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Progress report

Carcinoembryonic antigen (CEA)

The development of useful blood tests for cancer is of such clinical importance that it is hardly surprising that the finding of an apparently colonic cancer-specific antigen[1] and associated serum antibodies[2] resulted in an explosion of scientific effort. Unfortunately, the claim of specificity of both antigen and test has subsequently been seriously challenged and it seems appropriate to assess current information relating to this antigen, methods of preparation and assay, and results obtained by separate groups in patients suffering from a wide range of clinical disorders.

Carcinoma of the colon was initially chosen for these studies because of its localized character and non-invasiveness and also because of the large amount of normal colon simultaneously available at the time of surgical resection. This allowed examination of normal tissue antigens, and such absorption of antisera apparently produced a single immunologically tumour-specific antibody, confusion over tissue isoantigens being avoided.[3] The antigen was also found, though at lower concentrations, in other carcinomata of the digestive tract as well as in intestine, liver, and pancreas of the normal foetus, if taken within the first two trimesters of pregnancy. This encouraged Gold and Freeman to apply the term 'carcinoembryonic antigen' (CEA) to this material, although the broader and more loosely defined term, 'tumour-associated antigen' has recently gained much favour[4].

The first clinical results on patients, shown also from Gold's group claimed a remarkable specificity for the presence of CEA in the serum of patients with carcinoma of the colon.[5] They have further claimed to demonstrate specific antibody to CEA in patients with non-metastatic cancer of the large bowel and in pregnant women but in no others.[6] Unfortunately the goat which produced this highly specific antiserum has since died, and no animal either in Gold's or in any other laboratory has so far been able to raise such a highly specific antibody.

Immunological Specificity

Early studies involved absorption of crude tumour antisera with red cell antigens, plasma, and a standard amount of normal colonic mucosa. Examined against tumour extracts and their fractions by immunodiffusion, such absorbed antisera demonstrated single precipitin lines which gave reactions of identity between separate tumours.[1] Characterization of the antigen as a glycoprotein enabled preparation of purer material by extraction with perchloric acid,[7] and most recent reports from the Montreal group again demonstrate immunological purity.[8] This experience has not, however, been universal and others have demonstrated heterogeneity in apparently perfectly satisfactory preparations. Von Kleist and Burtin, after perchloric acid extraction and purification of tumour tissue, obtained a double line in the β position, and a third α-globulin crossreacting with foetal tissue.[9] More

recently, Kleinman, Harwell, and Turner also preparing their material by perchloric acid extraction obtained a double line on immunodiffusion of which at least one component gave a reaction of identity with Gold's antigen.[10] Antiserum was also found to break into two fractions (both IgG) by DEAE cellulose column chromatography, each of which gave double lines with their CEA preparations on immunodiffusion. Studies on this question are in progress and are concentrating particularly on the presence of a molecule with multiple antigenic determinants. Mach and Pusztaszeri have recently pointed to the presence of common antigenic determinants (NGP) occurring between tumour and normal tissue[11] and together with Von Kleist and Burtin[12] have postulated such common determinants (NCA) closely linked to others which may be tumour specific (CEA). If true, then removal or blocking of the common determinant might result in the development of a specific antiserum, and resolve some of the apparently contradictory findings on tumour specificity. The development of tumour specific antisera is, however, by no means an inevitable sequal to this search, depending as it does on the existence of a truly tumour-specific antigen, something for which there is little theoretical support. Hansen and his colleagues have described what they call a tumour-associated antigen reacting identically with Gold's CEA, present in a wide range of pathological sera, and always present, though in low concentration, in the sera of normal persons.[13]

