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Increased Take Rate of Human Tumour Xenografts after Carragheenan Treatment

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Abstract—*Experiments are reported in which attempts were made to improve the take-rate of human tumour xenografts by inhibiting macrophages. Carragheenan, a macrophage-toxic agent was used in a dose of* 5 mg/*mouse i.p. Results showed that Carragheenan inhibited the functional activity of peritoneal macrophages both in normal and immune-suppressed mice. The take-rate of three serially transplanted human colorectal xenograft lines increased when the transplantation was made into immune-suppressed mice treated with Carragheenan* 1 day *before tumour grafting.*

INTRODUCTION

SINCE the early 1970's there has been increasing interest in human tumours xenotransplanted either in congenitally athymic, 'nude' mice [1] or in artificially immune-deprived mice [2, 3]. In the last few years we have transplanted 12 human colorectal tumours into thymectomized, irradiated and bone-marrow reconstituted mice. As has often been observed, not all tumours grew [4–6] and in fact of the seven patients whose tissues gave positive grafts in the first passage only six could be repeatedly passaged. During serial passage the take-rates of these tumours ranged between 50–80%. In the different lines the take rate was almost constant from passage to passage. It is widely accepted that among other mechanisms macrophages play an important role in tumour growth [7]. The aim of our recent work was to increase the take-rate of human tumour xenografts using Carragheenan, a macrophage-toxic agent.

MATERIALS AND METHODS

Immune-suppression

Male CBA mice (Laboratory Animals Breeding Center, LATI, Gödöllő, Hungary) were thymectomized at about 6 weeks of age, and 2 weeks later they were given 920 rad whole body irradiation from a ^{60}Co source at a dose rate of 60 rad/min. Within 6 hr the mice were reconstituted with 5×10^6 syngeneic bonemarrow cells injected i.v.

Tumour material

Three human colo-rectal carcinomas were established as xenografts from surgical specimens and designated HT 6, HT 17 and HT 24. Histologically all of these lines proved to be well-differentiated adenocarcinoma. Tumours were transplanted s.c. and their volume measured as previously described [4].

Macrophages

Macrophages washed out from the peritoneal cavity with 3 ml of TC 199 medium were centrifuged for 10 min at 500 ***g*** and resuspended in the same medium. This procedure was repeated twice. The cells were identified by light- and electronmicroscopy. For the latter the cells were fixed in 2% OsO_4 in veronal buffer (pH 7.2), embedded in Durcupan, double-contrasted with uranylacetate and lead citrate and studied in a Jeol 100-B electronmicroscope (60 kV). The functional activity of macrophages was studied by their adherence, migration and phagocytosis, using slight modification of the methods described by Bullen and Losowsky [8] for adherence, by Stuart *et al.* [9] for migration and by Mims [10] for phagocytosis.

Carragheenan

Carragheenan Type 2, No. C-1138, Sigma Chemical Co., U.S.A., was dissolved in phy-

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siological saline at 80°C. Mice were treated (i.p.) 1 day before transplantation with a dose of 5 mg/mouse.

RESULTS

On the basis of the electron microscopic study, 70–80% of peritoneal cells proved to be macrophages (Fig. 1a). The rest were lymphocytes, neutrophils and mast cells. Functional activity of macrophages diminished after immune-suppression (Fig. 2).

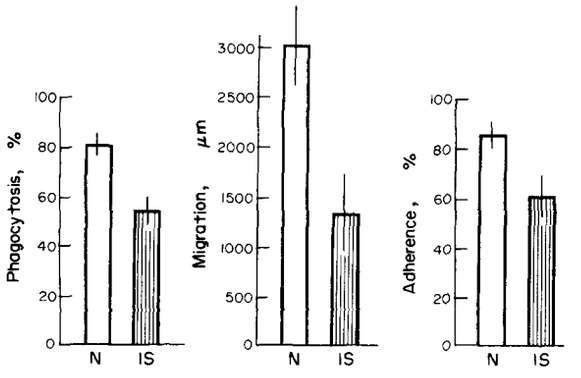

Fig. 2. Functional activity of peritoneal macrophages in normal (N) and immune-suppressed (IS) mice. Mean values ± S.D. (Phagocytosis was studied in vivo in 8 mice/group; migration in vitro—10 mice; adherence in vitro—8 mice).

