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.

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 diseases⁶ and bacterial infections⁷ 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 received renal allografts. The susceptibility of these patients to bacterial and viral infections, to endotoxaemia,⁸ and to subsequent development of malignancy is well known.

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Patients and Methods

Aggregated iodinated human serum albumin was prepared according to the method of Ilio and Wagner.⁹ Human serum albumin was diluted with sterile water to 3%, albumin radio-iodinated with ¹²⁵I (Radiochemicals, Amersham) was added, and the batch was alkalized to pH 10 with 0.2 M sodium hydroxide. Thereafter the solution was placed in a water bath at 90°C for three hours, during which time there was frequent shaking. We noted that the optical density of the aggregated solution increased by 0.03 units at 525 nm. Each iodinated aggregated batch was checked for free iodide content, which was always less than 1%. Methylhydroxybenzoate 0.1% was added as preservative and then the solution was stored at 4°C in 5-ml ampoules. Studies on paired controls showed no change in the half life of the stored material with length of storage.

Volunteer patients were injected with 5 mg/kg of iodinated aggregated albumin through a Venflon needle, and 2-ml blood samples were taken at precisely two-minute intervals for 12 minutes and then at 15 and 20 minutes into EDTA anticoagulant. In the early studies 0.5-ml plasma samples were counted, followed by trichloroacetic acid precipitates, and protein-free fractions. This did not improve the accuracy of counting, however, as the free iodide was so low, and thereafter 0.5-ml plasma samples were counted directly by means of a Nuclear Chicago gamma-counter. The isotope counts were plotted semi-logarithmically against time, as at this dose first-order kinetics were obeyed. Thus the half time of clearance of the albumin was calculated and also the phagocytic index (k).^{5,9} Most samples gave a single component, but a few had a double component decay and were used when a straight line could be drawn from the counts obtained from six to 15 minutes. A clinically adverse reaction was seen in only one normal volunteer: hypotension, tachycardia, and nausea were rapidly controlled by the intravenous injection of 100 mg of hydrocortisone.

Fifteen people volunteered to be controls for the test; 10 were patients recovering from an uncomplicated coronary thrombosis. In convalescent (third week) coronary patients the half life of the material was the same as in normal people. Twenty patients with chronic renal failure on haemodialysis were studied, five of whom had undergone splenectomy, and also six patients with active nephritis. The main study group comprised 35 patients with a renal allograft on whom 60 tests were performed. Of these 14 patients had had their allograft for over nine months. The others were patients with new renal allografts who were studied postoperatively, during early rejection, and after immunosuppressive treatment. The standard treatment was 40 mg of prednisone with 100-150 mg of azathioprine in the immediate post-transplant period, but this was supplemented by three intravenous daily doses of 1 g of methylprednisolone (Solu-Medrone) at the first sign of rejection. Outpatient maintenance treatment consisted of 15-20 mg of prednisone with 100-150 mg of azathioprine. Fifteen of the patients were also taking part in a trial of the effect of oral linoleic acid (sunflower seed oil) at a dose of 10 ml thrice daily.¹⁰ Oleic acid is known to cause reticuloendothelial depression.¹¹

Results

The table summarizes the results and the fig. shows the scatter of values in each group. Patients with chronic renal failure showed evidence of increased R.E.S. phagocytosis, even when they had had a splenectomy. Patients with active nephritis also had an increased R.E.S. capacity for aggregated albumin. In the early postoperative phase R.E.S. phagocytosis diminished, though it was comparable with the normal range shown by the controls. Even severe infection at this time, however, did not produce the expected increase of R.E.S. phagocytosis. On the days that high-dose methylprednisolone was given for rejection R.E.S. phagocytosis was much diminished, but by 24 hours after this treatment there was a rebound towards increased phagocytosis. The 14 long-term allograft survivors showed diminished R.E.S. phagocytosis.

