Br. J. Surg. Vol. 68 (1981) 402–404 *Printed in Great Britain*

Circulating immune complexes in patients with benign and malignant colorectal tumours

K. D. VELLACOTT, R. W. BALDWIN, T. W. BALFOUR AND J. D. HARDCASTLE*

SUMMARY

Circulating immune complexes were measured using a modified C1q binding assay in 100 preoperative patients with colorectal cancer and in 52 patients with benign polyps. Sixty-one per cent of the patients with cancer and 49 per cent of the patients with benign polyps had elevated levels, as did 92 per cent of those with malignant polyps. There was no relation to a, the stage of the disease, with 10 out of 13 Dukes' A tumours (77 per cent) having increased levels; b, the degree of differentiation of the cancers; c, the tumour mass. However, cancers on the right side of the colon had significantly higher levels than rectal cancers.

TUMOUR-associated antigens may be released from developing tumours and result in the formation of immune complexes in serum or other body fluids (1). Elevated serum levels of circulating immune complexes have been detected in many different malignancies (2–5), and Rossen et al. recorded the highest levels in patients who had residual tumour after surgical treatment and in those with rapidly progressing malignant disease (6). The measurement of immune complexes in the circulation may, therefore, be useful in the diagnosis and prognosis of malignant disease. The present study was designed to compare levels of immune complexes, using a modified C1q binding assay (7), in patients with colorectal cancer both before and after surgery and also in patients with benign colonic polyps which might be considered to be pre-malignant.

Patients and methods

Venous blood samples were obtained from 100 consecutive patients (51 male, 49 female) with proved colorectal cancer before surgery and then at 6 weeks, 3 months and subsequent 3 monthly intervals after surgery. Samples were also obtained from 52 patients (26 male, 26 female) with benign colonic polyps treated either by colonoscopic polypectomy ($n=46$) or surgical excision ($n=6$). Control samples were obtained from 40 healthy hospital personnel or patients (20 male, 20 female) admitted to hospital for minor surgical procedures and also from 10 patients (4 male, 6 female) with benign inflammatory disease of the bowel. The blood samples were allowed to clot at room temperature and were then separated and centrifuged at 2000 *g* for 20 min at 4 °C and then stored at −70 °C. C1q was purified from pooled human serum and labelled with radioactive iodine using a lactoperoxidase method (8). The immune complexes were quantitated using a C1q binding assay modified by the addition of heparin, which gives a clearer discrimination between normal and elevated levels. Ethylenediamine-tetracetic acid (EDTA) was then added to the samples and incubated at room temperature; this separates the endogenous C1q from the immune complexes in the sample to be tested. Subsequently the radiolabelled C1q was added and precipitated with polyethylene-glycol. After 1 h of incubation on ice, the tubes were centrifuged, the supernatant removed and the precipitate counted in a gamma counter. The results were expressed as C1q binding activity (7). All samples were tested in duplicate. Student's *t* test was used for statistical analyses.

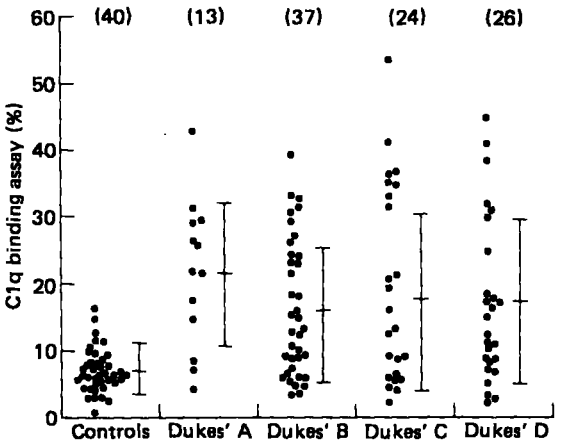

Fig. 1. Levels of immune complexes measured by C1q binding in 100 preoperative colorectal cancer patients.

Results

The age range was 20–76 years (mean 45·9 ± 17·0) for the 40 controls, 36–89 (mean 66·7 ± 11·1) for the 100 cancer patients, 36–90 (mean 62·2 ± 12·2) for the benign polyp group and for the inflammatory bowel disease group 23–65 (mean 49·6 ± 13·3). The C1q binding activity in the control group ranged from 2·91 to 16·39 (mean 7·06 ± 3·19). The normal level was taken as the mean ± 1 s.d. of the controls, i.e. 10·2.

Fig. 1 shows the results of the C1q binding activity in 100 patients with colorectal cancer, classified according to Dukes' staging, those with liver metastases being called Dukes' D. Sixty-one per cent of the patients with colorectal cancer had elevated levels of C1q binding. There was no obvious correlation with stage of disease, there being a high number of Dukes' A carcinomas, 10 out of 13, with elevated levels of C1q binding and similar high percentages in all stages of disease with no relationship to tumour mass. The mean value ± s.d. for stages A, B, C and D were 21·7 ± 11·04, 16·39 ± 10·11, 19·74 ± 14·92, 16·89 ± 11·11 respectively. The degree of differentiation of the tumours was 12 poorly, 70 moderately and 18 well-differentiated adenocarcinomas and there was also no relationship with C1q binding activity. The majority of the cancers occurred on the left side of the colon (83 per cent) but those on the right side had significantly higher levels than those in the rectum (*Table I*). All the Dukes' A tumours occurred in the rectum or sigmoid.

