Clin. exp. Immunol. (1984) **58**, 685–693.

Circulating immune complexes (CIC), carcinoembryonic antigen (CEA) and CIC containing CEA as markers for colorectal cancer

KERRY A. CHESTER & R. H. J. BEGENT *Cancer Research Campaign Laboratories, Department of Medical Oncology, Charing Cross Hospital, London, UK*

(Accepted for publication 13 July 1984)

SUMMARY

It has been suggested that circulating immune complexes (CIC) would provide a useful tumour marker system and that carcinoembryonic antigen (CEA) may form an antigen component of CIC found in patients with colorectal cancer. In this study the clinical usefulness of CIC and CIC containing CEA (CEA-IC) was investigated. Concentrations of CIC were measured in 30 patients with colorectal cancer. Fourteen patients were studied sequentially at approximately 1 month intervals after apparent curative resection of the primary tumour. Results were correlated with those obtained from serum CEA and compared to clinical status. CEA-IC were measured using a novel assay and compared with CIC and CEA values in 29 patients. CIC concentrations were elevated in patients with known disease and predicted clinical relapse in four of 14 patients. In two patients CIC remained elevated despite sustained remission. CEA-IC were not detectable in any of the patients studied. CIC estimations may augment CEA measurements as indicators of disease recurrence but lack of specificity makes them of little practical value as tumour markers in colorectal cancer. No evidence was found to support previous reports that CEA was an antigen component of CIC in this disease.

Keywords circulating immune complexes carcinoembryonic antigen colorectal cancer

INTRODUCTION

The frequent occurrence of elevated concentrations of circulating immune complexes (CIC) in malignant disease has been reviewed by Baldwin & Robins (1980). In many instances this is associated with advanced disease or relapse whilst there is a tendency for levels to be normal in remission (Baldwin & Robins, 1980). CIC concentrations correlate with tumour volume in some instances of both virally- and chemically-induced tumours of experimental animals (Jenette & Feldman, 1977; Jenette, 1980) and there is evidence that, in some cases, CIC contain a tumour product as an antigen component (for review see Baldwin & Robins, 1980). These observations have led to the suggestion that CIC may provide a useful tumour marker system.

Raised CIC values have been detected in patients with colorectal cancer (Rossen *et al.*, 1977; Vellacott *et al.*, 1981; Steele *et al.*, 1978, 1983). However, the relationship between tumour burden and CIC concentration is not clear. This is probably partly due to the non-specificity of currently available assays which depend on properties of the complexed antibody and it is possible that

Correspondence: Kerry A. Chester, Cancer Research Campaign Laboratories, Department of Medical Oncology, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK.

antigen directed CIC assays would provide a more specific and statisfactory means of tumour assessment.

There is evidence that carcinoembryonic antigen (CEA) described by Gold & Freedman (1965), may form the antigen component of CIC in some patients (Costanza *et al.*, 1973; Harvey *et al.*, 1978; Kapsopoulou-Dominos & Anderer, 1979a; Mavligit & Stuckey, 1983) and in one study the presence of CEA containing CIC (CEA-IC) appeared to be a useful indicator of clinical status (Staab *et al.*, 1980).

Progressively rising concentrations of CEA can predict clinical recurrence of colorectal cancer (Sugarbaker, Zamcheck & Moore, 1976; Mach *et al.*, 1978; Minton & Martin, 1978; Wanebo, Stearns & Schwatz, 1978; Tate, 1982). However, there are a number of clinical situations in which CEA alone does not provide a suitably sensitive disease indicator (Moertel, Schutt & Go, 1978; Goldenberg *et al.*, 1981).

We have examined the relationship between serum concentrations of CIC, CEA and CEA-IC in patients with colorectal cancer in order to assess the potential of CIC as a tumour marker system which would augment CEA and to investigate the possibility that complexed CEA may provide a specific indicator of clinical status.

MATERIALS AND METHODS

Patients and samples. Serum samples were obtained from 30 patients suffering from colorectal cancer and known to have active disease (age 47–87, median 69 years). Serial samples, taken at approximately monthly intervals, were obtained from 14 of these 30 patients. Control sera were donated by 43 healthy volunteers of both sexes (age 19–68, median 31 years) and 29 patients with rheumatoid arthritis.