Subsequently, perchloric acid extraction of pooled normal plasma produced material immunologically identical to true carcinoembryonic antigen.[14] This view is supported by Martin and Martin who were able to absorb CEA-antisera completely by adding large quantities of normal colonic mucosa.[15] This technique is not sensitive enough to confirm complete absorption, and immunofluorescent studies show the continued presence of anti-CEA activity.[16] Nonetheless, such absorbed antisera will stain not only tumour but also normal mucosa, thus supporting Martin's contention. Hence, although some preparations appear to be tumour specific[8,17] this question must for the moment remain open as also must the similar question of the reason for elevated serum concentrations in disease states. Thus Gold and Freedman postulate derepression of embryonic genes to allow synthesis of a foetal protein not present in normal adult tissues,[3] whereas Hansen claims that the antigen appears in the blood solely because of tissue disruption of the normal mucosal structure, and liberation of cellular proteins into tissue spaces rather than the intestinal lumen.[18] Something of a compromise between these opposing views is offered by Collins and Black[19] who suggest that CEA might be an uncovered surface antigen not normally exposed in the adult tissue—a so-called 'cryptic' site.

Tissue Localization

Immunofluorescent techniques have demonstrated a specific tumour pattern of distribution, strongly suggesting localization of the antigen to the luminal surface of the mucosal cell.[20] The use of ferritin conjugated antiserum and electron microscopy has confirmed these findings and suggested the site to be in the glycocalyx or fuzzy coat actually exterior to the cell membrane.[21] Measurements of CEA activity in tissues by radioimmunoassay[22] indicate higher values in metastases than in the primary lesion (both 0-230 ng/g) and very low concentrations in gastric carcinomata (0·1-3·0 ng/g), normal and

inflamed colonic mucosa (1-8 ng/g), and in foetal gut (2-3 ng/g). No activity could be detected in normal gastric mucosa. The antigen has also been extracted from cirrhotic liver and alcoholic cirrhotic serum[23] although this was apparently undetectable by the Z-gel assay.[13] Direct measurement of CEA in normal tissues by the double antibody test has proved negative,[17] and the use of immunofluorescence has also produced variable answers.[24,25,16]. From the latter method it is interesting to note the demonstration of CEA activity in polyps, ulcerative colitis, and haemorrhoids.[24] These studies may very well be confused by the presence in the tissues of blocking factors[26] which could prevent positive staining. Of equal importance, however, is early clarification of the specificity and cross reactions of CEA, NCA,[12] and NGP,[11] as antigenic presence in a tissue cannot be confidently claimed so long as the possibility exists of cross reactivity with a normal tissue. Carcinoembryonic antigen has also been well described in human faeces[27] and in meconium[28] consistent with immunofluorescent staining of the luminal contents of carcinomatous acini.[20]

Preparation of Carcinoembryonic Antigen

Several methods of preparation have been described, mostly utilizing the solubility of CEA in perchloric acid. The first clinical preparation was described by Gold[29] and involved initial extraction of tumour tissue with perchloric acid and purification of the soluble extract by paper block electrophoresis and chromatography at *p*H 4·5 on Sephadex G200. A preparation was obtained which seemed immunologically pure, showing a single β line on immunoelectrophoresis against an unabsorbed antisera raised to crude tumour extract; there was also a single peak on analytical ultracentrifugation. The method was lengthy and the same group have recently reported a modified method which handles larger quantities of material with greater yield,[8] in which perchlorate extraction is followed by Sepharose 4B chromatography, Sephadex G200 chromatography, and preparative gel electrophoresis on Sephadex G25. Kleinman, Harwell, and Turner[10] extracted CEA from perchlorate extract of tumour by pevikon electrophoresis, Sephadex G200 chromatography at *p*H 4·5, and isoelectric focusing. Their final preparation gave two lines on immunodiffusion against absorbed CEA antiserum, one line showing identity with an antisera to CEA supplied by Gold. Von Kleist and Burtin[9] also extracted CEA from tumour tissue with perchloric acid, and separated by Sephadex G200 chromatography at *p*H 8·2. Although their preparation showed only one β line on immunoelectrophoresis it was not homogeneous when examined in the ultracentrifuge, showing two peaks against a single one with Gold's. It was suggested that the material had undergone polymerization during the process of purification, although it is possible that the high *p*H of the chromatography might have altered the CEA molecule. Gold's chromatographies were performed at *p*H 4·5, the isoelectric point of carcinoembryonic antigen.