Because of this latter finding liver function tests were performed on long-term survivors. There were no changes on standard liver function tests, but of the eight patients who had a bromsulphalein test seven showed abnormal bromsulphalein retention. Three patients had values of 8% at 45 minutes and the others ranged from 2% to 7.5%. Thus there was evidence of minimal liver parenchymal cell dysfunction. All patients were negative for hepatitis B antigen.

There was no difference in R.E.S. phagocytosis among the long-term survivors according to whether they were receiving sunflower seed oil or placebo but the dose was relatively small.

R.E.S. Clearances in Various Conditions. Values are Mean Half Lives ($\pm$ S.D.) in Minutes

	No. of Tests Performed	R.E.S. Clearances (min)	Increase or Reduction	P Value*
Controls	15	14.1 $\pm$ 3.3		
Chronic renal failure	15	9.3 $\pm$ 2.7	Increased	<0.001
Chronic renal failure with splenectomy	5	6.1 $\pm$ 2.1	Increased	<0.001
Active nephritis	6	8.1 $\pm$ 2.3	Increased	<0.001
Early post-transplant	14	12.0 $\pm$ 4.0		
During methylprednisolone	17	10.7 $\pm$ 6.0	Reduced	<0.001
After methylprednisolone	11	10.2 $\pm$ 2.4	Increased	<0.005
Infection	4	1.1 $\pm$ 1.4		
Long-term allograft	14	15.1 $\pm$ 6.3	Reduced	<0.001

*Significance by Student's *t* test with Bessel's correction.

Distribution of R.E.S. clearances. Horizontal lines indicate means.

Discussion

The increased R.E.S. phagocytosis shown by patients with chronic renal failure on haemodialysis probably reflects induction by repeated exposure to infection. Those patients who had undergone splenectomy still had increased R.E.S. phagocytosis. In any case aggregated albumin is removed principally by the Kupffer cells of the liver, and there might be increased Kupffer cell activity after splenectomy.

In patients in the postoperative phase the previously enhanced phagocytosis was diminished, but this merely confirms reports that operations depress the R.E.S.¹² After high-dose corticosteroids there was a serious reduction of phagocytosis but the end of treatment was followed within 24 hours by a rebound increase. Thus, whereas high-dose corticosteroids produce R.E.S. depression, smaller doses seem to enhance phagocytosis. It is therefore the depression of phagocytosis in the long-term survivors on lower maintenance doses of 15-20 mg prednisone that requires explanation. This depression might explain the high susceptibility to infection, endotoxaemia, and certain malignancies so well recognized in renal allograft recipients. Our results show that these patients have minimal evidence of parenchymal liver cell dysfunction. Whether this and the Kupffer cell dysfunction are due to the long-term effect of immunosuppressive drugs or to undetected viruses is not yet known. Almost all of our patients have had cytomegalovirus infection.

If other studies confirm that impaired R.E.S. phagocytosis is a general feature of the successful long-term renal allograft recipient, then the test may have prognostic significance. Nevertheless, short-term recipients with impaired R.E.S. activity were extremely susceptible to infection. Our findings therefore highlight the fundamental fact that much more work on the biochemistry and pharmacology of the reticuloendothelial cell

system is required to enable patients to live in symbiosis with a foreign kidney and yet remain free of the risk of infection.

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SHORT REPORTS

Risk of Acute Renal Failure Associated with Disseminated Intravascular Coagulation

The mortality rates due to acute renal failure (A.R.F.) continue to be extremely high. Early and frequent dialysis, adequate caloric intake, and intensive care have not succeeded in lowering it. Disseminated intravascular coagulation (D.I.C.) has been found to be associated with A.R.F. in many cases. The aim of this report is to determine to what extent D.I.C. modifies the prognosis of A.R.F., and whether heparin treatment is valuable.

