PRELIMINARY COMMUNICATIONS

Colonic Carcinoma: Clinicopathological Correlation with Immunoreactivity

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Summary

The relation between tumour spread, histological differentiation, and in-vitro antitumour immunoreactivity was studied in 132 cases of carcinoma of the large bowel. Positive correlations were found between blood lymphocyte antitumour cytotoxicity and both tumour differentiation and absence of recurrence or metastatic spread.

Introduction

In 1971 we reported lymphocyte antitumour cytotoxicity in eight out of 24 cases of colonic carcinoma[1] and have since confirmed such reactivity in 34% of 263 cases. In 132 cases autologous blood lymphocyte antitumour reactivity was tested at the time of operation on the tumour, and complete pathological, clinical, and follow-up data were available. Forty-three of these patients showed lymphocyte cytotoxicity against their own tumour cells in vitro, and we analysed the data to see whether this reactivity correlated with histological differentiation of the tumour, tumour spread, or the recurrence rate.

Patients

The patients were selected only in so far as the operation specimen was accompanied by a blood sample taken immediately before the operation and data were available about clinical features, spread, and the histological characteristics of the primary tumour and regional lymph nodes. A total of 132 patients, who presented in 1970-4, fulfilled these criteria, survived the first six weeks after operation, and came for follow-up appointments—generally once during the first three months after operation, once during the next six months, and thereafter yearly. The patients' mean age was 61 years, and 67 were men and 65 women.

Histological confirmation of the clinical diagnosis was obtained on formalin-fixed paraffin sections stained with haematoxylin and eosin; all sections were reviewed by one of us (E.P.) without knowledge of the immunological data. Tumour spread was classified according to Kirklin's modification of Dukes's classification.[2,3] Seventy-nine tumours were confined to the wall of the colon and adjacent fat, and 53 had metastasized to the regional lymph nodes (see table I). All the tumours were adenocarcinomas; the histological differentiation in 25 cases in which tumours formed glands similar to those of the normal mucosa and with little cellular atypia was graded as good; in 33 in which the tumours formed only a few irregular glands with marked cellular atypia it was graded as poor; and in 74 in which tumour differentiation was intermediate it was graded as average (see table II). The criteria correspond with those of Dukes[2] for histological assessment of malignancy as low, average, and high grade.

TABLE I—*Relation between Tumour Spread and In-vitro Blood Lymphocyte Antitumour Cytotoxicity in Colonic Carcinoma*

	No. of Patients	Non-metastatic*				Metastatic†		
		A	B_1	B_2	Total	C_1	C_2	Total
Cytotoxic	43	1	5	28	34	0	9	9
Not cytotoxic	89	3	9	33	45	4	40	44
Total	132	4	14	61	79	4	49	53

*Localized tumours: A, confined to mucosa and submucosa; B_2, extending into muscle coats; B_2, extending beyond muscle coats.
†Tumours metastasized to lymph nodes: $C_1 = B_1$ + node deposits; $C_2 = B_2$ + node deposits.

Immunological Testing

Autochthonous tumour cells were reacted with blood lymphocytes in all 132 cases. Initially, in 48 cases, the lymphocyte cytotoxicity tests were in Pulvertaft Teflon ring chambers with a ratio of 2·5-10 lymphocytes per viable tumour cell and complement added.[1] Subsequently, in 84 cases, a microassay test system was used.[4] Briefly, tumour cell suspensions were plated into Falcon plastic microtest plates 3034,[5] 1000 per well, and cultured in medium 199 with 10% fetal calf serum for 24 hours at 37°C in air plus 5% CO_2. The plates were then washed with culture medium; adherent tumour cells were counted and usually numbered about 100 per well. Autochthonous or control lymphocytes were added at a ratio of 200 to one adherent tumour cell. After incubation for a further 48 hours tumour cells still attached in the wells were counted on an inverted phase-contrast microscope. Cytotoxicity was expressed as the percentage reduction in the mean number of surviving tumour cells in six tests (N_t) versus six controls (N_c)—that is,

$$\text{cytotoxicity} = \frac{N_c - N_t}{N_c} \times 100.$$

Student's t test was applied to assess the significance of differences between test and control wells; cytotoxicity was taken as positive at the level of $P<0·05$. Forty-three (33%) of the 132 patients gave a significant cytotoxic response.

Results

One death occurred two months after palliative operation. All other patients were followed up for three to 55 months (mean 18·5 months). The mean survival time in the 25 patients who died was the same (12·3 months) whether or not a cytotoxic lymphocytic response was present at the time of the operation. The same also held true for the 107 living patients (mean survival so far 19·8 months). Only three (9%) of the 34 patients with cytotoxic lymphocytes at the time of operation and still alive at the time of writing had clinical signs of recurrence whereas 17 (23%) of the 73 without such immunoreactivity had clinical recurrence.