Perchlorate is a very strong acid and might alter CEA during its extraction. Recently, a gentler extraction method has been described[30] using lithium di-iodosalicylate, but this preparation, which was homogeneous by acrylamide electrophoresis, was immunologically identical to Gold's.

It should be emphasized that tumour metastases are a more fruitful source of CEA than the primary tumour. Carcinoembryonic antigen has also been

prepared from non-malignant materials[14,23] but its concentration is extremely small.

Properties of Carcinoembryonic Antigen

Initial observations of preparations of CEA show that it is a glycoprotein, with a molecular weight of approximately 200000, and showing a single β line against unabsorbed crude CEA antiserum on immunoelectrophoresis. It is heat resistant up to a boiling time of 40 minutes, has no lipidic nature, and no demonstrable enzymic activity.[9] It is reported to contain a hexose content of 25 %[31] and a protein content ranging from 25 to 50 % has also been observed for various CEA preparations.[32] Sugar analyses of the carbohydrate portion of the molecule have been described, showing that CEA contains fucose which is not present in normal colonic tissue, and various laboratories have claimed that CEA can be characterized by the absence of N-acetyl galactosamine. Varying reports have been given of amino acid analyses[7,32] and sedimentation constant[11,2,4,12] determinations on carcinoembryonic antigen. Sedimentation constant values have ranged from 4·1S to 10·1S[7,8,9,17] and Todd's group[32] reported quite a different amino acid analysis to Gold's,[31] although both agree that aspartic acid, proline, glutamic acid, serine, leucine, and threonine are among the principal amino acid constituents of carcinoembryonic antigen.

Structural investigations have tried to elucidate whether CEA is a distinct molecular species. Todd[32] found that the amino-acid compositions of CEA preparations from five different tumours were very similar, and Gold[7] reported that CEA preparations from a number of different metastatic tumour samples all had single sedimentation constants lying in the range 6·9-8·0S and very similar sugar content, with the exception of sialic acid. Most groups who have prepared CEA[8,9,19,29] have obtained in their preparations at least one component with an S value lying in the range reported by Gold.[7] These findings would suggest that CEA is not just a common antigenic site on a variety of different molecules. However, others have prepared, in addition, CEA materials with S values of 10·1 and 4·1.[9,17] The 4·1S preparation might be explained by polymeric modification, but this could not explain the S value of the other material which differed also in its molecular size by gel filtration and polyacrylamide gel electrophoresis. Furthermore, additional purification of CEA by chromatography or epichlorohydrin triethanolamine cellulose[32] resulted in multiple peaks all containing CEA antigenic activity. Amino acid sequence studies on one of these peaks showed identical sequences with another CEA preparation that had not undergone this additional purification, suggesting that at least the polypeptide chain being sequenced would be a constant constituent of material with CEA activity.

There have recently been reports that CEA is related to blood group A substance. Lo Gerfo[33] considered that some CEA preparations were contaminated with blood group A substance but recently both Turner *et al*[34] and Gold *et al*[35] demonstrated the presence of A and specific CEA determinants on the purified ^{125}I CEA molecule itself, even though other laboratories have been unable to detect the presence in CEA of N-acetylgalactosamine—the key immunoreactive constituent of A substance. Simmons[36] believes CEA is a 'deficient' blood group substance. Working on a partially purified extract of CEA he demonstrated cross reaction between CEA

and blood group substance and found in tumours an inverse relationship between the occurrence of normal blood group substances and analagous tumour-specific glycoproteins. He too, believes that the CEA molecule contains antigenic sites cross-reacting with blood group substance and a CEA specific site.

