Short Communication

Fc RECEPTOR-BEARING AND PHAGOCYTIC CELLS IN SYNGENEIC TUMOURS OF C. PARVUM- AND CARRAGEENAN-TREATED MICE

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Attention has recently been drawn to the possible significance within tumours of cells with the biological characteristics of macrophages (Russell et al., 1976; Evans, 1977; Wood & Gollahon, 1977) including cells bearing receptors for IgG-coated erythrocytes (Fc receptors) (Milkstrom et al., 1968; Tønder & Thunold, 1973; Kerbel & Davies, 1974). It has been suggested that the incidence of these cells within tumours may bear a relationship both to the host’s immunological response, and to the growth, immunogenicity and metastatic spread of the tumour (Evans, 1972; Eccles & Alexander, 1974a; Kerbel et al., 1975; Russell et al., 1976).

The immune response is markedly affected by agents which influence macrophage activity (Halpern et al., 1964; Fowler & Thomson, 1978) such as C. parvum (Scott, 1974) and carrageenan (Thomson, 1978) and which have profound effects on tumour growth (Milas & Scott, 1978; Thomson & Fowler, 1977; Keller, 1976). In this preliminary study we have attempted to determine whether the influences of C. parvum and carrageenan on the growth of a transplantable syngeneic tumour are reflected in the proportions of Fc-receptor-positive and phagocytic cells within the tumour.

Eight to 12-week-old inbred female C3H He Mg mice derived from stock obtained from Bantin & Kingman Ltd, Grimston, Aldborough, Yorkshire, and weighing 20–25 g were used throughout.

The tumour used was a syngeneic adenocarcinoma which arose spontaneously as a mammary tumour in a virgin female mouse, and which was obtained originally from Dr D. Trevan, Imperial Cancer Research Fund, London. Transplantation was carried out using cell suspensions obtained from 10-day solid subcutaneous tumours, and 10⁶ viable cells were injected s.c. in the dorsolumbar region.

A formalin-killed suspension of C. parvum (Strain Number CN 6134, Batch BA 3935/A) was a gift from Wellcome Research Laboratories, Beckenham, Kent, and contained 7 mg dry wt/ml. Animals were injected i.p. with 1·4 mg at the same time as tumour-cell injection. Iota carrageenan (Auby Gel x52, Lot 1557) was prepared and injected as described previously (Thomson & Fowler, 1977). Mice received 4 i.p. injections of 1 mg over the weeks before tumour-cell challenge (Days –7, –5, –3 and –1).

At 7, 11 and 14 days after cell injection, tumour tissue was teased apart, and a cell suspension obtained by gentle homogenization using a loose-fitting rubber pestle. The cells were washed and allowed to stand in siliconized glassware for 1 h at room temperature in Eagle’s medium (MEM, Wellcome) containing 0·1% (w/v) pancreatic trypsin (Difco). Fragments were then allowed to settle and the cell suspension removed, centrifuged and resuspended in Eagle’s MEM. Cell numbers

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were then estimated by haemacytometry.

Carbon-ingesting cells were visualized by incubating the cells (2×10⁶/ml) in a 1:80 dilution of colloidal carbon (518, Günther Wagner, Pelikanwerke, Hanover, Germany) in MEM supplemented with 10% (v/v) foetal bovine serum (Gibco Bio-Cult) for 2 h at 37°C in a humidified atmosphere of 5% CO₂ in air. The incidence of carbon-ingesting cells and cell viability were then estimated by combined haemacytometry and phase-contrast microscopy. Cells bearing Fc receptors were enumerated by rosette formation, using ox erythrocytes sensitized with rabbit anti-ox RBC IgG (a gift from Dr A. E. Dewar, Department of Pathology, University of Edinburgh). Tumour cells (2×10⁶) were mixed with red cells at a ratio of 1:40, incubated for 10 min at 20°C, and then centrifuged at 150 g for 6 min before incubation for 2 h at 4°C. Two drops of 0.1% toluidine blue were added and the cells resuspended by end-over-end inversion of the tubes before estimation of the percentage of rosette-forming cells.

The significance of differences between means was estimated using Student’s t test.