Carragheenan treatment produced an increase in the number and a decrease in the functional activity of peritoneal macrophages both in normal and immune-suppressed mice with peak values at about 5–7 days after treatment. Both the adherence and phagocytotic capacity returned to normal level by 14 days (Fig. 3). Morphologically Carragheenan produced very severe cytoplasmic damage while the nucleus remained almost intact. The dilatation of lysosomes and the rupture of their membranes were the most pronounced alterations in the cytoplasm (Fig. 1b).

Table 1 shows the effect of Carragheenan treatment on the take rate of three xenograft lines. In each xenograft line it was found that Carragheenan increased the take-rate. In line HT 6 splenectomy did not appear to change the take rate. All tumours were rejected by normal mice but the decrease in tumour weight was slower in each of the prepared group (Fig. 4). The tumours transplanted bilaterally tended to show either double (both tumours grew) or no take, while single take was very rare.

In all cases tumours growing in pretreated mice showed a significantly shorter latent period (the time needed for tumours to reach a

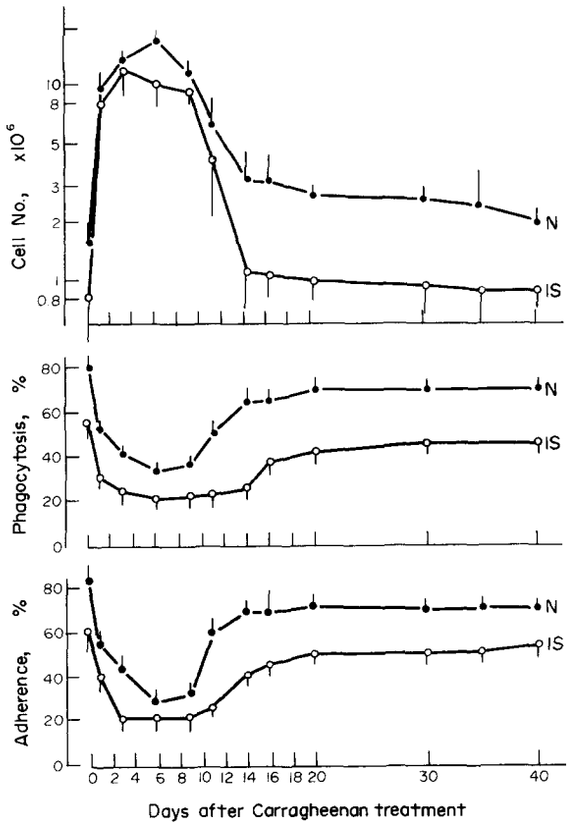

Fig. 3. Effect of Carragheenan on the number and functional activity of peritoneal macrophages in normal (N) and immune-suppressed (IS) mice. Mean values ± S.D. (4–4 mice/groups/time-points).

palpable size) but afterwards the growth rate was practically unchanged (Fig. 5). Carragheenan pretreatment did not produce detectable histological alterations in growing tumours.

Carragheenan also was able to make mice receptive to retransplantation following the rejection of an initial graft (Table 2). In these experiments mice that had rejected HT 6 tumour implants were retransplanted into the same site 8 weeks later. Giving Carragheenan 1 day before retransplantation raised the take-rate from zero to about 90%. Mice from which s.c. tumours had been excised were not resistant to a second graft made 1 week later.