Samples for measurement of CIC were snap frozen in liquid nitrogen immediately after separation and stored at −70°C until use; aliquots of the same sample were stored at 4°C for 1–3 days before measurement of CEA.

CIC. These were measured using the polyethylene glycol (PEG) assay described by Begent *et al.* (1982). CIC were precipitated from serum with a final concentration of 2% PEG and quantified as PEG precipitable IgG, estimated by radial immunodiffusion.

CEA. This was measured using a double antibody radioimmunoassay (Lewis & Keep, 1981).

CEA-IC. Eight hundred microlitres of 7% PEG in barbitone-buffered saline containing 60 mM EDTA, pH 7·6, was added to 800 μl serum. After 16 h at 4°C the samples were centrifuged for 20 min at 1,500*g* and 4°C and washed once in 3·5% PEG. The precipitate was dissolved in 80 μl of citrate–phosphate buffer pH 3, in order to dissociate the complexed CEA. CEA was extracted by addition of 80 μl of 2 M perchloric acid (PCA) to the dissolved precipitate. After centrifugation for 1 h at 1,500*g* and 4°C, the extracted CEA was exchanged from the supernatant into 800 μl of phosphate buffer, pH 7·3, containing 0·1% bovine serum albumin, using pre-calibrated columns of Biogel P-30. CEA concentrations in the extracts were assayed as described previously.

Model complexes. CEA was prepared by perchloric acid extraction of liver metastasis from colorectal tumours followed by exclusion chromatography on Sepharose 6B and Sephacryl S-300 (Keep, Leake & Rogers, 1978), antibody to CEA (anti-CEA) was raised in rabbits against a similar preparation. Model complexes were formed at two times antigen excess and two and eight times antibody excess by overnight incubation of the antisera with different quantities of CEA. Insoluble complexes were removed from the preparations by centrifugation for 30 min at 4°C and 1,500*g* after allowing to stand for 24 h at 4°C. Complexes were stored in liquid nitrogen until used or used immediately.

For experiments using radiolabelled CEA-IC a proportion of the CEA was iodinated by a modification of the chloramine-T method (Greenwood, Hunter & Gold, 1983), prior to incubation with the antisera.

Protein A binding of model CEA-IC. One hundred microlitres of sera containing ^{125}I-labelled CEA-IC was applied at 4°C to a column containing 1·5 ml of protein A–Sepharose. Unbound material was removed by washing with phosphate-buffered saline and bound CEA-IC were eluted

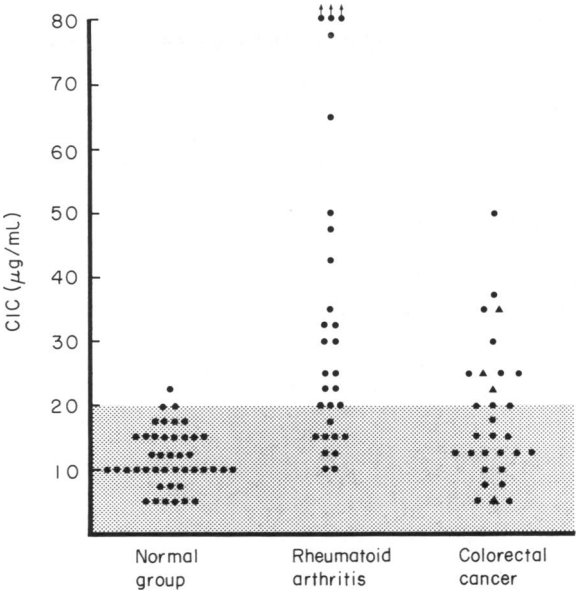

Fig. 1. CIC concentrations measured by the PEG precipitation assay in 26 patients with colorectal cancer known to have active localised (●) or metastatic (▲) disease. Shaded area = normal range.

with phosphate–citrate buffer at pH 3. The relative proportions of free and complexed ^{125}I-labelled CEA were estimated using a LKB Wallac 80,000 gamma sample counter (LKB Produckter AB, Sweden).