Measurement of Carcinoembryonic Antigen Levels

Quantitative measurement of the levels of CEA circulating in body fluids is performed by radioimmunoassay—a method based on the principle of co-precipitation-inhibition, capable of detecting quantities of CEA in ng. There are three principal assay systems at present: (1) the Gold assay[5] which uses the Farr technique; (2) Z-gel assay[13]; and (3) double antibody assay.[37] All three require (*a*) purified CEA, extracted usually from metastases of colonic cancer to the liver; (*b*) a sensitive antibody raised to purified CEA (usually by immunization of a goat or rabbit); (*c*) ^{125}I CEA prepared from purified CEA by the chloramine T method. Most people have used these assays to assess CEA levels in the blood although Todd's method of assay has recently been adapted for measurement of urine.[38]

Gold's assay uses 5 ml of serum and takes five days to perform. The serum is extracted with perchloric acid before it is assayed and the antibody-bound CEA is separated from 'free' CEA by ammonium sulphate precipitation. The Z-gel technique assays serum or plasma, extracted with perchloric acid, and it has the advantage of only requiring 1 ml of sample and takes one-and-a-half days for completion. The antibody-bound CEA is separated by zirconyl phosphate gel binding. The double antibody assay precipitates the CEA-antiCEA complex using a second antibody. This requires very careful quantitation of reagents, but uses the least amount of serum (0·2 ml), can detect down to 1 ng, and, unlike the other two methods, does not involve preliminary extraction with perchloric acid. This simplifies the whole procedure and reduces potential error by avoiding transfer of reagents from one container to another during the assay. It also reduces assay time, measurements on serum taking one day for completion. This assay, however, does have a different value for the upper limit of normal to the Z-gel and Gold assays, the cut off for abnormality being set at 12·5 ng/ml compared with 2·5 ng/ml. It is not known what causes this higher upper limit of normal, although it could be some cross-reacting substance or non-specific interference in serum, eg, increased viscosity, both of which would be removed by perchlorate extraction. However, in spite of the considerable technical differences the Todd assay does produce clinical results comparable to the Z-gel assay (see infra). Laurence *et al*[39] have reported that duplicate plasma samples assayed by both the double antibody and Z-gel systems showed qualitative agreement in 72% of cases studied.

It is important if the results of all three assays are to be comparable that the specificities of the different assay systems should be similar. Both the Z-gel and double antibody techniques use CEA prepared essentially by Gold's method,[8] immunochemically indistinguishable from the material used in the Gold assay. Hansen[4,18] believes that the Z-gel assay is detecting a different, ion-sensitive, antigenic site to the one measured in the Gold system. The Z-gel assay is performed in 0·05 M borate buffer which exposes this site, whereas the Gold assay is performed in 0·2 M borate buffer at which

ionic strength the CEA should be coiled up hiding the ion-sensitive site. In spite of this, Kupchik[40] has shown good quantitative agreement between Z-gel and Gold assays performed on the same blood sample.

Mach[11] has raised the question of specificity in the radioimmune assay of carcinoembryonic antigen. He suggests that no antiserum, even those raised to the most purified CEA preparation, is CEA specific before it is absorbed with normal tissues. He demonstrated that CEA had common antigenic determinants with a glycoprotein of smaller size extracted from normal adult tissue.

None of the radioimmunoassay systems in their present form lend themselves easily to routine use. The method required for preparation of CEA and antibody are complex and lengthy and the assay system itself is time consuming and expensive. Modifications of radioimmunoassay, such as a coated tube method, are at present being studied.

Go[41] has reported a modified Hansen assay which has eliminated the steps of preparation of the plasma (extraction and dialysis procedures) and only requires 0·1 ml of plasma. Laurence *et al* have successfully used the Todd assay counting only one isotope—^{125}I—instead of the three isotopes recommended by Todd. Other workers are investigating different methods of assay and automation. Darcy[42] has reported a modified immune diffusion technique using a second antibody which can detect 10 ng of CEA and Lange *et al*[43] have reported preliminary observations on a haemagglutination inhibition test for carcinoembryonic antigen.