*Department of Surgery and Cancer Research Campaign Laboratories, University of Nottingham.
Correspondence to: K. D. Vellacott, Department of Surgery, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH.

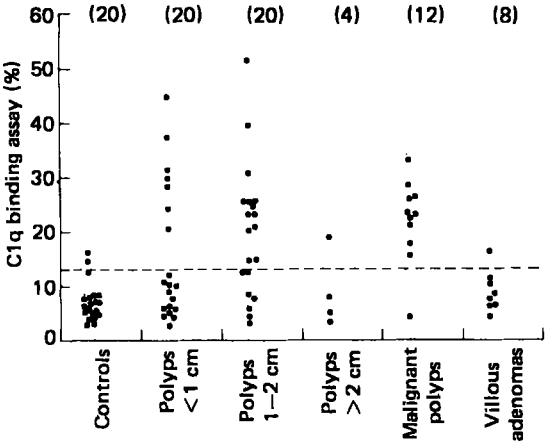

Fig. 2. Levels of immune complexes measured by C1q binding in 64 patients with benign and malignant polyps.

A further 64 patients with polyps, of which 12 were malignant, were also studied, the results being expressed according to size for the tubular adenomas, and histologically for the villous adenomas (*Fig.* 2). Forty-nine per cent of the benign polyps had increased levels of C1q binding and there was no correlation with the size of the polyp, although there were only 4 benign polyps greater than 2 cm in diameter. There were 8 villous adenomas in the group but only one of these had an increased level of C1q binding. However, of the 12 malignant polyps (9 tubular, 3 villous) all but one had increased levels of C1q binding. Three other patients, who had had polyps removed in the past, had increased levels of C1q binding, although no new polyps were detected on review colonoscopy.

The 10 patients with inflammatory bowel disease consisted of 9 with ulcerative colitis and 1 with Crohn's disease. Seven (70 per cent) had increased levels of C1q binding with a mean for the group of 24·02 ± 16·98. Five out of 6 further patients presenting with recurrent colorectal cancer had substantially increased levels of C1q binding. Two were treated with radiotherapy and the levels dropped following treatment.

Of the 100 patients with colorectal cancer, 16 (16 per cent) died within the first 6 months after surgery. There was no correlation with the level of immune complexes in the circulation and their prognosis. Of those that had a 'curative resection' and had elevated levels before surgery 46, two-thirds (31 per cent) had dropped to within normal limits within 3 months. By contrast, a proportion of the patients (9) had a rise of C1q binding activity at 6 weeks and this was probably due to the inflammatory healing process. Only 3 patients (3 per cent) in the group of cancer patients had other obvious reasons for elevated C1q binding such as associated asthma or hayfever.

Discussion

The nature of the complexes detected in cancer patients by non-specific methods is not known, although there is evidence that tumour-associated products are involved. The C1q and Raji cell binding assays may provide a measure of components consisting of antigens released or shed from tumour cells in combination with specific antibody (2).

We have shown increased levels of immune complexes in both benign and malignant colorectal tumours, there being no relationship to the size of polyps or stage of cancer. By contrast, studies using other tumour markers such as carcinoembryonic antigen (CEA) (9, 10) have shown that elevated levels are usually related to size and mass of the tumour and that elevations are unusual in early carcinoma. Of 13 Duke's A tumours in this study, however, 10 (77 per cent) had increased levels of circulating immune complexes (*Fig.* 1).

Some workers have found a relationship of increased CEA levels to the degree of differentiation of tumours (10) though this has not been confirmed in all studies (9). We were unable to find any association of circulating immune complexes with the degree of differentiation. Levels of CEA have also been shown to be higher in relation to left-sided colonic tumours, with a lower percentage of detectable levels in right-sided colonic and rectal tumours (11). We have found similar results in that rectal cancers have the lowest percentage of increased levels of immune complexes (48·5 per cent) but in reverse to CEA right-sided tumours had the highest percentage with increased levels of immune complexes.

Increased levels of immune complexes occurring irrespective of size (*Fig.* 2) in patients with polyps may mean that the polyp is secreting an auto-immune antigen into the circulation leading to immune complex formation, or there may be an inherent abnormality in the colonic mucosa which predisposes to the formation of polyps.

Immune complexes were found to be present in the group with benign inflammatory disease as has been demonstrated by other workers (12). The C1q binding method is not specific for malignant disease though increased levels have been demonstrated in a variety of non-colonic malignancies such as breast (3), lung (4) and bone (5). No immune complexes have been found in relation to ovarian cancers (13). However, in colorectal cancer the fact that tumour-associated products are involved is demonstrated by the fall in C1q binding activity after resection of the cancers.

