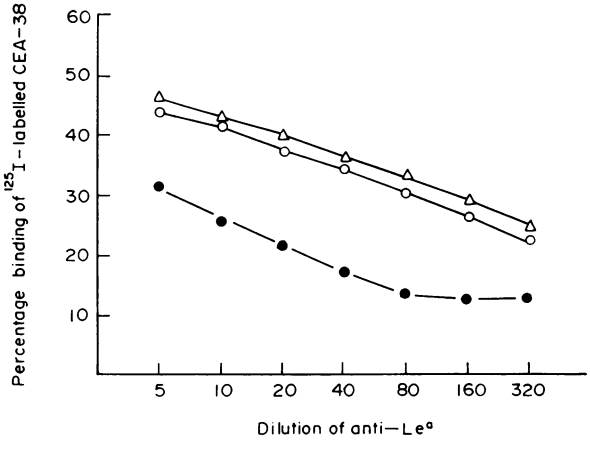

FIG. 2. The percentage binding of ^{125}I-labelled CEA-38 by (△)dilutions of anti-Le^a (Tuf.) unabsorbed and after absorption with (●)Le(a+) or (○)Le(a−) red cells.

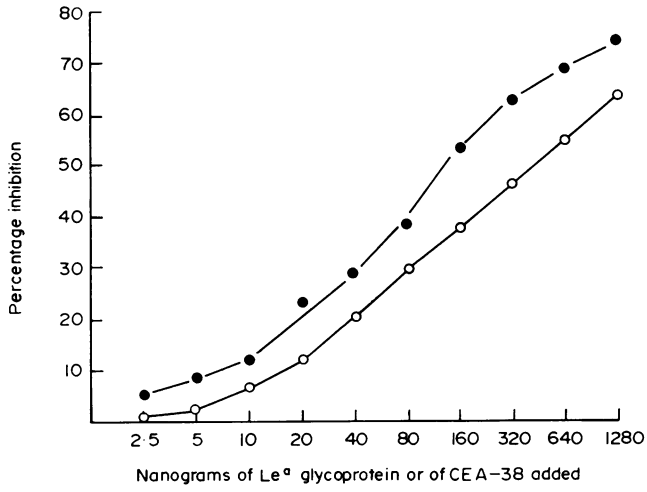

FIG. 3. The percentage inhibition of binding of ^{125}I-labelled CEA-38 by anti-Le^a (Tuf.) produced by the addition of increasing amounts of (○)unlabelled CEA and (●)Le^a glycoprotein.

were not identical (Fig. 3) but on a weight basis the inhibitory effect of Le^a glycoprotein was approximately twice that of CEA glycoprotein. Two other CEA preparations tested in this system gave weaker inhibition and two gave none. This confirmed that the inhibition of the reaction between anti-Le^a and CEA-38 obtained by Le^a glycoprotein was not due to the additional presence of CEA determinants on the Le^a glycoprotein.

BINDING OF ANTI-Le^a TO OTHER ^{125}I-LABELLED CEA PREPARATIONS

Quantitatively less extensive binding was obtained between anti-Le^a and two other ^{125}I-labelled CEA preparations, CEA-IV (41 per cent bound by anti-Le^a and 72 per cent by anti-CEA) and CEA-107 (43 per cent bound by anti-Le^a and 51 per cent by anti-CEA). When anti-Le^a was added to anti-CEA and tested against CEA-107 the per cent bound rose only from 51 per cent to 58 per cent. Of this rise, 5 per cent could be accounted for by the higher non-specific binding obtained by the addition of human antiserum to the assay system. The results therefore indicated that the bulk of the CEA and Le^a determinants present in CEA-107 shared the same carrier molecules.

ANTI-Le^b

Of eight iodinated CEA preparations tested with a crude IgM anti-Le^b concentrate, only one exhibited extensive binding. Seventy-one per cent of CEA-101 was bound by anti-Le^b while 85 per cent was simultaneously bound by anti-CEA. The shape of the antibody dilution curve suggested that a greater percentage would have been bound by a more potent anti-Le^b. Partial inhibition was obtained by absorption of the antibody with Le(a−b+) red cells (Fig. 4). Less extensive binding was also obtained with CEA-109, 52 per cent was bound by anti-Le^b and 45 per cent by anti-CEA. Mixing of the two antibodies did not result in a significant increase in the percentage of binding of CEA-109, suggesting that most Le^b and CEA determinants share the same carrier molecules.