DISCUSSION

The take-rate of human tumours xenotransplanted into immune-suppressed mice may be influenced by several factors, for instance the speed of vascularization of tumour graft, the residual immune-response of the host, host cells with non-specific cytotoxicity. Inadequate vascularization can be an important factor at the first implantation of surgical

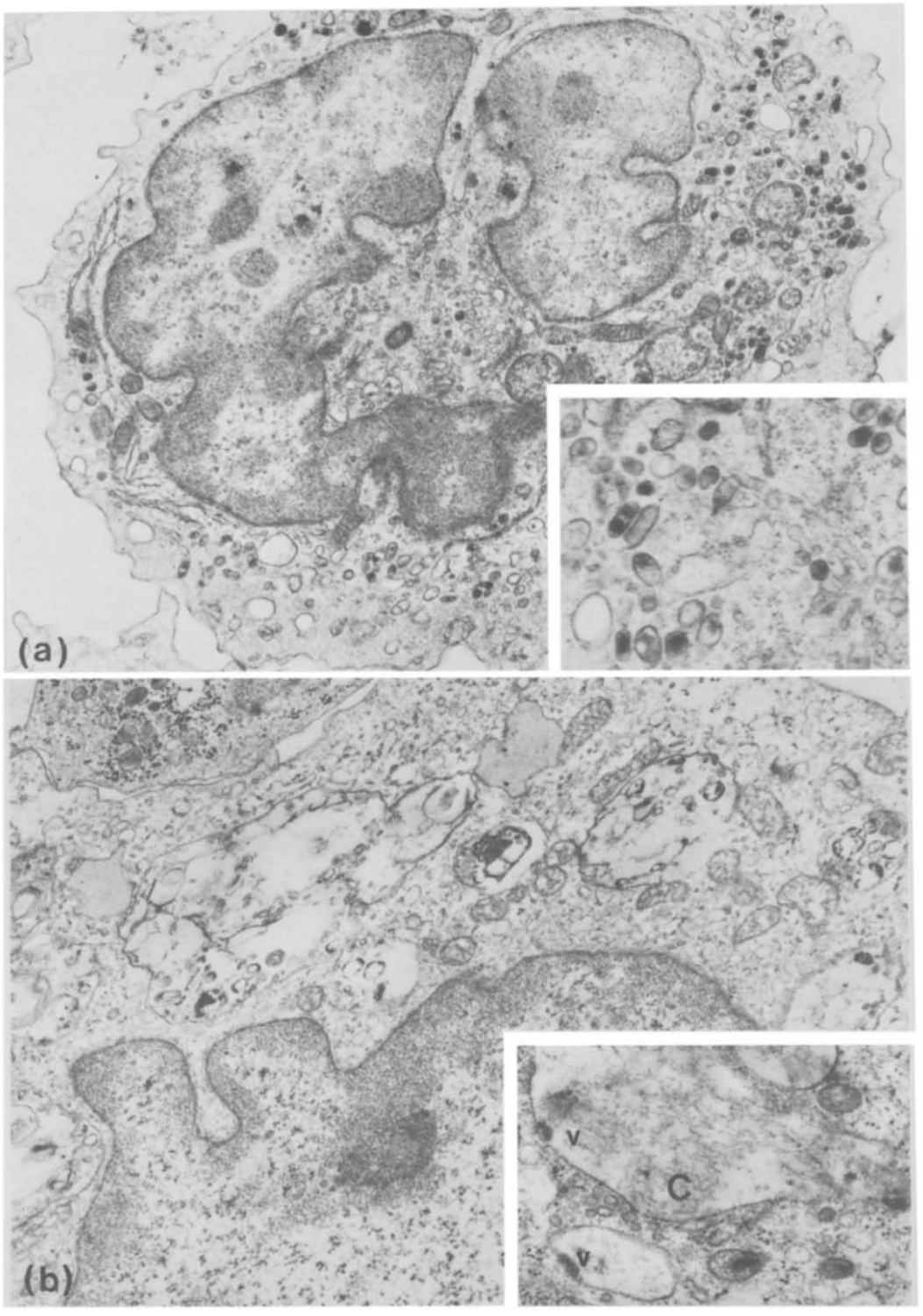

Fig. 1(*a*). *Peritoneal macrophage from immune-suppressed mouse* (2 weeks *after immune-suppression*) *with lobulated nucleus and compact nucleolus. The cytoplasm is rich in organelles, mainly in lysosomes* (×12500—*No.* 129718). *Insert: primary lysosomes of normal macrophage* (×26400—*No.* 131866).