RESULTS

CIC and CEA-IC concentrations were measured pre-operatively and sequentially post-operatively in patients who had undergone apparently curative resection of primary carcinoma of the colon or

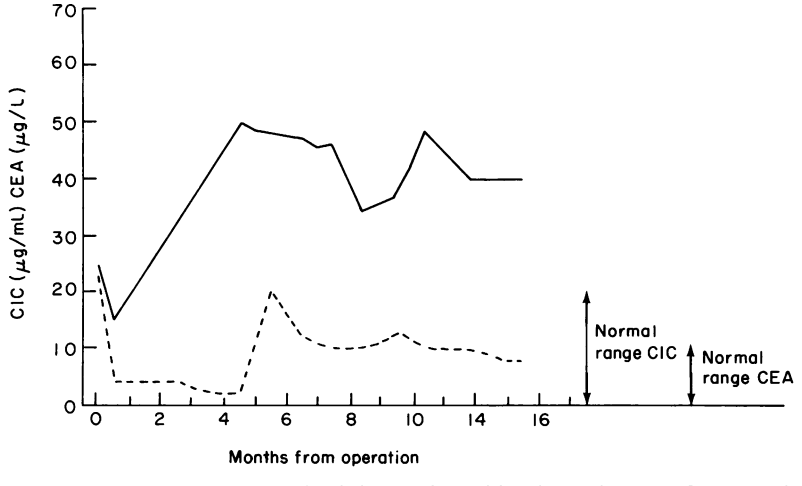

Fig. 2. Serum CIC (——) and CEA (– – –) levels in a patient with colorectal cancer. Concentrations of both markers were raised pre-operatively, post-operatively CIC concentrations increased and remained elevated whilst CEA concentrations stayed below the pre-operative level and generally remained in the normal range. Relapse was detected clinically 5·5 months post-operatively.

Fig. 3. Serum CIC (——) and CEA (– – –) levels in a patient with colorectal cancer. CIC concentrations were consistently elevated and CEA values remained normal. The patient was in remission throughout the study.

rectum. Results were correlated with those obtained from serum CEA and compared with clinical status based on histological grading, clinical examination and conventional radiology including computerized tomography where appropriate.

CIC

Results for 30 patients known to have active disease (26 localised and four metastatic) are shown in comparison with those obtained for the normal and positive control groups (Fig. 1). Values above 20·6 μg IgG/ml of serum (mean + 2 s.d.) were considered abnormal.

Raised values were present in seven of 26 patients pre-operatively. Post-operatively, CIC concentrations were elevated, as defined by two or more consecutively raised values, in six of 14 patients who were studied sequentially; four of these six relapsed clinically (for example see Fig. 2) 3–12 months after the first raised CIC value and in two of these CIC concentrations remained raised until relapse. Relapse was predicted at this point by elevated CEA values in only one of four. Two of six patients remained in clinical remission although their CIC concentrations were constantly elevated (for example see Fig. 3).

The remaining eight of 14 patients did not have elevated values for CIC, of these two patients subsequently relapsed accompanied by elevated CEA concentrations and six remained in remission.

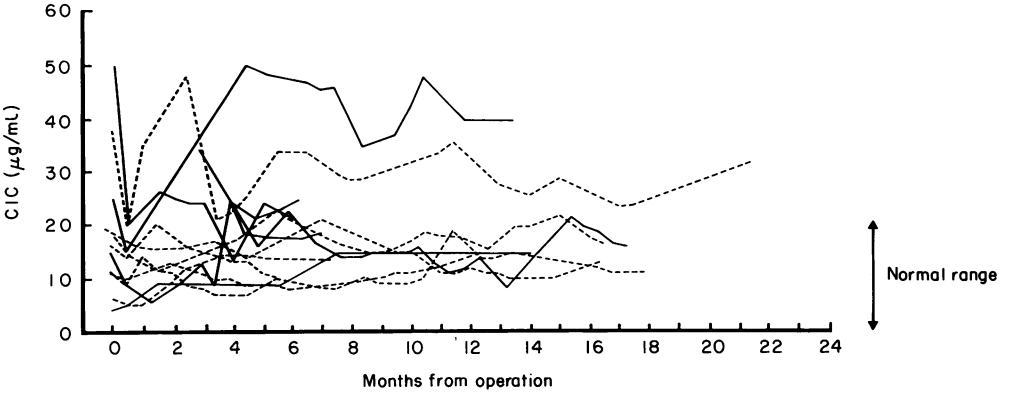

Fig. 4. CIC values obtained sequentially on 13 patients, six of 13 relapsed (——) and seven of 13 remained in remission (– – –). A further patient studied cannot be shown as the surgical procedure took place 11 years prior to this study. This patient remained in remission with consistently elevated CIC concentrations.

