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Evaluation of Intratumoral Immunostimulants in the Treatment of a Transplantable Rat Mammary Carcinoma

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Abstract—*One* C. parvum *preparation and two BCG (Bacillus Calmette-Guérin) strains have been evaluated for their effects, when injected intratumorally, on established growths of a transplantable rat mammary carcinoma of spontaneous origin. It was found that whilst the anti-tumor effect of intratumoral injection varied from experiment to experiment certain conclusions could be drawn. Thus for maximal effect the injection should be given as early as possible and at a high dose; furthermore, multiple intratumoral injections appeared to offer no advantage over a single injection. It was also apparent that the anti-tumor effect following intratumoral injection of* C. parvum *or BCG was not improved when the tumor-bearing animals were presensitised to these immunostimulants. Intratumoral* C. parvum *was also used as an adjunct to surgery in the treatment of metastases; however, in this model it did not improve the effect of surgery alone. It is concluded that under certain defined circumstances intratumoral injection can bring about tumor regression. However, the conditions under which it is effective may render this form of treatment of limited application.*

INTRODUCTION

THE INTRATUMORAL injection of immunological adjuvants such as *C. parvum* and BCG has been widely used in animal systems to induce primary tumor regression [1–6] and in some cases eradication of microscopic lymph node metastases [7, 8]. Furthermore, on the basis of such experimental studies it is being used in the treatment of human cancer [9, 10]. Most of the experimental studies on intratumoral injection have been carried out using tumors that have artefacts attached to their induction or maintenance; for example, tumor induction by carcinogen [4], tumors arising in high cancer strains of mice [6], and tumors maintained in tissue culture [3]. Such defects might be expected to weaken the status of the experimental systems as models of human cancer [11, 12].

Recently, Greager *et al.* [13] reported that a transplantable rat mammary carcinoma of spontaneous origin in a low cancer strain of rats was amenable to treatment by intratumoral injection with immunostimulants, especially *C. parvum*. In this report we have attempted to extend this observation and also to define the conditions for a maximal anti-tumor effect in this system. In addition, the use of intratumoral *C. parvum* as an adjunct to surgery has been evaluated for the treatment of metastases.

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MATERIALS AND METHODS

Rats

All experiments were carried out in female Wistar-derived Nottingham (WAB/NOT) rats from the Cancer Research Laboratories, University of Nottingham. Animals were housed in plastic cages and fed standard laboratory diet (Oxoid) with water *ad libitum*.

Tumor

Adenocarcinoma SP4 arose without deliberate induction in the mammary tissue of a female WAB/NOT inbred rat in 1966. Since then it has been transplanted by trocar graft to syngeneic recipients of the same sex as the primary host. The characteristics of this tumor have been reported elsewhere [14–17]. Briefly,

the cell inoculum producing progressive growth in 50% of normal rats (TD-50) is approximately 1×10^3 cells; it is immunogenic, appropriately immunised rats rejecting cell inocula up to 2×10^4 cells; it metastasises to regional lymph nodes and viscera. Only tumors of less than 25 *in vivo* passages were used in these experiments.

In all experiments 2×10^4 freshly trypsinised Sp 4 tumor cells [18] were injected into the mammary tissue of the right pectoral region of WAB/NOT rats. By 9–13 days the tumors had grown to about 5 mm diameter, this being the earliest point at which intratumoral injection was practicable. Following an intratumoral injection tumor growth was measured weekly by computing a mean tumor diameter from two measurements of the tumor at right angles and the rats were killed when either the primary tumor or regional (axillary) lymph node reached 3.5 cm mean diameter. The day on which rats were killed was termed their survival time. Experiments were terminated when the remaining rats had rejected their tumors completely or had no more than a small nodule at the site of tumor injection which had been stable for a few weeks. Such animals were termed long-term survivors and for computation of a mean survival time they were assigned a nominal survival time of 130 days from the time of tumor injection.

Tumor excision was performed as described elsewhere [16]. In the experiment involving surgery the rats were killed when distressed due to the effects of metastatic tumor growth. The day on which they were killed was termed the survival time.

