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Comparative Antitumor Effects of *Corynebacterium parvum, Bordetella pertussis, Bacillus Calmette-Guérin,* and Levamisole Alone or in Combination with Cyclophosphamide in the CaD_2 Murine Mammary Adenocarcinoma System[1]

Dallas M. Purnell,[2] Gerald L. Bartlett,[3] John W. Kreider, Timothy G. Biro, and Joseph Kontra

Department of Pathology [D. M. P.], School of Medicine, University of Maryland, Baltimore, Maryland 21201, and the Departments of Pathology [G. L. B., J. W. K., T. G. B., J. K.] and Microbiology [G. L. B., J. W. K.] and Specialized Cancer Research Center [G. L. B., J. W. K.], The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

ABSTRACT

The antitumor efficacy of various immune stimulants [*Corynebacterium parvum, Bordetella pertussis,* and *Bacillus Calmette-Guérin* (BCG)] and levamisole alone or in conjunction with cyclophosphamide (CY) was studied in the CaD_2 mammary adenocarcinoma system using schedules developed previously with *C. parvum* and CY.

Weekly systemic treatment with *C. parvum, B. pertussis,* or BCG was effective in controlling tumor growth and had equivalent antitumor effects, but weekly treatment (or a single treatment) with levamisole was ineffective. Weekly treatment with *B. pertussis* was better than treatment given only once, but repeated treatment with *C. parvum* or BCG was not more effective than a single treatment with these agents. When administered as a single systemic injection, *C. parvum* was superior to *B. pertussis* in controlling tumor growth, but a single systemic injection of BCG was as effective as *C. parvum.* Systemic administration of immune stimulants had variable effects on survival, which were sometimes not correlated with effects on tumor size.

Combined treatment with BCG and CY was significantly more effective than CY treatment alone in controlling tumor growth in most trials, as was combination treatment with *C. parvum* and CY. Combination treatment with *B. pertussis* and CY was not better in prolonging survival than CY alone. Levamisole rarely improved the antitumor effect of CY chemotherapy and had no effect on survival compared to the effect of CY alone.

INTRODUCTION

We have described the antitumor activity of systemically administered killed *Corynebacterium parvum* alone (8) or in conjunction with CY[4] (6) in a transplantable murine mammary adenocarcinoma (CaD_2) system, and we have reported the influence of dose and schedule of immune stimulant on the efficacy of combination treatment with these agents in the CaD_2 system (5). Those studies showed that combination treatment with *C. parvum* and CY was significantly more effective in controlling tumor growth than either the immune stimulant or cytotoxic drug treatment alone. Optimal effects of combined *C. parvum*-CY treatment in this system were obtained when 443 to 1400 μg of *C. parvum* per mouse were injected 2 to 3 days after CY (45 mg/kg) and when combination treatment was continued on a weekly basis (5). In order to extend those observations to other immune stimulants, we have now studied the antitumor activity of *Bordetella pertussis,* LMS, and several strains of BCG, alone and in combination with CY using schedules developed in the *C. parvum*-CY system. In most experiments, the results were compared to those obtained with *C. parvum* alone or with *C. parvum*-CY treatment.

MATERIALS AND METHODS

Mice. Female BALB/c × DBA/2 F_1 mice (hereafter called $CD2F_1$) were obtained from Charles River Breeding Laboratory, Wilmington, Mass., through the auspices of the Drug Research and Development Branch of the National Cancer Institute, Bethesda, Md.

Tumor. The poorly differentiated, spontaneous mammary adenocarcinoma CaD_2 was obtained from The Jackson Laboratory, Bar Harbor, Maine. The CaD_2 tumor is maintained in our laboratory by serial s.c. passage of tumor cell suspensions (10^6 cells/0.1 ml) in female $CD2F_1$ mice. Cell suspensions of CaD_2 were prepared enzymatically as previously described (7). Cell concentrations for injection were determined by hemocytometer counts of trypan blue-excluding cells; viability was usually >95%. Inocula (s.c.) of 5×10^4 viable tumor cells were tumorigenic in 100% of mice; tumors usually became palpable 5 to 7 days after inoculation.