Sequential CIC values obtained for all patients with disease recurrence are shown in comparison with results obtained for patients without detectable recurrence in Fig. 4.

CEA-IC

Model studies. The PEG/PCA assay was applied to model complexes formed at seven different antigen–antibody ratios from eight times antigen excess to eight times antibody excess and to control sera containing equivalent amounts of free CEA. For antibody excess complexes 51–64% of the CEA added was recovered and at equivalence 44%. At antigen excess the amount of CEA recovered was the same in each instance, presumably reflecting the optimal binding capacity of the anti-CEA added, and represented 26, 14 and 7% of the CEA used to form two, four and eight times antigen excess complexes, respectively. CEA recovery from the controls was less than 1% in all cases.

Model complexes formed at two times antigen and two and eight times antibody excess were used as standards in six individual tests, recovery of the CEA added was $31 \pm 8\%$, $49 \pm 5\%$, $45 \pm 12\%$ respectively (errors given as 1 s.d.). This represented 51% (two times antigen excess), 77% (two times antibody excess) and 68% (eight times antibody excess) of the total CEA-IC as estimated by protein A binding of radiolabelled complexes.

Application to patient sera. Results for sera obtained from 29 patients with known disease are shown in Table 1. CEA-IC were not detected in any of these nor in six patients tested sequentially at

Table 1. Relationship between disease status, CEA, CIC, and CEA-IC in patients with colorectal cancer

Patient number	Dukes' grading	CEA (μg/l)	CIC (μg/ml)	Complexed CEA (μg/l)
1	A	10	18	<1
2		<2	13	<1
3		<1	13	<1
4		<2	13	<1
1	B	2	38	<1
2		18	30	<1
3		5	25	<1
4		62	18	<1
5		<2	18	<1
6		1,070	15	<1
7		7	13	<1
8		<2	13	<1
9		5	9	<1
10		17	4	<1
1	C	8	40	<1
2		3	34	<2
3		13	29	<1
4		11	25	<1
5		7	21	<1
6		<2	20	<1
7		10	15	<1
8		9	15	<1
9		4	11	<1
10		<2	11	<1
11		68	10	<2
12		7	10	<1
13		3	8	<1
1	D	29	11	<1
1	unresectable	5	25	<2

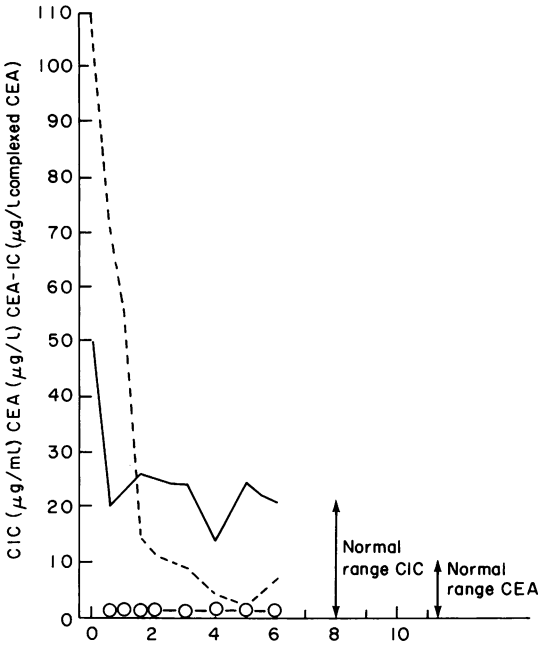

Fig. 5. Sequential CIC (——), CEA (- - -) and CEA-IC (o——o) concentrations in a patient with colorectal cancer who relapsed 8 months post-operatively. Values for both markers were raised prior to surgery, CIC concentrations were elevated at some points post-operatively and CEA levels returned to normal. CEA-IC were not detected.