Patients, Methods, and Results

Of the 86 patients treated in our department for A.R.F. from January 1969 to May 1974 27 had associated D.I.C. The aetiology was obstetric in 21 cases, surgical in 20, traumatic in five, medical in 37, not identified in two, and as a result of cardiac catheterization in one. The diagnosis of D.I.C. was based on three criteria: a platelet count $\leq 150 \times 10^9/l$, prothrombin time ≥ 15 seconds, and fibrinogen ≤ 1.6 g/l. Patients with hepatic disease were excluded from the study. In every patient the usual treatment for A.R.F. was followed. In 12 of 27 patients with D.I.C. intravenous sodium heparin was given. The average dose for the first 24 hours was 300 mg every four hours. The dose of heparin was considered to be correct if the thrombin time or the coagulation time were twice the control. Statistical analysis was performed by the χ^2 test.

Mortality Rates in 86 Patients With A.R.F.

	No. Dead	No. Alive	Mortality Rate (%)
Not studied haemostatically	19	13	59.4
With D.I.C.	16	11	59.2
Without D.I.C.	12	15	44.4
Total patients with A.R.F.	47	39	54.65

In 54 patients haemostatic studies were performed. The overall mortality rate was 55%. The mortality rate was higher in patients with D.I.C. than in those without, but this difference was not statistically significant ($\chi^2 = 1.21$). Mortality did not decrease (58%) in patients treated with heparin as compared with the 15 non-treated patients (60%). Excluding minor haemorrhage, there were serious therapeutic complications in 10 of the 12 treated patients. Nine patients suffered gastrointestinal haemorrhages and heparin had to be stopped. After three days of treatment one patient developed a retroperitoneal haematoma which required surgical drainage. The incidence of gastrointestinal haemorrhage was less in the non-treated patients (five out of 15). The analysis of cause of death in 27 patients with A.R.F. and D.I.C. showed that gastrointestinal haemorrhages contributed to death in eight cases (five alone, two associated with septic shock, and one associated with generalized bleeding). Septic shock alone caused four deaths, and the other four deaths were attributed to miscellaneous factors. One year after the acute episode both the five survivors of the heparin-treated group and the six survivors of the non-treated group had a glomerular filtration rate of > 60 ml.

In one patient from the latter group a renal biopsy showed patchy cortical necrosis.

Discussion

The association of A.R.F. and D.I.C. was found in 31% of the patients with A.R.F. The same frequency has been reported in Kleinknecht (28.9%),¹ while Zech found it in 5% of cases,² and Conté in 14.6%.³ These differences were due to the different causes of A.R.F. in each series and the different diagnostic criteria for D.I.C. D.I.C. is often associated with A.R.F., especially in obstetric patients.

Both Kleinknecht¹ and Zech² considered that D.I.C. worsened prognosis. In our series the mortality rate was higher for patients with D.I.C., but the difference was not significant. Conté³ suggested that D.I.C. does not influence the prognosis of A.R.F., but if only his "toxic-infectious shock" cases are considered, the results are closer to those in the other studies.

The dramatic complications of the heparin treatment must be emphasized: 10 out of 12 treated patients suffered serious haemorrhages. Gastrointestinal haemorrhages are a well established factor of morbidity and mortality in the course of A.R.F., and heparin might aggravate their evolution in patients with already critical disease.

Heparin treatment of D.I.C. has been unhesitatingly recommended by many workers, though it still remains a point of controversy. Some workers¹⁻⁴ have reported that the mortality rate has not been modified by heparin. Treatment of the primary disease is essential and early care of acidosis, sepsis, and shock checks haemostatic disturbances without anticoagulant treatment.¹⁻⁵ Cautious administration of heparin should be reserved only for selected cases, in which the catalytic mechanism of D.I.C. perpetuates the coagulation disorder, or when heparin treatment is the only possible means of diagnosis. On the other hand, it has been claimed that heparin prevents cortical necrosis,²⁻³ but we can draw no valid conclusions from our findings. In the non-treated group, however, one case of cortical necrosis was observed.

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