C. parvum *preparations*

The bacterial suspension designated *C. parvum* CN6134 (Batch No. PX383) was obtained from Wellcome Research Laboratories, Beckenham, Kent, U.K., as a heat-killed preservative-free suspension at 7 mg dry weight of organisms/ml. This preparation has been shown to be superior to other *C. parvum* preparations in an experimental immunotherapy model [19].

BCG preparations

Connaught BCG, Lot 292-1, freeze dried, was a gift from Professor S. Landi, Connaught Medical Research Laboratories, Willowdale, Ontario, Canada. As determined in this laboratory [20] the dry weight concentrations was 14 mg/ml, the organisms had a viable unit count of 5×10^7/ml and were 2% viable.

Pasteur BCG liquid suspension, Immuno Pasteur F, Lot 30 was purchased from Institut Pasteur Production, Paris, France. The dry weight concentration was 14 mg/ml, the organisms had a viable unit count of 3.6 $\times 10^8$/ml and were 15% viable (unpublished data). These preparations have been shown to be superior to other BCG preparations in an experimental immunotherapy model [20].

Sensitisation to C. parvum *and BCG*

Prior to experiments involving intratumoral injection, separate experiments were performed to find out when the rats were maximally sensitive to *C. parvum* and BCG. Sensitivity to *C. parvum* was induced in rats by injecting 100 μg *C. parvum* s.c. into the upper aspect of the left hind foot. The degree of sensitivity to *C. parvum* was measured by injecting 100 μg *C. parvum* s.c. into the upper aspect of the right hind foot and measuring with a caliper gauge the footpad thickening after 24 hr. Results are expressed as the difference in right footpad thickness between sensitised rats and normal rats receiving only the right footpad injection of *C. parvum*. It can be seen from Fig. 1 that rats were maximally sensitive to *C. parvum* between 15 and 20 days following sensitisation.

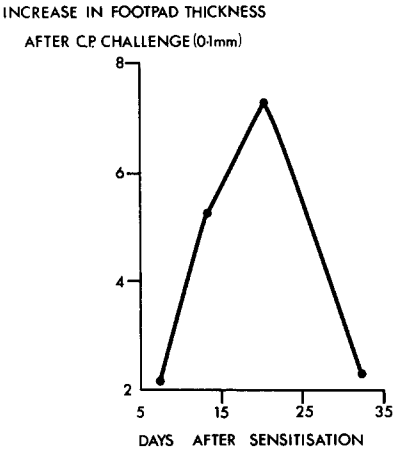

Fig. 1. Delayed-type hypersensitivity to C. parvum *(CP). At the times shown a challenge dose of* C. parvum (100 μg) *was injected into the contralateral footpad of rats previously sensitised to* C. parvum *and the footpad thickness measured at* 24 hr. *Results are expressed as the difference between sensitised rats and unsensitised rats receiving only the challenge dose of* C. parvum. Y-axis *units are* 0.1 mm *of difference in footpad thickening.*

Sensitivity to BCG was induced in rats by injecting 0.05 ml (4 μg dry weight, 1×10^7 viable units) of BCG TMC 1011, Batch A4 (Trudeau Institute, Saranac Lake, NY) s.c. into the right flank. Assessment of the state of

sensitivity to BCG was carried out by injecting 1000 U (20 μg) of PPD (Purified Protein Derivative of BCG—Evans Medical Ltd., Speke, Liverpool, U.K.) s.c. into the left ears of sensitised or unsensitised rats with a 30 gauge needle. It was observed that in sensitised rats the ears injected with PPD were highly erythematous by 24 hr. Uninjected ears of sensitised rats and injected ears of unsensitised rats were normal. Furthermore, injection of 20 μg of a protein unrelated to PPD (i.e., bovine serum albumin) into the ears of rats sensitised to BCG caused no erythema. Methods involving injection of antigen into ears of experimental animals have been found to be a very sensitive measure of the degree of sensitivity to that antigen [21] and this was found to be so in our experiments in which rats sensitised to BCG were subsequently found to exhibit a positive reaction to PPD on every day tested, controls being uniformly negative (data not shown). However, using a different method to assess sensitivity to BCG [22], rats were shown to be maximally sensitive 2–3 weeks after sensitisation.