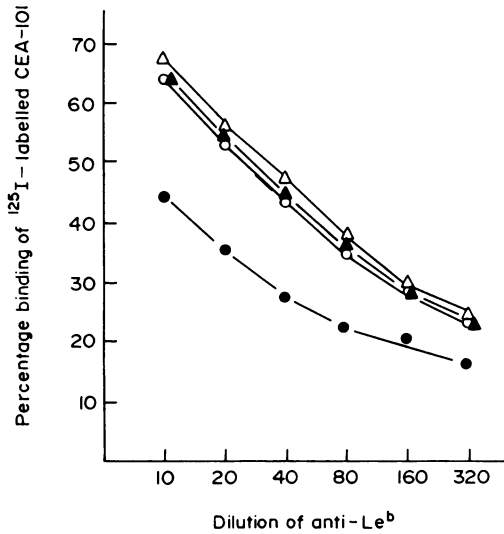

FIG. 4. The percentage binding of ^{125}I-labelled CEA-101 by anti-Le^b (Bar.) (△)unabsorbed and after absorption with group (○)Le(a+b−), (●)(a−b+) or (▲)(a−b−) red cells.

ANTI-A AND ANTI-B

Preliminary results were obtained with a potent anti-A antiserum (Gar.) from a group O donor. This antiserum gave extensive binding of four ^{125}I-labelled CEA preparations from three different patients. In each case, anti-A antiserum and goat anti-CEA antiserum bound CEA to approximately the same extent, amounting to 70–80 per cent of the total radioactivity added. A typical antibody dilution curve is shown in Fig. 5. The anti-A antiserum (Gar.) was exhaustively absorbed with a panel of two group A_1, two A_2, three B and three group O adult red cells of known Rhesus, P, Lewis, MNS, Duffy and Kell blood groups. Three other blood group antisera were used; an anti-A from a group B

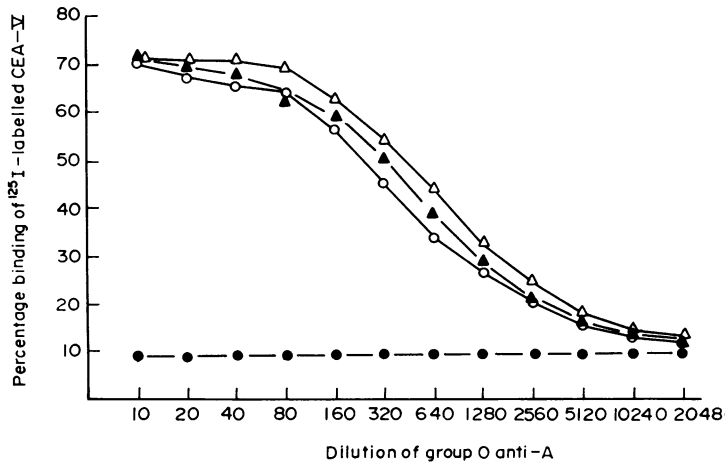

FIG. 5. The percentage binding of ^{125}I-labelled CEA-V by anti-A (Gar.) (△) unabsorbed and after absorption with group (●) A, (○) B or (▲) O red cells.

donor and two anti-B antisera, one from an A and one from an O donor (Table 1). Two patterns of results emerged.

TABLE 1

THE TITRES OF THE BLOOD GROUP ANTIBODIES AND THE METHODS EMPLOYED FOR THEIR DETECTION

Donor	Blood group of donor of antibody	Specificity of antibody	Serological technique and antibody titres	
			Saline agglutination	Anti-IgG
Gar.	O	Anti-A	2048	4096
		Anti-B	256	512
Bre.	O	Anti-A	512	256
		Anti-B	2048	1024
Rea.	B	Anti-A	n.t.*	64
Zgo.	A	Anti-B	512	256
			Anti-complement globulin	
Tuf.	A	Anti-Le^a	256	
		Anti-Le^b	16	
Far.	AB	Anti-Le^a	256	
		Anti-Le^b	256	
Bar.	AB	Anti-Le^a	0	
		Anti-Le^b	256	
			Saline agglutination	
Ste.	A	Anti-I	25,000 at 20°	
Shi.	B	Anti-i	250 at 4°	
Pet.	A	Anti-i	100 at 4°	

* N.t. = not tested.