1 or 2 month intervals for periods ranging from 3–12 months. An example of one of these patients is shown in Fig. 5.

DISCUSSION

The results demonstrate that CIC were present at elevated concentrations in patients with colorectal cancer as compared with normal controls. Also the presence of elevated CIC concentrations did not tend to correlate with the presence of elevated CEA levels and therefore a higher percentage of relapse prediction was achieved when both CIC and CEA were considered rather than CEA alone (50% *vs* 100% in the 14 patients studied sequentially). This is consistent with the work of Dent *et al.* (1980) who reported that the measurement of C1q binding activity could augment that of CEA as a prognostic indicator in patients with carcinoma of the bronchus.

Raised CIC concentrations were also present in patients who remained clinically well, probably due to the inability of the PEG assay to distinguish between CIC which were related to the presence of the tumour and those which were not. This may, in part, be because the PEG assay used detected only those CIC which contained IgG immunoglobulins. Where antibodies to CEA, or CIC containing CEA, have been reported the antibody present has frequently been IgM (MacSween, 1975; Harvey *et al.*, 1978; Kapsopoulou-Dominos & Anderer, 1979a). The antigen directed assay for CEA-IC developed for the current work should detect CEA containing CIC regardless of the class of immunoglobulin involved. Results from this assay indicated that CEA-IC were not part of the CIC populations in the patients investigated but it is possible that CIC containing other tumour associated products and antibodies other than IgG were present. These CIC would not be detected by the PEG assay.

The mechanism by which PEG precipitates CIC is not known. It is commonly accepted that PEG works by steric exclusion and generally precipitates proteins according to their molecular

weight, high molecular weight molecules being precipitated at lower PEG concentrations than low molecular weight molecules (Zubler *et al.*, 1977). Thus, at suitable PEG concentrations monomeric IgG can be separated from complexed IgG. Clearly PEG precipitation does not specifically isolate CIC and therefore results from PEG based tests can be difficult to interpret, especially if the total precipitated protein is taken to be a measure of CIC and no attempt is made to quantify known CIC components in the precipitate. Assays such as the 2% PEG test used in our study, where the amount of IgG in the PEG precipitate was taken to be a measurement of CIC, offer greater specificity for CIC but can be affected by co-precipitation of monomeric IgG which is present in much greater concentrations than CIC. This is particularly important when it is considered that pathological sera often have abnormal IgG concentrations. However, it has been demonstrated that 2% PEG successfully discriminates between aggregated and monomeric IgG (Soltis & Hasz, 1983) and results from the 2% PEG assay used in our study support this since there was no correlation between IgG concentrations and CIC values in the samples studied. Nevertheless the possibility that other factors, such as abnormal Clq concentrations, could lead to non-specific IgG precipitation cannot be excluded. However, the major disadvantage of the PEG assay is probably the lack of specificity which arises because the assay is antibody directed. Other assays for CIC appear to have similar drawbacks. For instance, Hobbiss *et al.* (1983) have recently reported that CIC concentrations measured by three different techniques (C1q, Raji and L1210 binding assays) did not differentiate between benign, inflammatory and neoplastic colorectal disease. Furthermore no correlation was found between CIC values and stage of disease in patients with colorectal carcinoma (Hobbiss *et al.*, 1983).

False positives present a serious limitation to the use of CIC as a diagnostic or prognostic tool in malignant disease, as illustrated in the comparison of results obtained for sequential studies shown in Fig. 4. No difference was observed between values obtained for patients who remained in remission and for those who subsequently relapsed. Unfortunately, elevated CIC concentrations appear to be present in a wide range of conditions rather than in a limited number of easily recognised disease states (Theofilopoulos & Dixon, 1979; Begent *et al.*, 1982). For example, in a recent report 20·1% of patients admitted to a general hospital were found to have significantly elevated concentrations of CIC (Abuelo *et al.*, 1982).