Clinical Application

All tests described for the assay of CEA have been applied to considerable numbers of patients' sera and studies are being continually reported. Most reports relate to the radioimmunoassay of Thompson, Krupey, Freedman, and Gold[5] using reagents either supplied directly from the Montreal laboratory, or prepared according to their method. Reports relating to the Z-gel assay developed by Hansen and his colleagues are now appearing,[13,44,45] and the double antibody technique has been applied in Britain with reagents supplied from California.[39,46] Other methods and data exist, but most of the available information centres around these studies (Table I)[5,13,39,44,46,47,48,49]. Positive results are regularly found in patients with carcinoma of the large bowel confirming the earlier observations, but the degree of positivity has diminished, and values of 70 to 80% are more common than the 97% originally reported. There has been an even greater change in the proportion of positive results obtained from non-colonic carcinomata and other disease states, and most authors find as many positive reactors amongst patients with carcinoma of the stomach, pancreas, and liver as with colon and rectum. In addition, non-alimentary carcinomata also appear to be associated with many positive reactions, although not quite to the same degree as bowel lesions. A positive rate of 50% is common for these lesions, with carcinoma of the lung giving rather higher percentages. These differences are not explainable by the method used as very similar results are being currently reported from the three techniques. In a recent report of the combined Canadian study,[49] results are reported from the Montreal laboratory which gives the lowest positive rates of all for endodermal cancer. In non-malignant disease, Moore, Kupchik, Marcon and Zamcheck[47] first

Radioimmunoassay	*Gold (Farr)*				*Z-gel*		*Double Antibody*	
	Thompson *et al* (1969)	Moore *et al* (1971)	Le Bel *et al* (1972)	Joint Canadian (1972)	Lo Gerfo (1971)	Reynoso *et al* (1972)	Laurence *et al* (1972)	Booth *et al* (1972)
Malignant Diseases								
Carcinoma of colon and rectum	97	91	72	64	86	83	69	75
Other gastrointestinal cancer								
Total	9	70	74	60	84	82	63	78
Stomach	—	3/3	—	3/5	73	2/5	2/5	13/19
Pancreas	—	13/13	—	4/7	2/2	3/3	3/3	5/6
Liver	—	0/2	—	3/3	6/6	1/1	1/1	—
Non-gastrointestinal cancer								
Total	0	46	55	51	70	39	44	39
Lung	—	6/8	—	1/1	77	7/10	70	4/12
Breast	—	0/1	—	50	63	—	48	36
Reticuloendothelial	—	0/2	5/7	1/1	4/7	2/20	42	6/15
Non-malignant Diseases								
Gastrointestinal								
Peptic ulcer	—	—	—	—	0/18	—	7/21	1/17
Inflammatory bowel	—	1/8	67	30	30	—	32	9
Cirrhosis	—	45	1/8	48	0/15	—	4/5	16/21
Chronic lung disease	—	—	—	—	11	—	45	3/7
Chronic renal disease	—	7/9	—	—	0/13		—	—
Benign breast lesions	—	—	—	—	—	—	8	0/9
	200	279	393	503	674	346	775	405

Table I *Comparison of the percentages of positive results in different clinical states by separate groups using the currently available methods*

Small numbers of results are given individually, rather than as a percentage.

pointed to positive assays in non-cancerous patients, particularly in those with cirrhosis, gastrointestinal, and renal disease. Other authors have confirmed these observations although there is a wide variation in the proportion of positive results.[13,39,46,48,49] In patients with peptic ulcer, for example, one study has given a 33% positive rate in patients with peptic ulceration,[39] whereas there was only one positive in 39 patients reported from two other groups.[34,46] Similarly, whereas most studies report a substantial number of positive results in cirrhosis[39,46,47,49] there were no positives in 15 tests using the Z-gel assay.[13] A possible explanation for this variability lies in the relationship to alcohol intake, and in Bostonian patients positive rates in alcoholic and non-alcoholic cirrhotics were shown to be 45% and 0% respectively.[50] In view of the known differences in the contribution of alcoholism to chronic liver disease in different countries, this question should also be examined elsewhere.