CEA-III and CEA-V

These CEA preparations were separately prepared from hepatic metastases of a single patient of blood group A, and the results obtained were identical. Binding by anti-A (Gar.) was totally inhibited by absorption of the serum with either A_1 or A_2 cells but not by cells of group B or O (Table 2). Extensive binding was obtained with another anti-A from a group B donor. No binding was obtained with an anti-B from a group A donor. Weak binding was obtained with a group O anti-B which was specifically inhibited by absorption with A cells and was evidently due to anti-A in this serum. The

TABLE 2

THE PERCENTAGE BINDING OF ^{125}I-LABELLED CEA BY ANTI-CEA AND BY ABSORBED AND UNABSORBED ANTI-A (GAR.)

	Percentage binding of ^{125}I-labelled CEA			
	CEA-III	CEA-V	CEA-101	CEA-Montreal
Anti-CEA	76	71	73	81
Anti-A				
Unabsorbed	77	72	73	79
Absorbed by A cells	12	10	17	16
Absorbed by B cells	76	69	13	11
Absorbed by O cells	78	72	73	76
Non-specific binding	12	10	15	14
Blood group of donor of CEA	A	A	B	Unknown

presence of the A antigen in these two CEA preparations was therefore confirmed. CEA-VI, which was further purified from CEA-V by precipitation with specific anti-CEA followed by extraction with perchloric acid (Martin, personal communication), was also extensively bound by anti-A.

CEA-101 and CEA-Montreal

These CEA preparations were obtained from two different donors. The tumour donor of CEA-101 was blood group B, that of CEA-Montreal was unknown. The binding activities of group O anti-A antiserum (Gar.) against each were totally inhibited by absorption with A_1 and A_2 cells and, surprisingly, also by B cells but not by O cells (Table 2). Extensive binding of each was obtained with two potent anti-B antisera. The binding activities of the anti-B antisera were specifically inhibited by absorption with B cells but not by A_1 or O cells. No binding was obtained by group B anti-A. These results indicated the presence of the B antigen on these two CEA preparations and the absence of the A antigen.

These experiments did not, however, elucidate the specificity of the reaction obtained between the group O anti-A (Gar.) and CEA-101 and CEA-Montreal, which could be abolished by absorption of the antiserum with either A or B cells. Serological investigation of this antiserum failed to reveal a blood group antibody having specificity outside the ABO system and the results obtained by absorption of the antiserum by a panel of cells of known blood groups excluded involvement of other blood group systems. Results obtained with serum fractions confirmed that the specificity differed from that observed between anti-A (Gar.) and CEA-III and CEA-V. Binding of CEA-III and CEA-V was found in both IgG and IgM fractions of anti-A (Gar.); significant binding of CEA-101 and CEA-Montreal was, however, found only in the IgG fraction of this antiserum.

CROSS-REACTING ANTI-AB

In addition to anti-A and anti-B, group O antisera contain a population of antibody molecules, which are capable of agglutinating both group A and B red cells, (Landsteiner and Witt, 1926) and which are believed to be directed against that part of the structure of the saccharide chain which is common to the A and B antigens (Owen, 1954; Kabat, 1956). Serological examination of the group O anti-A (Gar.) revealed the classical findings associated with the presence of cross-reacting anti-AB. Absorption of the antiserum by A cells reduced the titre with B cells and eluates from A cells agglutinated B cells and eluates from B cells agglutinated A cells. (Moss, 1910; Landsteiner and Witt, 1926). Eluates of this antiserum from A_1, A_2, B and O cells were also tested for their ability to bind ^{125}I-labelled CEA preparations. Significant binding of CEA-III and CEA-V (containing A antigen) was obtained by eluates from B cells, as well as from A cells, but not from O cells. Significant binding of CEA-101 and CEA-Montreal (containing the B antigen) was obtained by eluates from A_1 and A_2 cells and this binding may also have been due to cross-reacting anti-AB binding to the B antigen.

BINDING OF ANTI-A TO OTHER ^{125}I-LABELLED CEA PREPARATIONS

In addition to the four CEA preparations that were bound as extensively by anti-A (Gar.) as by anti-CEA, three other CEA preparations, CEA-14, CEA-105 and CEA-107,

which were all from group A patients, were partially bound by anti-A (40 per cent, 37 per cent and 29 per cent respectively bound by anti-A as opposed to 70 per cent, 95 per cent and 51 per cent bound by anti-CEA). The A specificity of the binding to CEA-105 and CEA-107 was established by absorption experiments. Antibody dilution curves with CEA-14 and CEA-105 indicated that the binding had reached a plateau. These results suggest that in some CEA preparations only a proportion of the CEA molecules present bear A determinants.