The lack of correlation between values obtained for CEA and those obtained for CIC does not exclude the possibility that CEA was present in complexed form. Levels of CIC are probably more influenced by host factors than those of CEA and the two parameters may vary independently whilst still reflecting the presence of tumour. Also it is possible that the small antigen excess complexes likely to form in the presence of a large tumour burden may be undetected by the 2% PEG assay. Furthermore if CEA was present, but complexed with antibody it was unlikely to be detected using the double antibody radioimmunoassay (unpublished observation).

For detection of CEA-IC, a higher concentration (3·5%) of PEG was used since this did not cause precipitations of free CEA and it was considered that a larger proportion of CIC, including smaller complexes, would be precipitated by 3·5% compared to 2% PEG. Non-specific precipitation of IgG, which might occur within the higher PEG concentrations, was not of consequence in this assay since the antigen portion of the complex was quantified. Results obtained using model CEA-IC demonstrated that the assay was reproducible and capable of detecting less than 10 μg/l of complexed CEA. When the assay was applied to sera of patients with colorectal cancer complexed CEA was not present at detectable quantities in any of the sera studied. Similar findings have been reported by Steele *et al.* (1978) who failed to detect anti CEA reactive material in CIC obtained from patients with colorectal adenocarcinoma or to raise CEA reactive antisera by immunization with CIC components.

Our work does not support that of Kapsopoulou-Dominos & Anderer (1979a, 1979b) and Staab *et al.* (1980) who reported the presence of CEA-IC in a high percentage of patients with disease recurrence. These workers described a high molecular weight anti-CEA reactive material present in sera of patients with gastrointestinal carcinoma. However they did not establish directly that this material was CIC containing CEA combined with specific antibodies (Kapsopoulou-Dominos & Anderer, 1979a). The assay subsequently developed to measure CEA-IC was similarly indirect; the high molecular weight material was shown, in contrast to CEA, to be PCA insoluble (Kapsopou-

lou-Dominos & Anderer, 1979b) and therefore it was claimed that the portion of CEA which became precipitated by PCA was CEA-IC. It is possible that the anti-CEA reactive material measured by this group was of a different nature from the complexed CEA examined in this study.

Mavligit & Stuckey (1983) have recently reported that increases in concentrations of CEA occurred in sera from patients with metastatic colorectal cancer when the sera were treated with $MgCl_2$ (to split CEA-IC) and the released, putative, anti-CEA antibody was removed by precipitation with protein A containing *Staphylococcus aureas*. The increases were thought to be due to CEA released from CEA-IC but the authors do not describe the effect this treatment would have on CEA values in control sera to which CEA had been added at concentrations similar to those found in patients' sera. In one patient thought to have CEA-IC, IgG eluted from the *S. aureus* was shown to bind ^{125}I-labelled CEA with a specific binding, after adsorption with group A red blood cells, of 19·4% of that obtained with commercial anti-CEA. IgG from another patient, not thought to have CEA-IC, showed no specific binding after similar treatment. However, serum from a normal donor investigated showed 12·9% specific binding. A larger number of observations would have to be made before conclusions would be drawn about the specificity of this reaction.

It is arguable whether CEA is immunogenic in man. Many workers have reported the presence of CEA reactive IgM and IgG antibodies in normal serum and in serum of patients with colonic neoplasms (Gold, Freedman & Gold, 1972; MacSween, 1975; Pressman, Chu & Grassberg, 1979). However, the immunological specificity of this reaction is not clear. It is possible that these antibodies are directed against a blood group A site on the CEA molecule or towards other tissue antigens, such as non-specific cross-reacting antigen, which share common determinants with CEA (Pompecki, 1980).

In summary, the detection of elevated CIC concentrations was able to augment CEA as a tumour marker system but this was of limited clinical value due to the presence of high values in patients who did not subsequently show disease recurrence. It is possible that a proportion of the CIC detected contained tumour specific antigens, but an antigen specific assay directed against CEA suggested that CEA was not one of these.

This work underlines the need for further characterization of CIC and tumour antigens in the search for new tumour marker systems.