Positive results appearing in patients with inflammatory bowel disease are particularly important for two major reasons. First, these conditions represent the principal differential diagnoses for carcinoma of the colon and positive results are a major limitation in the value of the test. Secondly, ulcerative colitis has been clearly demonstrated as a precancerous lesion,[51] and it is extremely important to determine whether or not positive reactors have already developed cancer or early precancerous changes.[52] The recent demonstration of CEA by immunofluorescence in the tissues of patients with ulcerative colitis and haemorrhoids[24] is extremely important and requires confirmation and further examination. There is considerable variation in the degree of positivity reported for these patients, and although the most usual rate is 30%[13,39,49,53] a single rate of 67% exists,[48] together with three others of approximately 10%.[46,47,54] The reasons for these differences will no doubt

be carefully examined. Other important measurements in non-malignant conditions include 11 and 45% positive rates for chronic lung disease[13,39] and 0% and 8% for benign breast disease.[39,46] A figure of 42% for chronic pancreatic disease[55] appeared, as in cirrhosis, to be largely related to alcohol intake.

A recent report of CEA activity in urine related to bladder carcinoma,[38] and in amniotic fluid to meconium and foetal distress,[28] open up important new applications of the test.

Return to normal values of elevated CEA concentrations after successful resection of carcinomata reported initially by Gold's group[5] has been amply confirmed and the change correlated with the known clinical result. A fall in concentration to normality results from total resection with no change after unsuccessful surgery.[56] In those where the surgical result was in doubt, subsequent clinical progress correlated very closely with the degree of fall in CEA levels occurring postoperatively. A further variation in this pattern has also been described[57] where the rate of fall was related to initial concentration. If the preoperative value was between 3 and 15 ng/ml (Z-gel assay) return to normal took about three days. With higher concentrations, however (20-35 ng/ml), the fall was much slower to take effect, and did not commence before six days, being usually complete by 16 days. These authors suggest that the persistently positive results may be due to small pockets of residual tumour, and further that the delayed fall may represent immunological rejection by circulating antibodies. A much earlier rise in antibody concentration has, however, been demonstrated[58] and if confirmed, this suggestion would become less likely.

The relationship between rates of positivity and the type of tumour has recently excited much interest, but the degree of differentiation of colonic, breast, or bronchial tumours appears to have no bearing on the finding of a positive serum result.[39] But the spread of the lesion is quite closely correlated with positivity for serum CEA, and most authors have studied Duke's classification (Table II).[39,44,49,56,59] Results compounded from these studies give positive rates for serum CEA of 40%—Dukes A, 65%—Dukes B, 75%—Dukes C, and 90%—Dukes D. Thus, approximately half of the patients with operable lesions and good clinical results are CEA positive, whereas the rate is almost 100% in those with distant metastases.

Whilst the presence of CEA-positive serum in some groups of patients not suffering from malignant disease considerably reduces the test's clinical value, concentrations in these sera are generally lower than in those with a car-

Author	*Duke's Classification*			
	A	*B*	*C*	*D*
Lo Garfo *et al* (1972)	7/19	14/23	21/28	46/54
Kleinman and Turner (1962)	8/10	5/8	12/12	13/13
Dhar, Moore, Zamchek, and Kupchik (1972)	3/16	9/17		18/18
Joint Canadian Study—Montreal Measurements (1972)	2/11	28/47	13/20	34/43
Laurence *et al* (1972)	13/29	22/29	6/10	20/20
Totals	33/85	69/107	52/70	131/148
Percentage (rounded to nearest 5%)	40	65	75	90

Table II *Positive CEA results related to Duke's classification*

cinoma.[13,39,46,49] Studies using the Z-gel assay first illustrated this point, and whereas standard positive rates for ulcerative colitis and carcinoma of the colon were 30% and 86% respectively, these values became 0% and 36% if a higher cut-off was used. The recent Canadian report[49] also shows major differences in concentration between the separate groups, but without a higher level which might be diagnostically useful. The double-antibody technique is proving somewhat more promising in this regard, and a second level of 40 ng/ml appears to limit virtually all results from non-malignant sera. Even with the limitation of this higher level approximately 40% of patients with colonic cancer are CEA positive and 26% of those classified as Dukes A or B.[39] Although less than ideal, and clearly inapplicable to population screening, this information could be of considerable value to the clinician with a difficult diagnostic problem.