It is assumed that the sensitivity reactions measured are delayed-type hypersensitivity reactions, although this has not been formally demonstrated. However, it is known that using a similar system in mice sensitivity to *C. parvum* can be abrogated by thymectomy [23], and that BCG TMC 1011 is a potent inducer of delayed-type hypersensitivity reactions [24]. Furthermore, it is known that in our system sensitivity to PPD can be adoptively transferred with peritoneal cells from BCG immune rats (unpublished data).

Statistics

The prolongation of survival compared to controls in rats treated by intratumoral injection was assessed for significance by the non-parametric, one-tailed Wilcoxon Rank Sum test [25]. In addition, the difference in incidence of long-term survivors in treatment and control groups was compared by the Fisher Exact test [26] [$P > 0.05$—Not Significant (NS)].

Evaluation of anti-tumor effect of immunostimulants

In order to control for variability immanent in this *in vivo* system evaluation of immunostimulant dose regime and pre-treatment (Tables 1–4) was carried out by incorporating in each experiment a particular treatment group to act as internal standard. This treatment group was a single, early, intratumoral injection of 1.4 mg *C. parvum*. In all experiments this treatment resulted in prolongation of survival and a proportion of long term survivors. The degree of significance of survival prolongation or the incidence of long term survivors compared to controls gave a useful gauge against which to compare the efficacy of other treatment groups within an experiment.

RESULTS

Survival in mammary carcinoma Sp 4-bearing rats treated with intratumoral C. parvum

In the first experiment rats were injected with 2×10^4 freshly trypsinised SP 4 tumor cells in the mammary pad on Day 0 and observed until tumors were barely palpable. At this point they were randomised into three groups which received: no treatment (Group I); 0.2 ml intratumoral physiological saline Day 9 (Group II); or 1.4 mg *C. parvum* in 0.2 ml physiological saline Day 9 (Group III). Tumor growth was followed and survival time

Table 1. *Intratumoral injection of* C. parvum: *effect on survival time*

Group	Treatment	Survival time in days (mean survival time)*	Significance compared to untreated controls: Survival prolongation—Wilcoxon rank sum test	Significance compared to untreated controls: Incidence of long term survivors—Fisher exact test
I	None	37, 37, 37, 37, 44, 44, 86, 100, $2 \times 130^+$ (68.2)	—	—
II	Intratumoral saline (0.2 ml) Day 9	30, 30, 37, 37, 51, 58, 72, 128, 128, 130^+ (70.1)	NS	NS
III	1.4 mg intratumoral *C. parvum* (0.2 ml) Day 9	44, 65, 86, 86, 93, $4 \times 130^+$ (98.0)	$P < 0.05$†	NS

*Tumor cells (2×10^4) injected Day 0 into mammary pad.
†Gp III significantly different to Gp II ($P < 0.05$).

Table 2. Evaluation of intratumoral injection: effect of varying dose and time of administration of bacterial preparations

Group	Treatment	Survival time in days (mean survival time)*	Significance compared to untreated controls: Survival prolongation—Wilcoxon rank sum test	Significance compared to untreated controls: Incidence of long term survivors—Fisher exact test
I	None	29, 36, 36, 50, 91 (48.4)	—	—
II	1.4 mg intratumoral *C. parvum* Day 9	36, 50, 64, 78, $3 \times 130^{+}$ (88.2)	$P<0.05$	NS
III	200 μg intratumoral *C. parvum* Day 9	29, 71, 71, 71, 71, 130^{+} (74.0)	NS	NS
IV	1.4 mg intratumoral *C. parvum* Day 17	36, 50, 50, 71, 78, 91, 130^{+} (72.3)	NS	NS
V	1.4 mg intratumoral BCG †Day 9	43, 91, $5 \times 130^{+}$ (112.0)	$P<0.01$	$P=0.05$
VI	200 μg intratumoral BCG †Day 9	36, 36, 43, 64, 64, 130^{+} (62.2)	NS	NS

*Tumor cells (2×10^4) injected Day 0 into mammary pad.
†BCG Immuno Pasteur F—liquid suspension.