Treatment of mice with C. parvum and iota carrageenan had marked but opposing effects on tumour growth (Fig. 1). Injection of C. parvum impaired growth, whereas pretreatment with carrageenan potentiated growth of the tumour. Evidence that both these agents affected activity of the reticuloendothelial system was clearly demonstrated by increases in spleen weight above those in tumour-bearing controls (Fig. 2). Splenomegaly was most pronounced in C. parvum-treated mice, a spectacular increase in weight occurring during the 2 weeks after injection.

Fc-receptor-bearing and carbon-ingest-
ing cells represented minor cell populations within the tumour; their incidence was related, in untreated animals, to time after tumour cell injection (Figs. 3 & 4) and consequently to tumour size. The former cells were present consistently in greater proportion than phagocytic cells, indicating that many of the former were non-phagocytic.

In C. parvum-treated animals the incidence of Fe-receptor-bearing cells on Days 11 and 14 were significantly higher ($P<0.05$) than in controls, whereas in carrageenan-treated mice the incidence of these cells was significantly decreased on Days 7 and 11 ($P<0.05$ and $P<0.025$ respectively). In contrast, the incidence of carbon-ingesting cells within tumours was not significantly affected by either C. parvum or carrageenan.

Our results clearly demonstrate that C. parvum and carrageenan exert opposing effects on growth of a transplantable tumour within syngeneic hosts, and are consistent with the reported antagonistic effect of carrageenan and other antimacrophage agents on the anti-tumour activity of microbial adjuvants (Hopper et al., 1976; Keller, 1977).

Although splenomegaly is an overt manifestation of the reticuloendothelial-stimulating properties of C. parvum, the significant increase in spleen weight induced by carrageenan is also accompanied by a relative increase in phagocyte activity within this organ, with a concomitant decrease in activity of the liver (Fowler & Thomson, 1978). However, it is clear from this study that although increased spleen weight accompanies the arrest of tumour growth in the case of C. parvum, the inverse correlation exists for carrageenan treatment. This implies that the systemic effects of these agents may be of greater relevance to tumour growth than their influences on the spleen alone.

The low incidence of Fe-receptor-positive and phagocyte cells within tumours in this study is consistent with the scarcity
of histologically recognizable lymphoreticular cells within this particular tumour (Thomson et al., unpublished). Comparable proportions of cells with functional macrophage characteristics have been found within certain other animal tumours, although considerably higher incidences have been reported both in animal and human tumours (Evans, 1972; Eccles & Alexander, 1974a, b; Russell et al., 1976; Wood & Gollahon, 1977). The decline in the proportion of Fc-receptor-bearing cells with increased tumour size observed in the present study is consistent with work on other tumours by Szymaniec & James (1976) and Moore & Moore (1977).

Fc-receptor-bearing cells within tumours may constitute a heterogeneous population, since a variety of cells, other than macrophages, including B or T lymphocytes, neutrophils, eosinophils and mast cells may display this characteristic (see Korn et al., 1978). The relatively higher incidence of Fc-receptor-positive over carbon-ingesting cells, which we have observed, may be explained by the presence of non-phagocytic monocytes, such as those described by Haskill et al. (1976). In the latter study these cells constituted the major effector-cell population, and it is monocytes rather than mature macrophages which would be increased by C. parvum.

It is difficult to reconcile the well-documented effects of C. parvum and carrageenan on macrophage activity in vivo with their failure to influence more markedly the incidence of cells with macrophage characteristics within the tumour, particularly in view of the pronounced effects of these agents on tumour growth. However, Szymaniec & James (1976) found that C. parvum occasionally had an appreciable antitumour effect without increasing the proportion of Fc-receptor-positive cells within the tumour. In contrast, Eccles & Alexander (1974a) showed that BCG induced a small decrease in metastasis which was associated with a slight increase in tumour macrophages. We are aware of no studies other than the present one on the effect of carrageenan on the macrophage content of experimental tumours. Although our study is based on observations on only one tumour model, it is clear that the marked effects of C. parvum and carrageenan on reticuloendothelial function in vivo are not necessarily reflected in similarly pronounced alteration in the proportion of cells with functional macrophage characteristics within solid tumours. Further studies in progress with C. parvum and carrageenan, aimed at analysing the initial interactions between host lymphoreticular and tumour cells, may help resolve this apparently paradoxical situation.

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