(*b*) *Peritoneal macrophage from immune-suppressed mouse* 24 hr *after Carragheenan treatment. Nucleus is less lobulated and the number of cytoplasmic organelles decreased. Besides the damaged mitochondria the most striking alteration is the dilatation of lysosomes and the rupture of their membrane* (×13800—*No.* 12709). *Insert: dense materials close to the lysosome membrane are presumed to be Carragheenan particules* (×26400—*No.* 131869).

Table 1. *Take rate of human colorectal tumour xenografts in immune-suppressed mice*

	Take rate			
	Observed Growing/ transplanted tumours	(%)	Corrected* Growing/ transplanted tumours	(%)
HT 6/8 Immune-suppressed CBA	44/74	58.1	44/72	61.1
	18/34	52.9	18/34	52.9
+splenectomy	20/38	52.6	20/36	55.5
+Carragheenan†	24/30	80.0	24/26	92.3
	39/42	90.5	38/38	100.0
HT 17/3 Immune-suppressed CBA	12/20	60.0	12/18	66.6
+Carragheenan	16/20	80.0	16/16	100.0
HT 24/4 Immune-suppressed CBA	9/20	45.0	9/18	50.0
	12/20	60.0	12/18	66.6
+Carragheenan	16/20	80.0	16/18	88.9
	16/20	80.0	16/18	88.9

*When the tumours were larger than 2.5 cm^3 the mice were killed and checked for presence of thymus tissue. Corrected take rate is indicated only for those mice which had no residual thymus.

†Carragheenan was given i.p. in a dose of 5 mg/mouse 1 day before transplantation.

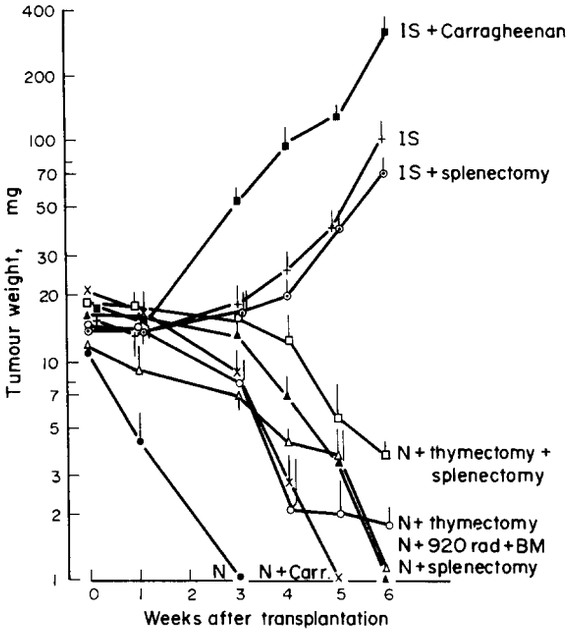

Fig. 4. *Growth of HT* 6/8 *human tumour xenografts in normal* (*N*) *and immune-suppressed* (*IS*) *mice. Mean values* ± S.D. (8–8 *tumours/groups/time-points*).

material because of either the weak stimulus from the tumour towards the host to induce the proliferation of capillaries into the graft (tumour angiogenesis factor) or the relatively high sensitivity of tumour cells to hypoxia during the temporary avascular phase after grafting. As tumours become serially transplantable, host factors may have more importance in rejection than for instance the heterogeneity of tumour pieces. This is indicated by the increasing tendency to observe double or no takes after bilateral implantation. Remnants of thymus or reinjection of T lymphocytes at bone-marrow reconstitution may also lead to rejection. Steel *et al.* [11] reported when the number of bone-marrow cells used for reconstitution was reduced the tumour take-rate substantially increased. They also found that treatment with cyclophosphamide before transplantation also enhanced the take-rate. We have found that inadequate thymectomy is an unequivocal reason for rejection. In every mouse where thymus was retrospectively found to be present the tumours failed to grow. In our work, incomplete thymectomy occurred in less than 5% of mice and the effect of excluding these mice is indicated in Table 1. Splenectomy of immune-suppressed mice did not increase the take-rate of colorectal xenografts although Giovanella *et al.* [12] reported the stimulatory effect of splenectomy on the growth of human mammary tumours in nude mice.