Immune Stimulants. Suspensions of killed *C. parvum* CN6134) containing 7 mg (dry weight) bacteria per ml were provided by Burroughs Wellcome and Co., Research Triangle Park, N. C. Pertussis vaccine ("fluid") containing 4×10^{10} killed *B. pertussis* bacteria per ml was obtained from Eli Lilly and Co., Indianapolis, Ind. Frozen suspensions of BCG were purchased from the Trudeau Institute, Saranac Lake, N. Y. The strains used were: Phipps strain (TMC-1029, Lot NC-11); Tice strain (TMC-1032, Lot NC-4474); Pasteur strain (TMC-1011, Lot NC-741); Connaught strain (TMC-266-1, Lot NC-41774); and Glaxo strain (TMC-1024, Lot NC-2574). Vials were shipped and stored at −70° and were warmed rapidly to 37° just before use. Lyophilized Tice strain [Lot IL74(s)65] was purchased from the Institution for Tuberculosis Research, Chicago, Ill., and was reconstituted according to the manufac-

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[2] To whom requests for reprints should be addressed.

[3] Recipient of USPHS Career Development Award K04-CA70948 from The National Cancer Institute.

[4] The abbreviations used are: CY, cyclophosphamide; LMS, levamisole; BCG, *Bacillus Calmette-Guérin*; KO, killed organisms.

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turer's instructions. LMS was the gift of Janssen Research and Development, Inc., New Brunswick, N. J. Dilutions of immune stimulants, when required, were prepared in sterile 0.9% NaCl solution.

Cytotoxic Drug. CY powder was purchased from Mead Johnson and Company, Evansville, Ind. The stock solution and dilutions of CY were prepared in sterile 0.9% NaCl solution immediately prior to use.

Tumor Mensuration. The length and width of tumors were measured 2 to 3 times/week with calipers, and tumor size was expressed as the mean product ±S.E. of 2 diameters in sq mm.

Statistical Tests. Tumor sizes in experiments were analyzed when tumors in control groups were ~200 sq mm. One-way analysis of variance and the Newman-Kuels or Dunette test for comparison of groups in multigroup experiments were used for this purpose. Survival of tumor-bearing mice was expressed as the mean survival time in days, and differences in the mean survival time of different groups were assessed by the Mann-Whitney *U* test.

RESULTS

Systemic Treatment with Immune Stimulant Alone

B. pertussis. We first studied the antitumor activity of BCG, *B. pertussis*, and LMS as sole treatment for the CaD_2 tumor. This was done in order to find suitable doses of these agents for use in combination treatment experiments. In most cases, *C. parvum* was included in these experiments for comparative purposes. Table 1 shows the influence of systemic treatment with *B. pertussis* on the growth of CaD_2 tumors. Weekly i.p. injections of 8×10^9 KO *B. pertussis* significantly slowed tumor growth in comparison to tumor growth in mice given no treatment. However, a single i.p. injection of 8×10^9 KO *B. pertussis* or weekly treatment with 8×10^8 or 8×10^7 KO *B. pertussis* had no significant effect on tumor growth. Either single or weekly treatment with *C. parvum* (1400 μg/mouse) significantly retarded tumor growth in comparison to untreated mice. The latter treatment was equivalent to weekly *B. pertussis* (8×10^9 KO) in its effect on tumor growth. Single or weekly treatment with *C. parvum* significantly improved survival of tumor-bearing mice compared to untreated mice, as did weekly treatment with 8×10^9 or 8×10^7 KO *B. pertussis*.

BCG. We next compared the antitumor activity of several strains of BCG when administered as single i.v. or i.p. injections of 2×10^7 colony-forming units/mouse (Table 2). Tumor growth was significantly retarded by single i.v. injections of viable lyophilized Tice and frozen Phipps BCG and by a single i.v. injection of *C. parvum*. In contrast, heat-killed lyophilized Tice BCG and the other frozen BCG strains had no significant effect on tumor growth compared to untreated mice. Mice treated i.p. with viable or heat-killed, lyophilized Tice BCG developed larger tumors than did those treated i.v. with viable lyophilized Tice BCG, but they did not differ from untreated mice. *C. parvum* was not significantly better in controlling tumor growth than was either of the effective BCG strains. None of the treatments had any significant effect on the survival of tumor-bearing mice (cf. 1–3). To obtain additional information on the importance of route and frequency of injection to the effectiveness of BCG treatment, we determined the effect of frozen Phipps or lyophilized Tice BCG on tumor growth when administered as a single injection (i.v. or i.p.) or as weekly i.p. injections (Table 3). The results indicated that the tumor-inhibitory effect obtained by a single i.v. injection of either BCG strain was not significantly different from the effect obtained by