Other factors examined for an effect on CEA concentration include age, blood group, and smoking. Although their data are limited, the Canadian study claimed no relationship to age in carcinoma patients, though values tended to be higher in older than in younger patients suffering from non-malignant disease.[49] It is, of course, probable that the incidence of occult neoplasia increases with age and more information is necessary. No correlation with blood group was found in 175 patients. Laurence and his colleagues[39] recently showed that of 14 patients with pulmonary infections who were CEA-positive, 10 smoked cigarettes, whereas positive results did not occur in otherwise normal smokers. They concluded that it may be the combination of chest infection and smoking which is primarily responsible for the raised values in these patients.

Immunity to Carcinoembryonic Antigen

The possible existence of a defence reaction of a cancer patient against his own malignant tumour is of great clinical interest, since it would offer possibilities of immunotherapy for the patient and a new approach to the diagnosis of cancer. Several attempts have therefore been made to demonstrate the autoantigenicity of CEA in patients with cancers of the digestive tract. Gold,[6] using a semi-purified preparation of CEA and a modified bis-diazotized benzidine (BDB) haemagglutination procedure, tested 212 human sera for antibody to carcinoembryonic antigen. Seventy per cent of patients with non-metastatic cancer of the digestive system were found to be positive, together with the majority of pregnant and postpartum women studied, although this percentage fell sharply in the third trimester. All patients with metastatic dissemination and all other patients and normal healthy controls were negative. Gold concluded that patients suffering from cancer of the digestive system are capable of producing antibodies to CEA, and pregnant women can develop an antibody response to CEA transferred via the placenta from the foetal digestive system in the first two trimesters of development. The presence of metastases led to removal of circulating antibodies from the system. Patients with non-metastatic cancer, whose results were negative, became positive after removal of the tumour mass.

Collatz *et al*[60] were unable to confirm Gold's results. They examined the sera of 190 patients by immunoadsorption, and immunofluorescence and passive haemagglutination techniques. All their results using immunoadsorption and immunofluorescence were negative. They were unable to

make the bis-diazotized benzene passive haemagglutination technique used by Gold work for technical reasons. Using tannic acid and glutaraldehyde as coupling agents between colon tumour extracts and sheep red cells, they obtained positive results in sera of colonic cancer patients, two control groups of healthy subjects and patients with non-cancerous conditions, but they then demonstrated that this activity was due to the presence of antibodies directed against normal tissue proteins in perchlorate extracts of tumours. This could also explain Gold's results because he too used crude perchlorate extract as antigenic material and had therefore not clearly demonstrated the specificity of his antibody.

Lo Gerfo *et al*[61] were also unable to detect circulating antibodies to carcinoembryonic antigen. They examined 265 sera by radioimmunoassay—a more sensitive technique than those used by either of the previous workers. The sera of 110 patients with non-metastatic colonic cancer, of 122 with other gastrointestinal, breast, and pulmonary neoplasms, of 20 pregnant women, and of 13 healthy volunteers were all negative. Twenty-five sera were also examined by Sepharose 6B column chromatography after the addition of ^{125}I CEA for antibodies, but these were also negative. Lo Gerfo, like Collatz, suggested that Gold might have been detecting antibody activity against normal tissue antigens or alternatively blood group antibodies.

The results of Collatz *et al*[60] and Lo Gerfo *et al*[61] are not unexpected in view of the fact that CEA is present in normal plasma.[14] Gold,[36] however, has recently reported further experiments detecting human CEA antibodies in patients with metastatic cancer, using the technique of radioimmunoelectrophoresis. He comments on the need to examine the problem by several techniques before stating absolute conclusions on the presence or absence of antibodies. His results remain to be confirmed.

The presence of circulating CEA-antibody complexes has not yet been investigated.