Table 3. Evaluation of intratumoral injection: effect of varying number of injections and time of administration of bacterial preparations

Group	Treatment	Survival time in days (mean survival time)*	Significance compared to untreated controls: Survival prolongation—Wilcoxon rank sum test	Significance compared to untreated controls: Incidence of long term survivors—Fisher exact test
I	None	20, 20, 27, 27, 27 (24.2)	—	—
II	1.4 mg intratumoral *C. parvum.* Day 9	27, 55, $5 \times 130^{+}$ (104.6)	$P<0.01$	$P=0.05$
III	1.4 mg intratumoral *C. parvum.* Days 9, 15, 23, 29	55, 84, 91, 97, $3 \times 130^{+}$ (102.4)	$P<0.005$	NS
IV	1.4 mg intratumoral *C. parvum.* Days 15, 23, 29	20, 27, 46, 77, 84, 130^{+} (64.0)	NS	NS
V	1.4 mg intratumoral BCG†. Day 9	41, 56, 56, 84, 91, $2 \times 130^{+}$ (84.0)	$P<0.005$	NS
VI	1.4 mg intratumoral BCG†. Days 9, 15, 23, 29	56, 70, 70, 77, $3 \times 130^{+}$ (94.7)	$P<0.005$	NS

*Tumor cells (2×10^4) injected Day 0 into mammary pad.
†BCG Connaught multiple puncture—lyophilised.

assigned to individual rats as described in Materials and Methods.

The results in Table 1 show that intratumoral injection of *C. parvum* can indeed produce a significantly increased survival time in tumor-bearing rats; however, the incidence of long term survivors was not significantly different to controls in this experiment. An intratumoral injection of saline was without effect. It was noted that occasionally tumors receiving no treatment at all could regress and while this was a rare phenomenon it was not confined solely to this experiment (see also Table 5).

Effect of dose schedule on intratumoral injection of mammary carcinoma Sp 4

Although intratumoral injection of *C. parvum* at an early stage of tumor development appeared to be of some benefit (Table 1) it was of interest to see whether the anti-tumor effect could be improved by altering the dose schedule or by using live BCG instead of

Table 4. Effect of C. parvum *presensitisation on intratumoral injection of* C. parvum

Group	Treatment	Survival time in days* (mean survival time)	Significance compared to untreated controls: Survival prolongation—Wilcoxon rank sum test	Significance compared to untreated controls: Incidence of long term survivors—Fisher exact test
I	None	27, 27, 27, 27, 34, 34, 97 (38.4)	—	—
II	1.4 mg intratumoral *C. parvum*. Day 10	34, 55, 85, 97, 118, $3 \times 130^+$ (97.4)	$P<0.005$	NS
III	*C. parvum* presensitised†. Day 6	27, 27, 27, 34, 34, 34, 130^+ (39.9)	NS	NS
IV	*C. parvum* presensitised. Day −6 1.4 mg intratumoral *C. parvum*. Day 10	55, 69, 69, 69, 69, 90, $2 \times 130^+$ (85.1)	$P<0.005$‡	NS

*Tumor cells (2×10^4) injected Day 0 into mammary pad.
†100 μg *C. parvum* SC left foot.
‡Gp IV compared to Group III, $P=0.01$; Gp IV compared to Group II, NS.

Table 5. Effect of BCG presensitisation on intratumoral injection of BCG

Group	Treatment	Survival time in days* (mean survival time)	Significance compared to untreated controls: Survival prolongation—Wilcoxon rank sum test	Significance compared to untreated controls: Incidence of long term survivors—Fisher exact test
I	None	40, 40, 40, 40, 40, 45, 50, 70, 130^+ (55.0)	—	—
II	1.4 mg intratumoral BCG†. Day 13	45, 120, $7 \times 130^+$ (119.4)	$P<0.005$	$P=0.01$
III	BCG presensitisation‡. Day −6	45, 45, 45, 77, 91, 91, 98, 130^+ (77.8)	NS	NS
IV	BCG (presensitisation‡. Day −6 1.4 mg intratumoral BCG†. Day 13	68, 82, 105, 120, $4 \times 130^+$ (111.9)	$P<0.005$§	NS