We would like to thank Dr Gordon Rogers and Dr Pat Keep for their advice and for the provision of CEA and anti-CEA, Professor R.N. Maini for the donation of sera from patients with rheumatoid arthritis and Hugh Mitchell and colleagues who performed the assays for CEA. We are indebted to Dr Norman Zamcheck, of Harvard Medical School, for suggesting the principle of the assay for immune complexes containing CEA. The work was supported by the Cancer Research Campaign.

REFERENCES

ABUELO, J.G., TOLKAN, S.R., DISCIULLO, S.O., PEZZULO, J.C. & SAMBANDAM, S.T. (1982) Circulating immune complexes in unselected patients admitted to the medical service of a general hospital. *J. clin. lab. Immunol.* **7**, 27.

BALDWIN, R.W. & ROBINS, R.A. (1980) In *Cancer Markers* (ed. by Sell.) p. 507. Humana Press, Clifton.

BEGENT, R.H.J., CHESTER, K.A., WALKER, L.C. & TUCKER, D.F. (1982) Circulating immune complexes as markers and response to chemotherapy in malignant teratomas and gestational trophoblastic tumours. *Br. J. Cancer*, **45**, 217.

COSTANZA, M.E., PINN, V., SCHWARTZ, R.S. & NATHANSON, L. (1973) Carcinoembryonic antigen–antibody complexes in a patient with colonic carcinoma and nephrotic syndrome. *N. Engl. J. Med.* **289**, 520.

DENT, P.B., LOUIS, J.A., MCCULLOCH, P.B., DUNNETT, C.W. & CEROTTINI, J.C. (1980) Correlation of elevated CIq binding activity and carcinoembryonic antigen levels with clinical features and prognosis in bronchogenic carcinoma. *Cancer*, **45**, 130.

GOLDENBERG, D.M., NEVILLE, A.M., CARTER, A.C. *et al.* (1981) CEA (carcinoembryonic antigen). Its role as a marker in the management of cancer. *J. Cancer Res. clin. Oncol.* **101**, 239.

GOLD, P. & FREEDMAN, S.O. (1965) Demonstration of tumour specific antigen in human colonic carcinomata by tolerance and absorption techniques. *J. exp. Med.* **121**, 439.

GOLD, J.M., FREEDMAN, S.O. & GOLD, P. (1972) Human antiCEA antibodies detected by radioimmuno-electrophoresis. *Nature*, **239**, 60.

GREENWOOD, F.G., HUNTER, W.M. & GLOVER, J.S. (1963) The preparation of ^{131}I-labelled growth hormone of high specific radioactivity. *Biochem. J.* **89**, 114.

HARVEY, S.R., VAN DUSEN, L.R., DOUGLASS, H.O., HOLYOKE, E.D. & CHU, T.M. (1978) Identification of a macromolecule containing an anticarcinoembryonic antigen-reactive substance and immunoglobulin M in human pancreatic cancer. *J. Natl. Cancer Inst.* **61,** 1199.

HOBBISS, J., COOPER, K.M., MOORE, M., GOWLAND, E. & SCHOFIELD, P.F. (1983) Limitation of immune complex measurements in colorectal disease. *Br. J. Surg.* **70,** 473.

JENETTE, J.C. & FELDMAN, J.D. (1977) Sequential quantitation of circulating immune complexes in syngeneic and allogeneic rats bearing Molony sarcomas. *J. Immunol.* **118,** 2269.

JENETTE, J.C. (1980) Consistent fluctuations in quantities of circulating immune complexes during progressive and regressive phases of tumour growth. *Am. J. Pathol.* **100,** 403.

KAPSOPOULOU-DOMINOS, K. & ANDERER, F.A. (1979a) Circulating carcinoembryonic antigen immune complexes in sera of patients with carcinomata of the gastrointestinal tract. *Clin. exp. Immunol.* **35,** 190.

KAPSOPOULOU-DOMINOS, K. & ANDERER, F.A. (1979b) An approach to the routine estimation of circulating carcinoembryonic antigen immune complexes in patients with carcinomata of the gastrointestinal tract. *Clin. exp. Immunol.* **37,** 25.

KEEP, P.A., LEAKE, B.A. & ROGERS, G.T. (1978) Extraction of CEA from tumour tissue, foetal colon and patients sera, and the effect of perchloric acid. *Br. J. Cancer,* **37,** 171.