Cell-mediated immunity has attracted similar interest and several studies have been reported. As with antibody measurements, results have been variable and positive responses have occurred only with impure materials. Neither skin reactivity[62,63] nor lymphocyte transformation[64] were demonstrable to Gold's pure CEA, although the use of cruder fractions induced clear responses in the same studies.[62,63] Positive results were also obtained with extracts of colonic cancer cells from tissue cultures, though not from mesodermal tumours. In addition, foetal intestinal and liver extracts taken during the first two trimesters also gave positive skin tests.[62] The authors have suggested that humoral immunity might be directed to antigenic determinants in the carbohydrate portion of the CEA molecule, whereas cellular reactions would be expected more to the proteinous part. Damage in preparation of purified CEA might then reduce the size of the proteinous section, thus destroying the haptenic group specifically responsible for the induction of delayed hypersensitivity.

Using a different technique, Hellström and his colleagues have also demontrated cell-mediated immunity to tumour extracts with the same type of carcinoembryonic specificity.[65,66] Culturing tumour cells from patients with cancer of the colon, breast, and lung, Wilm's tumour, and neuroblastoma, they demonstrated inhibition of colony formation when using lymphocytes, either autologous or allogeneic, but taken from patients with the same tumour type.[65] Positive reactions were not obtained from lymphocytes taken from

normal persons or from those with tumours of breast, lung, or kidney. Similar inhibition of growth occurred in colonies of foetal gut and liver cells but not foetal kidney, thus supporting the carcinoembryonic nature of the haptenic group.[66]

Cell-mediated immunity is thus demonstrable to tumour extracts in patients with carcinoma of the digestive tract, and does not disappear after removal of the tumour.[67] In answer to the important question, 'Why, therefore, is the tumour not destroyed', Hellström has recently undertaken some extremely interesting studies on the presence of blocking substances. Serum from cancer patients was found to inhibit the phenomenon of colony inhibition[68] and more recently an inhibitory substance has been eluted at acid *p*H from human tumour tissues, though not so far from colonic carcinoma.[69] By further analysis and separation, it was possible to demonstrate two fractions, one of molecular weight greater than 100 000, the other of less. Sjögren and his colleagues showed that this material did contain IgG2 and suggested that the so-called 'blocking factor' might be antigen-antibody complexes. Of very considerable clinical interest, they could not find the serum blocking factor in a patient undergoing spontaneous remission in the face of widespread metastases.

It is important to consider the ability of patients to respond normally to an antigenic stimulus whenever assessing the significance of this type of observation, and diminished cutaneous reactions have been shown to immunization with streptokinase-streptodornase in patients with metastatic malignant disease.[70] Whether this implies a general reduction in reactivity of these patients remains to be determined.

Conclusions

At the present time it is inappropriate to regard CEA as a tumour-specific but rather as a tumour-related antigen, possibly containing both common tissue and tumour-specific antigenic determinants. Recent developments in this area may lead to a clarification of the immunological nature of CEA and hopefully to a more specific test. Work is increasing on chemical composition with particular interest relating to the carbohydrate section of the molecule and possibly to blood group substances.

Very high tumour concentrations are reflected in the plasma, the latter rising with dissemination of malignancy. Positive results are obtained in certain non-malignant clinical conditions, particularly cirrhosis, pancreatitis, inflammatory bowel disease, and cor pulmonale. These values detract from the test's diagnostic usefulness but concentrations are usually lower than in cancerous sera, and it may prove possible to set a second concentration, above which malignancy becomes highly probable. From the clinical standpoint, the significance of a given elevated CEA concentration must be clarified in defined situations and extensive study is necessary of changing levels with time, particularly in apparently non-malignant states. For the moment, the major clinical application appears to lie in the assessment of patients suspected of having carcinoma of the large bowel, stomach, and possibly breast, and in the long term follow up after apparently successful surgery. To this might be added screening for bladder carcinoma in urine samples, and determination of foetal distress in amniotic fluid. The redevelopment of a

truly cancer-specific serum test such as originally described[5] remains one of the most important challenges to the clinical immunologist.

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