Blood lymphocyte antitumour cytotoxicity was significantly commoner in patients with localized tumours (43%) than in those who already had metastases (17%) (χ^2 test after Yates's correction, $\chi^2 = 8·7$; $P<0·01$; table I). Peripheral blood lymphocyte antitumour cytotoxicity was also significantly commoner in patients with well-differentiated tumours (64%) than in those of average or poor differentiation (25%) ($\chi^2 = 12·2$; $P<0·001$; table II). Only four of the 25 patients with well-differentiated tumours had lymph node metastases, in contrast to 49 of 107 of those with tumours of poorer differentiation ($P<0·05$).

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TABLE II—*Relation between Histological Differentiation and In-vitro Blood Lymphocyte Antitumour Cytotoxicity in Colonic Carcinoma*

	No. of Patients	Histological Differentiation Grade		
		Good	Average	Poor
Cytotoxic	43	16	18	9
Not cytotoxic	89	9	56	24
Total	132	25	74	33

Discussion

We have shown that metastatic spread of carcinoma of the large bowel to regional lymph nodes is associated with a significantly lower incidence of lymphocyte antitumour cytotoxicity than when the tumour is confined to the wall and adjacent fat. One explanation of this, that tumour growth and spread lead to specific immunological paralysis, agrees with our findings that colon carcinoma antigens inhibit the cytotoxic lymphocytes.[6-8] A similar inverse relation between tumour spread and both skin immunoreactivity to dinitrochlorobenzene and blood lymphocyte count has been reported.[9]

Our finding that well-differentiated tumours are associated with antitumour lymphocyte cytotoxicity, more than those of poorer differentiation, may be a function of the greater spread by the latter. Alternatively or in addition, poorly differentiated tumours readily metastasizing might be weakly antigenic and not stimulate the host's immune response. We cannot, however, exclude the possibility that lymphocyte antitumour cytotoxicity may help to maintain good tumour differentiation and restrict growth. Whatever the sequential relation between the clinicopathological status of the tumour and immune response it is highly likely that the positive correlations between lymphocyte antitumour cytotoxicity and histological differentiation, localized growth, and less recurrence will be reflected in a better long-term prognosis.

Our present analysis of immunoreactivity has been confined to lymphocyte cytotoxicity against autochthonous tumour cells because a priori it might be expected to be of major biological significance, and our data were complete. We are, however, aware that the host's immune response to cancer is complex and includes various cellular and humoral components. Several of these were examined during the study—for example, serum antibodies to tumour cells and blocking factors. At this stage no clinicopathological correlation with these has emerged. Histopathological studies (to be published) showed a positive correlation between leucocytic infiltration of tumour stroma and recurrence-free survival. There was also an association between regional lymph node cortical-paracortical hyperplasia and node lymphocyte cytotoxicity. This accords with a recent report,[10] derived from five-year survival data in 18 cases of colonic carcinoma, that regional lymph node hyperplasia is associated with a favourable prognosis.

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Reticuloendothelial Function in Renal Allograft Recipients

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Summary

The phagocytic capacity of the reticuloendothelial system (R.E.S.) was assessed in patients with chronic renal failure and in renal transplant recipients. R.E.S. phagocytosis was increased in the former group. Soon after transplantation R.E.S. phagocytosis was moderately reduced (though levels were comparable with those of normal controls) but was particularly reduced after high-dose corticosteroid treatment for rejection. In long-term allograft recipients R.E.S. phagocytosis was also depressed though steroid maintenance doses were small.

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Introduction

The phagocytic capacity of the reticuloendothelial system (R.E.S.) is important as the body's first system of defence against infection. Phagocytosis of antigen by macrophages is the first step in the induction of an immune response.[1] Primary antibody responses may be inhibited by immunosuppressive agents at this initial stage, but secondary antibody responses are more resilient. Macrophage phagocytosis is also important in the resistance of a host to malignancy.[2] For these reasons numerous studies have been made in animals and man of the capacity to clear colloids or lipid emulsions from the circulation.[3 4] The most popular test is the clearance of heat-aggregated iodinated human serum albumin.[5] In patients with immunological diseases[6] and bacterial infections[7] R.E.S. phagocytosis is increased but in patients with malignancy it is often depressed.[2] R.E.S. depression may also result from the use of large doses of immunosuppressive drugs, cortisone, or irradiation but may be enhanced by oestrogens.

We therefore assessed reticuloendothelial clearances in patients before and after they recieved renal allografts. The susceptibility of these patients to bacterial and viral infections, to endotoxinaemia,[8] and to subsequent development of malignancy is well known.