ANTI-I AND ANTI-i

Six ^{125}I-labelled CEA preparations were tested for the presence of these antigens. All were negative.

DISCUSSION

The blood group antigens, A, B, Le^a, or Le^b were detected in nine CEA preparations from ten cases of colonic carcinoma by direct binding of ^{125}I-labelled CEA by blood group antibodies (Table 3). The extent of binding of four preparations of CEA by blood

TABLE 3

THE EXTENT OF BINDING BY BLOOD GROUP ANTIBODIES OF ^{125}I-LABELLED CEA PREPARATIONS OBTAINED FROM THE COLONIC TUMOUR OF TEN DIFFERENT PATIENTS

CEA preparation		Tumour site	Blood group of patient	Percentage of ^{125}I-labelled CEA bound by blood group antibody	
				> 50 per cent	< 50 per cent
Lausanne	CEA- 14	Metastases	A	—	A
	CEA- 38	Metastases	O	Le^a	—
	CEA-101	Metastases	B	B, Le^b	—
	CEA-105	Metastases	A	—	A
	CEA-107	Primary	A	—	Le^a, A
	CEA-108	Primary	O	—	—
	CEA-109	Primary	O	Le^b	—
Dijon	CEA-III and CEA-V	Metastases	A	A	—
	CEA-IV	Metastases	A	—	Le^a
Montreal	CEA-Montreal	Metastases	Unknown	B	—

group antibodies precludes simple contamination of CEA by blood group glycoprotein. In other CEA preparations contamination could not be excluded, but in those preparations in which CEA was partially bound by blood group antibody there was evidence that there may be two populations of CEA molecules, some bearing the CEA determinant alone and others bearing both CEA and blood group determinants. A similar relationship has been demonstrated between the blood group A and Le^a antigens in blood group glycoproteins (Watkins and Morgan, 1959).

In five of the ten cases, either Le^a (three cases) or Le^b (two cases), antigens were detected. The extent of the binding of CEA by anti-Lewis antibodies never amounted to that obtained by anti-CEA but the shape of the antibody dilution curves indicated that antibody excess was not achieved. Complete inhibition of the reaction between anti-Le^a and CEA-38 was not obtained by absorption with Le(a+) red cells or by the addition

of Le(a+) serum. By serological tests with red cells human anti-Le^a appears, with only one known exception, to be solely IgM (Mollison, 1972). However, the present two anti-Le^a antisera exhibited binding of ^{125}I-labelled CEA and also of ^{125}I-Le^a glycoprotein in both their IgG and IgM fractions (unpublished observations). Marcus, Bastani, Rosenfield and Grollman (1967) observed that goat IgG anti-Lewis antibody is poorly absorbed by human Le(a+) or Le(b+) red cell and it may therefore not be possible to inhibit totally the reaction between anti-Lewis antisera and the Lewis determinant in glycoprotein by absorption of antisera with red cells. Complete inhibition of the reaction between the anti-Le^b preparation, an IgM concentrate, and CEA-101 was not achieved by absorption with Le(b+) red cells, but this may have been due to the presence of IgG antibody molecules in the crude IgM concentrate.

Red cells acquire their Lewis antigens by absorption of glycolipid molecules from lipoprotein carrier molecules in the plasma (Sneath and Sneath, 1955; Marcus and Cass, 1969). It was not, however, possible to achieve total inhibition of anti-Le^a antiserum by the addition of serum from a Le(a+) donor, but the experimental conditions restricted the amount of Le(a+) serum that could be added and failure to achieve complete inhibition may have been due to failure to achieve antigen excess.

Complete inhibition of the binding of ^{125}I-labelled CEA-38 by anti-Le^a was obtained by the addition of Le^a glycoprotein. However, blood group glycoproteins are known to carry multiple antigenic determinants (Watkins, 1972). Le^a glycoprotein contains an antigen that cross-reacts with antisera to type-XIV pneumococcal polysaccharide (Watkins, 1966). The complete inhibition of anti-Le^a may therefore have been mediated, at least in part, by the type-XIV antigen.

A or B antigens were detected in CEA preparations from six patients (Table 2). In no case was the blood group activity detected incompatible with the host's blood groups, as has been described for a sulphoglycoprotein extracted from gastric carcinoma (Häkkinen and Virtanen, 1967). Only one CEA preparation obtained from five group A patients had no trace of A antigen.