*Tumor cells (2×10^4) injected Day 0 into mammary pad.
†BCG Connaught multiple puncture—lyophilised.
‡0.05 ml BCG TMC 1011 (1×10^7 viable units, 4 μg dry wt.) SC left flank.
§Group IV compared to Group III, $P=0.02$; Group IV compared to Group II, NS.

killed *C. parvum*. To investigate the effect of BCG and also the effect of altering the timing and amount of immunostimulant used for intratumoral injection the following experiment was performed. Rats were injected with 2×10^4 freshly trypsinised Sp 4 tumor cells in the mammary pad (Day 0) and when tumors were barely palpable, the rats were randomised into six groups: Gp I—no treatment; Gp II—1.4 mg *C. parvum* in 0.2 ml physiological saline Day 9; Gp III—200 μg *C. parvum* in 0.2 ml physiological saline Day 9; Gp IV—1.4 mg *C. parvum* in 0.2 ml physiological saline Day 17; Gp V—1.4 mg BCG Immuno Pasteur F (0.2 ml) diluted with physiological saline Day 9; Gp VI—200 μg BCG Immuno Pasteur F (0.2 ml) diluted with physiological saline Day 9. Tumor growth was followed and survival time assigned to individual rats as described in Materials and Methods.

It can be seen from Table 2 that an early (Day 9) intratumoral injection of *C. parvum*

(1.4 mg) gave significant survival prolongation compared to controls ($P<0.05$) whereas the same dose given on Day 17 and a small dose (200 mg) given on Day 9 did not. However, survival times in these treatment groups (II, III and IV) were not significantly different from each other. A single intratumoral injection on Day 9 of BCG Pasteur F (1.4 mg) was also tried in this system and proved to be highly effective, giving a significant incidence of long-term survivors compared to controls ($P=0.05$). The low dose of BCG did not result in significant survival prolongation, and on this occasion survival time was significantly lower ($P<0.05$) than for the higher dose of BCG.

A further experiment was carried out in order to examine whether multiple intratumoral injections were superior to a single injection. Rats were again injected with 2×10^4 freshly trypsinised Sp 4 tumor cells in the mammary pad (Day 0) and when their tumors were palpable the rats were randomised into six groups: Gp I—no treatment; Gp II—1.4 mg *C. parvum* in 0.2 ml physiological saline Day 9; Gp III—1.4 mg *C. parvum* in 0.2 ml physiological saline Days 9, 15, 23, 29; Gp IV—1.4 mg *C. parvum* in 0.2 ml physiological saline Days 15, 23, 29; Gp V—1.4 mg BCG Connaught Multiple Puncture in 0.2 ml physiological saline Day 9; Gp VI—1.4 mg BCG Connaught Multiple Puncture in 0.2 ml physiological saline Days 9, 15, 23, 29, Tumor growth was followed and survival time assigned to individual rats as described in Materials and Methods.

It can be seen from Table 3 that all these treatments except the one in which the start of intratumoral injection was delayed (Gp IV) gave significant survival prolongation compared with controls. Furthermore, in this experiment intratumoral injection of 1.4 mg *C. parvum* on Day 9 gave a significantly higher incidence of long term survivors than controls ($P=0.05$). When comparisons were made between survival times of groups treated with single or multiple injections of immunostimulant (II vs III, V vs VI) no significant differences were evident at the numbers of rats used (7 per group).