LEWIS, J.C.M. & KEEP, P.A. (1981) Relationship of serum CEA levels to tumour size and CEA content in nude mice bearing colonic-tumour xenografts. *Br. J. Cancer,* **44,** 341.

MACH, J.P., VIENNY, H., JAEGER, P., HALDERMANN, B., EGELY, R. & PETTAVEL, J. (1978) Long-term follow-up of colorectal carcinoma patients by repeated CEA radioimmunoassay. *Cancer,* **42 (suppl. 3),** 1439.

MACSWEEN, J.H. (1975) The antigenicity of carcinoembryonic antigen in man. *Int. J. Cancer,* **15,** 246.

MAVLIGIT, G.M. & STUCKEY, S. (1983) Colorectal carcinoma: evidence for circulating CEA–anti-CEA complexes. *Cancer,* **52,** 146.

MINTON, J.B. & MARTIN, E.W. (1978) The use of serial CEA determinations to predict recurrence of colon cancer and when to do a second-look operation. *Cancer,* **42,** 1422.

MOERTEL, C.G., SCHUTT, A.J. & GO, V.L.W. (1978) Carcinoembryonic antigen test for recurrent colorectal carcinoma. *JAMA,* **239,** 1065.

POMPECKI, R. (1980) Presence of immunoglobulin G in human sera binding to carcinoembryonic antigen (CEA) and non-specific cross-reacting antigen (NCA). *Eur. J. Cancer,* **16,** 973.

PRESSMAN, D., CHU, T.M. & GROSSBERG, A.L. (1979) Carcino-embryonic antigen-binding immunoglobulin isolated from normal human serum by affinity chromatography. *J. Natl. Cancer Inst.* **62,** 1367.

ROSSEN, R.D., REISBERG, M.A., HERSH, E.M. & GUTTERMAN, J.U. (1977) The C1q binding test for soluble immune complexes: clinical correlations obtained in patients with cancer. *J. Natl. Cancer Inst.* **58,** 1205.

SOLTIS, R.D. & HASZ, O.E. (1983) The effect of serum immunoglobulin concentration on immune complex detection by polyethylene glycol. *J. Immunol. Meth.* **57,** 275.

STAAB, H.J., ANDERER, F.A., STUMPF, E. & FISCHER, R. (1980) Are circulating CEA immune complexes a prognostic marker in patients with carcinoma of the gastrointestinal tract? *Br. J. Cancer,* **42,** 26.

STEELE, G., SONIS, S., STELOS, P. *et al.* (1978) Circulating immune complexes in patients following clinically curative resection of colonic cancer. *Surgery,* **83,** 648.

STEELE, G., LAHEY, S., RODRICK, M. *et al.* (1983) Circulating immune complexes in patients with colorectal cancer. *Am. J. Surg.* **145,** 549.

SUGARBAKER, P.H., ZAMCHECK, N. & MOORE, F.D. (1976) Assessment of serial carcinoembryonic antigen (CEA) assays in post-operative detection of recurrent colorectal cancer. *Cancer,* **38,** 2310.

TATE, H. (1982) Plasma CEA in the post-surgical monitoring of colorectal carcinoma. *Br. J. Cancer,* **46,** 323.

THEOFILOPOULOS, A.N. & DIXON, F.J. (1979) The biology and detection of immune complexes. *Adv. Immunol.* **28,** 89.

VELLACOTT, K.D., BALDWIN, R.W., BALFOUR, T.W. & HARDCASTLE, J.D. (1981) Circulating immune complexes in patients with benign and malignant colorectal tumours. *Br. J. Surg.* **68,** 402.

WANEBO, H.J., STEARNS, M. & SCHWATZ, M.K. (1978) Use of CEA as an indicator of early recurrence and as a guide to a selected second-look procedure in patients with colorectal cancer. *Ann. Surg.* **188,** 481.

ZUBLER, R.H., PERRIN, L.H., CREIGHTON, W.D. & LAMBERT, P.H. (1977) Use of polyethylene glycol (PEG) to concentrate immune complexes from serum or plasma samples. *Ann. Rheum. Dis.* **36, (Suppl.)** 23.