Gold *et al.* (1972) recently identified the blood group antigen A in a CEA preparation by immunological means. The antigen was termed 'A-like', in view of the failure by three laboratories to demonstrate *N*-acetyl-D-galactosamine, the immuno-dominant sugar of the A determinant, in this CEA preparation. The 'A-like' antigen was identified by the binding of ^{125}I-labelled CEA obtained by an anti-A concentrated from a group O serum by absorption to, and elution from, group A stroma. Cross-reacting anti-AB may, however, elute from A stroma with anti-A and the use of such eluates may then result in misidentification of the B as the A antigen. Yokayama and Fudenberg (1964) showed that cross-reacting anti-AB can either be IgG, IgM and probably also IgA. Our antibody was located only in the IgG fraction and may, therefore, not be typical of anti-AB as detected by agglutination tests.

We obtained supporting evidence for the presence of the A antigen in CEA from carbohydrate analysis. Traces of *N*-acetyl-D-galactosamine were detected in CEA-III, CEA-V and CEA-VI, in which the A antigen was detected immunologically, but not in CEA-101 or in CEA-Montreal, in which only the B antigen was detected (Burkhard, Jaquet, de Rham, Fritsché, Holburn and Mach, 1973).

Our findings are in conflict with those of Simmons and Perlmann (1973), who were unable to demonstrate the presence of A, B or H antigens in tumour extracts by haemagglutination inhibition. The explanation may lie in greater sensitivity of the radio-

immunoassay technique. The presence of only a single blood group antigen site on each CEA molecule may be all that is necessary to ensure detection in the radioimmunoassay. Simmons and Perlmann (1973) were, however, able to detect blood group I and type XIV activity by a precipitation technique, but with one exception, only after subjecting tumour extracts to acid hydrolysis.

There would appear to be two possible explanations for the presence of blood group and CEA antigenic determinants on the same carrier molecules.

First, that there is accidental insertion of blood group antigens into a glycoprotein that does not normally bear them. Blood group antigens are synthesized by the stepwise transfer of sugars from nucleotide-bound sugars to precursor substances under the influence of sugar transferase enzymes (Morgan and Watkins, 1969). The transferase enzymes that initiate and elongate carbohydrate chains are highly substrate specific, requiring not only the correct terminal sugar in the correct anomeric linkage but also the correct penultimate residue (Kobata, Grollman and Ginsberg, 1968; Hearn, Smith and Watkins, 1968). Transferase enzymes are not, however, always unfailing with regard to substrate specificity (Gottschalk, 1969).

The alternative explanation is that CEA is the glycoprotein that normally bears tissue blood group antigens. In this case, one must account for the relative paucity of blood group antigens in most examples of CEA. The A antigen was strongly represented in only one CEA preparation from five group A donors. Le^a and Le^b were strongly represented in only one CEA preparation each, although the Lewis gene has a high incidence in Caucasians (McConnell, 1961). Hakamori and Jeanloz (1970) have demonstrated that there is loss of expression of A and B antigens accompanied by accumulation of Lewis antigens in glycolipid extracts of gastrointestinal adenocarcinomas. Lewis antigens may act as precursors for the synthesis of A and B antigens (Watkins, 1966). The accumulation of Lewis antigens may, therefore, represent an accumulation of incomplete saccharide chains consistent either with an interruption of biosynthesis (perhaps due to deranged transferase enzyme synthesis) or with enhanced glycosidase activity. We were unable to demonstrate an accumulation of Lewis antigens in CEA preparations but the major site of biosynthetic arrest could be at an earlier stage.

Simmons and Perlmann (1973) proposed that CEA determinants appear in blood group glycoproteins as a result of the same derangement in carbohydrate synthesis that results in the disappearance of A and B antigens; namely, incomplete synthesis of saccharide chains and accumulation of shortened chains. At the terminal of these short chains, antigenic determinants, which are normally buried in the adult by the addition of further sugars, are revealed. This hypothesis would imply that there are many similarities between CEA and blood group glycoproteins. Carbohydrate analysis of CEA reveals, however, the presence of moderate amounts of mannose, which is absent from blood group glycoproteins (Banjo *et al.*, 1972). In addition, *N*-acetyl-D-galactosamine is absent in most examples of CEA (Banjo *et al.*, 1972; Mach and Pusztaszeri, 1972; Burkhard *et al.*, 1973). This suggests that the *O*-glycosidic linkage between *N*-acetylgalactosamine and serine or threonine which is present in blood group glycoproteins (Watkins, 1970) is uncommon in CEA.

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