Among the cell populations involved in host defence mechanisms against tumours the role of macrophages operating both by specific or non-specific pathways is documented [13, 14]. Macrophage blocking agents or antimacrophage serum could increase the take-rate of syn-, iso- or allogeneic tumours or decrease the cytotoxic effect of macrophages [15–20]. Carragheenan is a macrophage-toxic agent that operates by damage of lysosomes [15, 21]. It has been shown that Carragheenan

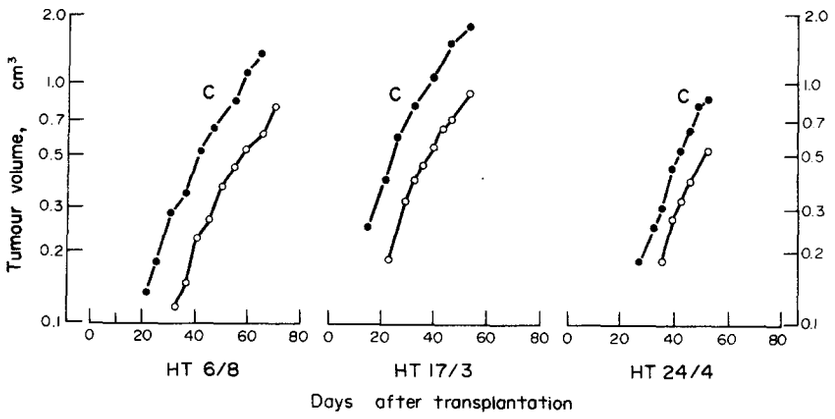

Fig. 5. Growth of human colorectal xenografts in immune-suppressed mice with or without Carragheenan pretreatment. Symbols represent median values. (No. of tumours per group: HT 6/8 ● 38 tumours—○ 18; HT 17/3 ● 10—○ 8; HT 24/4 ● 16—○ 9). The latency period was significantly shorter after Carragheenan pretreatment as indicated by the time needed to reach 0.3 cm³ tumour volume: HT 6/8 $P<0.01$; HT 17/3 $P<0.001$; HT 24/4 $P<0.001$.

Table 2. Results of retransplantation of mice grafted with HT 6 tumours

Experiment No.	Results of first implantation	Carragheenan* pretreatment	Take-rate on retransplantation (takes/No. of implants)
1	growth†	–	14/14‡
2	growth	–	10/10
3	rejected	–	0/18
4	rejected	–	0/16
5	rejected	+	6/8
6	rejected	+	8/8

*Given 1 day before retransplantation.
†Initial grafts were excised 1 week before retransplantation.
‡This column represents the take-rate of retransplants.

administered at optimal time and dose could enhance the take of isogeneic tumours [19, 20]. Macrophages derived from congenitally athymic or artificially immune-deprived mice should have some influence on tumour growth as well according to the studies with *Corynebacterium parvum* [22, 23].

These results prompted us to look for the effect of Carragheenan on the take-rate of xenogeneic human tumours. In all three transplantable tumour lines that we have studied Carragheenan clearly increased the take-rate, shortened the latency period but had no effect on the growth rate or histological structure of tumours nor induced metastases. In almost all cases, when the Carragheenan pretreatment failed, remnants of thymus were observed.

It can be concluded, that the inhibition of functional activity of macrophages in immune-suppressed mice promotes the grafting of human tumour xenografts even in those mice which rejected the same tumour previously. It is not certain whether all macrophages are involved in the graft rejection or only a subpopulation of them. The question is open, whether macrophages need cell-to-cell contact for their thymus-independent effect, or whether they work through humoral substances. Whatever the mechanism, Carragheenan pretreatment offers a way to

reduce the resistance of immune-suppressed mice against human tumours and so may enhance the take-rate of serially transplanted as well as primarily implanted human tumour xenografts.

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