Effect of pre-sensitisation with C. parvum *or BCG on intratumoral injection of mammary carcinoma Sp 4*

The antitumor effect of immunostimulants such as *C. parvum* or BCG in animals sensitised to these organisms depends both on the tumor/host system (compare refs. 2 and 23) and on the type of sensitivity induced [27]. It was therefore of interest to see if the response rate after intratumoral injection of mammary carcinoma Sp 4 with *C. parvum* or BCG could be improved by performing the intratumoral injections in tumor-bearing rats sensitised to these organisms. To this end rats were challenged with *C. parvum* and BCG at various times after sensitisation to find out when they were maximally sensitive to these immunostimulants. As explained in Materials and Methods, this was gauged to be in the third week following sensitisation. Since it is known that following injection of 2×10^4 Sp 4 cells tumors are palpable and suitable for intralesional injection after 9–13 days, rats were pre-sensitised to *C. parvum* or BCG six days (Day −6) before injection of tumor cells on Day 0 so that intratumoral injection was performed when the tumor-bearing rats should have been maximally sensitive to the immunostimulants (Days 10–13). The treatment groups used in these experiments are shown in Table 4. (*C. parvum*) and Table 5 (BCG).

It can be seen from Tables 4 and 5 that pre-sensitisation either to *C. parvum* or BCG did not improve the anti-tumor effector of an intratumoral injection of these immunostimulants. This was particularly striking in the case of *C. parvum* pre-sensitisation where intratumoral injection of *C. parvum* in normal rats gave approximately 50% long term survivors (3/8) but pre-sensitisation could not improve on this.

Effect of intratumoral injection plus surgery on survival of rats bearing mammary carcinoma Sp 4

Although early intratumoral injection of *C. parvum* was consistently successful in significantly prolonging the survival time of mammary carcinoma Sp 4-bearing rats and yielding one-third or more long term survivors, when the intratumoral injection was delayed there was only a slight, and at the numbers of rats used, insignificant prolongation of survival (Tables 2 and 3). It was therefore decided to see if infiltration of *C. parvum* into the primary tumor, at a time when it could affect primary tumor growth to a small extent but did not cause a substantial number of regressions, could influence the development of metastases. A similar apprach has been discussed for the treatment of human breast cancer [9]. In this experiment rats were injected with 2×10^4 freshly trypsinised Sp 4 tumor cells in the mammary pad on Day 0 and then on Day 15 one-half of the tumor-bearing rats were treated with intratumoral

saline, and the other half with intratumoral *C. parvum*. Subsequently (Days 32–35) one-half of each group had their primary tumors excised. Thus, the experimental groups were: I—0.2 ml intratumoral physiological saline Day 15; II—1.4 mg intratumoral *C. parvum* in 0.2 ml physiological saline Day 15; III—0.2 ml intratumoral physiological saline Day 15, excision of primary tumor Day 32–35; IV—1.4 mg intratumoral *C. parvum* in 0.2 ml physiological saline Day 15, excision of primary tumor Day 32–35. Survival time was assigned to individual rats as described in Material and Methods.

It can be seen from Fig. 2 that *C. parvum* (Day 15) alone was able to inhibit to some extent the growth of the primary tumor but did not produce any long term survivors. The inhibition of tumor growth was reflected in a smaller mean tumor diameter at the time of surgery (intratumoral saline, mean tumor diameter 3.0 cm; intratumoral *C. parvum* mean tumor diameter 2.7 cm): the difference, however, was not significant. Furthermore, intratumoral *C. parvum* alone produced a significantly increased survival time compared to rats treated with intratumoral saline (Fig. 2A, $P<0.02$). However, although intratumoral *C. parvum* did prolong survival significantly compared to intratumoral saline, intratumoral *C. parvum* combined with surgery was not better than intratumoral saline combined with surgery (Fig. 2B).