Table 1

Effect of systemic treatment with B. pertussis or C. parvum on growth of CaD_2 tumors

Treatment beginning on Day 3[a]					
Single	Weekly	Dose	No. of mice	Tumor size (sq mm) on Day 20	Mean survival time (days)
No treatment			10	209 ± 21[b]	31
C. parvum		1400 μg	10	77 ± 28[c]	34[d]
	C. parvum	1400 μg	10	27 ± 7[c]	46[d]
B. pertussis		8×10^9 KO	8	131 ± 21	30
	B. pertussis	8×10^9 KO	9	76 ± 18[c]	40[d]
	B. pertussis	8×10^8 KO	10	165 ± 29	31
	B. pertussis	8×10^7 KO	8	140 ± 26	36[d]

[a] All mice received 5×10^4 CaD_2 cells s.c. on Day 0.
[b] Mean ± S.E.
[c] $p < 0.05$ (Newmann-Kuels test) compared to untreated mice.
[d] $p < 0.05$ (Mann-Whitney *U* test) compared to untreated mice.

Table 2

Effect of different BCG preparations on growth of CaD_2 tumors

Treatment beginning on Day 3[a]	Route of injection	No. of mice	Tumor size (sq mm) on Day 21	Mean survival time (days)
No treatment		10	261 ± 25[b]	33
C. parvum	i.v.	10	124 ± 15[c]	28
B. pertussis	i.v.	8	194 ± 23	27
LY[d] Tice	i.p.	8	291 ± 23[e]	25
LY Tice HK	i.p.	8	280 ± 22[e]	29
LY Tice	i.v.	9	164 ± 17[c]	31
LY Tice HK	i.v.	7	244 ± 16	28
FF Tice	i.v.	9	182 ± 17	37
FF Phipps	i.v.	9	163 ± 24[c]	33
FF Pasteur	i.v.	8	210 ± 10	25
FF Connaught	i.v.	8	208 ± 17	27
FF Glaxo	i.v.	8	246 ± 16	27

[a] All mice received 5×10^4 CaD_2 cells s.c. on Day 0. BCG dose in all cases was 2×10^7 colony-forming units/mouse; *C. parvum* dose was 1400 μg/mouse; *B. pertussis* dose was 8×10^9 KO/mouse.
[b] Mean ± S.E.
[c] Tumors significantly ($p < 0.05$; Newmann-Kuels test) smaller than in untreated controls.
[d] LY, lyophilized; HK, heat killed (60° for 1 hr); FF, fresh frozen.
[e] Tumors significantly ($p < 0.05$; Newmann-Kuels test) larger than in mice treated i.v. with LY Tice.

Table 3

Effect of single or repeated treatment with BCG on growth of CaD_2 tumors

Treatment beginning on Day 3[a]					
Single	Weekly	Route	No. of mice	Tumor size (sq mm) on Day 21	Mean survival time (days)
No treatment			10	225 ± 14[b]	30
FF[c] Phipps BCG		i.v.	10	76 ± 11[d]	41[e]
FF Phipps BCG		i.p.	8	119 ± 15[d]	51[e]
LY Tice BCG		i.v.	10	75 ± 13[d]	44[e]
LY Tice BCG		i.p.	10	128 ± 26[d]	38[e]
	FF Phipps BCG	i.p.	9	111 ± 20[d]	35
	LY Tice BCG	i.p.	10	102 ± 20[d]	37

[a] All mice received 5×10^4 CaD_2 cells s.c. on Day 0. BCG dose was 2×10^7 colony-forming units/mouse.
[b] Mean ± S.E.
[c] FF, fresh frozen; LY, lyophilized.
[d] $p < 0.05$ (Newmann-Kuels test) compared to untreated mice; no other comparisons were significant.
[e] $p < 0.05$ (Mann-Whitney *U* test) compared to untreated mice.

a single i.p. injection of these BCG preparations. Weekly i.p. treatment with these BCG strains was no better than single i.v. or i.p. treatments in controlling tumor growth. With the exception of weekly BCG treatment, all treatments significantly improved survival of tumor-bearing mice.