Monoclonal antibodies to the antigens associated

Table I: COMPARISON OF C1q BINDING ACTIVITY WITH SITE OF COLORECTAL CANCERS

	Total	Mean of C1q BA (±s.d.)	No. with Increased C1q BA	Increased C1q BA (%)
Right side of colon	12	23·01 ± 13·17*	11	91·6
Transverse colon	5	13·38 ± 10·57	2	40·0
Left side of colon	41	19·11 ± 12·21	28	68·3
Rectum	42	15·47 ± 10·72*	20	48·5

*$P = < 0{\cdot}05$.

with these tumours are being produced so that it will be possible to identify their relationship to both polyps and early carcinomas. If it is possible to show that the antigens being secreted by the polyps are the same as those associated with malignant tumours, this will supply increasing evidence in support of the 'polyp to cancer transition'. Identification of these tumour-associated antigens will provide futher encouragement to continue looking for possible tumour markers in relation to early colorectal cancer, it should help in selecting those patients who have had polypectomy and should be followed up carefully and may result in a simple method that can be used as a screening test for early colonic cancer.

Acknowledgements

Mr K. D. Vellacott is supported by the Wellcome Trust Foundation and the studies were also supported by the Cancer Research Campaign. We are indebted to Miss S. Crosdale, who was supported by the Special Trustees for Nottingham University Hospitals, for her considerable technical assistance.

References

1. PRICE M. R. and BALDWIN R. W.: Shedding of tumour cell surface antigens. *Cell Surface Rev.* 1977; **3**: 424–71.
2. BALDWIN R. W. and ROBINS R. A.: Circulating immune complexes in cancer. In: SELL S. (ed.): *Cancer Markers: Development and Diagnostic Significance*. Clifton, N. J.: Humana Press, 1980; **19**: 507–31.
3. HOFFKEN K., MEREDITH I. D., ROBINS R. A. et al.: Circulating immune complexes in patients with breast cancer. *Br. Med. J.* 1977; **2**: 218–20.
4. LOWE J., SEGAL-EIRAS A., ILES P. B. et al. Circulating immune complexes in patients with lung cancer. *Thorax* 1981; **36**: 56–9.
5. SEGAL-EIRAS A., ROBINS R. A., BALDWIN R. W. et al.: Circulating immune complexes in patients with bone tumours. *Int. J. Cancer* 1980; **25**: 735–9.
6. ROSSEN R. D., REISBERG M. A., HERSH E. M. et al.: The C1q binding test for soluble immune complexes: clinical correlations obtained in patients with cancer. *J. Natl. Cancer Inst.* 1977; **58**: 1205–15.
7. DOYLE P. J., ROBINS R. A., WEBB P. G. et al.: A modified C1q binding assay in breast cancer. *Clin. Exp. Immunol.* 1981. (In the press.)
8. HEUSSER C., BOESMAN M., NORDIN J. H. et al.: Effect of chemical and enzymatic radioiodination on in vitro human C1q activity. *J. Immunol.* 1973; **10**: 820–8.
9. COOPER M. J., MACKIE C. R., SKINNER D. B. et al.: A reappraisal of the value of carcinoembryonic antigen in the management of patients with various neoplasms. *Br. J. Surg.* 1979; **66**: 120–3.
10. MARTIN E. W., KIBBEY W. E., DIVECCHIA A. et al.: Carcinoembryonic antigen. Clinical and historical aspects. *Cancer* 1976; **37**: 62–81.
11. LIVINGSTONE A. S., HAMPSON L. G., SHUSTER J. et al.: Carcinoembryonic antigen in the diagnosis and management of colorectal carcinoma: current status. *Arch. Surg.* 1974; **109**: 259–64.
12. KEMLER B. J. and ALPERT E.: Inflammatory bowel disease associated circulating immune complexes. *Gut* 1980; **21**: 195–201.
13. PRICE M. R., LAUGHLIN P. J., ROBINS R. A. et al.: Tumour markers with unknown functions in gynaecological neoplasm. *Arch. Gynecol.* 1980; **229**: 325–31.

Paper accepted 18 December 1980.

Fifty years ago (from the *British Journal of Surgery* (1931–32) 19: 517)

First edition

The Management of Abdominal Operations. By RODNEY H. MAINGOT, F.R.C.S., Surgeon, Royal Waterloo Hospital, London. Crown 8vo. Pp. 312 + xii. 1931. London: H. K. Lewis & Co. Ltd. 7s. 6d. net.

THE author of this book has undoubtedly filled a gap in surgical literature. It would have been better, in a work so full of views on which much criticism could be made, had he indicated the appropriate origin of such opinions under the proper chapter heading, instead of lumping his numerous co-editors together in the Preface. We should also have thought better of the book if the author had omitted a description of operations (many of them in our opinion inaccurate) and had concentrated on pre- and post-operative treatment.

Beginning with 'anæsthesia', he discusses in succeeding chapters (there are sixty-five) every possible kind of complication, and, in addition, gives an excellent account of pre- and post-operative treatment. Many pages are left blank for notes to be added by the reader.

The book is good in every way. It is a veritable 'gold mine' for a house surgeon or a senior student. It is a very useful reference book for an operating surgeon, and an excellent guide for the general practitioner and for nurses. The author has managed, by avoiding 'padding', to compress an enormous amount of information into singularly little space. He writes well and has chosen his co-editors with much judgement.