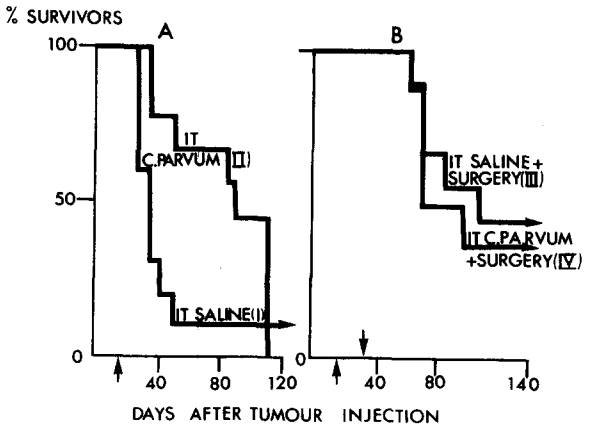

Fig. 2 (A and B). Effect of intratumoral (IT) injection and surgery on survival time of rats bearing Sp 4 tumors. Rats treated with IT injection only (Fig. 2A) were killed when their primary tumor or axillary lymph node metastases reached 3.5 cm mean diameter. Rats treated by IT injection and tumor excision (Fig. 2B) were killed when distressed due to the growth of metastatic tumor (either axillary lymph node or visceral). Eight to ten rats per group. Surviving rats were tumor-free at the termination of the experiment. ↑ Intratumoral injection (either C. parvum or saline), day 15. ↓ Excision of primary tumor growing in mammary pad, days 32–35.

DISCUSSION

A discussion of intratumoral injection in relation to cancer therapy seems to resolve itself into two topics: under what conditions, and with which tumors is it effective? The original report [13] that intratumoral injection could be effective against the transplantable spontaneously arising mammary carcinoma Sp 4 described 100% incidence of long term survivors after multiple intratumoral injections of *C. parvum*. Using slightly different dose regimens we have confirmed that palpable growths of mammary carcinoma Sp 4 can be induced to regress following intratumoral injection although in these experiments the incidence of long term survivors varied from 28 to 77%. Furthermore, it was observed that BCG preparations (Connaught Multiple Puncture and Immuno Pasteur F) were as effective as *C. parvum*, multiple intratumoral injections offered no advantage over a single injection, intratumoral injection was most effective when administered early in primary tumor development, and a high dose of BCG was more effective than a low dose. The difference between a high and low dose of *C. parvum* was not so clear cut. In addition, presensitisation either to BCG or *C. parvum* did not enhance the effect of intratumoral injection of these immunostimulants. This agrees with the findings of Scott [23]; however, these negative results may not apply to all tumors [2].

The recommendations on dosage of BCG are similar to those worked out by investigators using the guinea pig/line-10 hepatoma system [28], another tumor that on rare occasions is known to regress for no apparent reason [29]. Furthermore, when expressed as mg/kg body weight, the dosage of *C. parvum* that produced an anti-tumor effect in these studies was comparable to that reported by Scott in mice [1]. This report also demonstrated that there was an optimal dose of *C. parvum* for intratumoral injection. The results on dosage of immunostimulant appear to severely circumscribe the unity of intratumoral injection as a single treatment. However, animal studies on the combination of intratumoral injection with chemotherapy [6] or surgery [7, 8] are worthy of further consideration to see if such combinations are additive or even synergistic. Unfortunately, we were not able to confirm the encouraging report [7] that a judicious combination of intratumoral *C. parvum* and surgery was able to eradicate lymph node metastases even though the initial report also concerned a rat

mammary carcinoma, albeit carcinogen-induced. Thus, in Fig. 2, we see that intratumoral *C. parvum* plus surgery was no more effective than intratumoral saline plus surgery, despite the fact that intratumoral *C. parvum* alone gave a significantly greater survival time than intratumoral saline alone. This we interpret to mean that whilst *C. parvum* can inhibit the growth of the tumor into which it is injected, in this model it has no inhibitory effect on the development of metastases.

The utility of intratumoral injection is further restricted when one considers the type of tumor against which intratumoral injection is effective. Thus, of four transplantable spontaneously arising tumors studied, only one, mammary carcinoma Sp 4, responded to intratumoral injection [13, 17]. In another study which utilised a range of tumors of varying immunogenicities it was again found that only immunogenic tumors responded to intratumoral injection of BCG [5]. More direct evidence for the involvement of immune mechanisms in the regression of tumors following intratumoral injection of *C. parvum* comes from the observation that regression only takes place in animals with an intact thymus [3].

In conclusion, intratumoral injection of immunological adjuvants such as *C. parvum* and BCG can bring about regression of experimental animal tumors under certain defined conditions. However, whether this form of therapy will be of any benefit to cancer patients will depend on which, if any, human tumors possess the requisite degree of immunogenicity.

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