LMS. The antitumor activity of LMS was studied by administering different doses of this agent to tumor-bearing mice as single i.p. injections. Seventy mice were given 5×10^4 CaD_2 cells s.c. and divided into 7 groups of 10 mice each. All treatments were given after 3 days of tumor growth. One group of mice received no treatment. A second group was treated with *C. parvum* (1400 μg), and the remaining groups of mice were treated with LMS at dose levels of 6, 12, 24, 48, and 72 mg/kg. As in other experiments, *C. parvum* significantly retarded tumor growth. In contrast, low doses of LMS (6 to 24 mg/kg) had no effect on tumor growth, and higher doses (48 and 72 mg/kg) were lethal within 5 to 10 min of injection (data not shown).

Direct Comparison of Immune Stimulants. In the next 2 experiments (Table 4), we compared the antitumor effectiveness of LMS, *B. pertussis*, BCG, and *C. parvum* as sole treatment for CaD_2 tumors. Tumor growth in the untreated mice was slower than usual in these experiments. In both experiments, all the immune stimulants except LMS significantly slowed tumor growth in comparison to growth in untreated mice. *B. pertussis*, BCG, and *C. parvum* caused equivalent tumor inhibition in each experiment. With the exception of *B. pertussis* in the first experiment, none of the treatments significantly improved survival of tumor-bearing mice over those given no treatment.

Systemic Combination Treatment with Immune Stimulant and CY

***B. pertussis*-CY.** We selected a dose of 8×10^9 *B. pertussis* KO, which was an optimal dose for single-agent treatment, and used it to study the antitumor activity of *B. pertussis* combined with CY (Table 5). In the first experiment, all treatments caused significant slowing of tumor growth compared to mice receiving no treatment. Combination treatment with *B. pertussis* and CY, with immune stimulant given first, produced significantly greater tumor inhibition than treatment with either agent alone. Combination treatment with these agents in the reverse sequence was more effective than *B. pertussis* alone, but it was not significantly better than CY alone. CY treatment given on Day 3 and both combination treatments significantly improved survival of tumor-bearing mice in comparison to mice given no treatment, but other treatments had no effect on survival. Survival of mice given combination treatment was not significantly different from that of mice given CY alone. In the second experiment (Table 5), mice treated with either *B. pertussis*, CY, or with both agents had significantly smaller tumors than did untreated mice, but combination treatment was not significantly more effective than CY treatment alone. Survival of mice given combination treatment was significantly prolonged compared to mice given either treatment alone.

BCG-CY. Using a dose of 2×10^7 colony-forming units/mouse of Tice BCG, which was optimal as single-agent treatment for CaD_2 tumors, we studied in 2 separate experiments the relative effectiveness of treatment with *C. parvum* or BCG combined with CY (Table 6). In the first experiment, combination treatment with BCG and CY, with immune stimulant given first, caused significantly more tumor inhibition than did treat-

Table 4

Direct comparison of 4 immune stimulants as sole treatment for CaD_2 tumors

Weekly treatment begun on Day 6[a]	Tumor size (sq mm) Experiment 1 on Day 28	Tumor size (sq mm) Experiment 2 on Day 29	Mean survival time (days) Experiment 1	Mean survival time (days) Experiment 2
No treatment	204 ± 21[b]	178 ± 56	41	44
LMS	174 ± 32	77 ± 25	43	57
B. pertussis	80 ± 25[c]	60 ± 17[c]	45[d]	50
BCG	84 ± 15[c]	9 ± 6[c]	34	31
C. parvum	47 ± 15[c]	28 ± 13[c]	44	57

[a] All mice received 5×10^4 CaD_2 cells s.c. on Day 0. LMS, 12 mg/kg i.p.; *B. pertussis*, 8×10^9 KO/mouse i.p.; BCG (lyophilized Tice), 2×10^7 colony-forming units/mouse i.p.; *C. parvum*, 1400 μg/mouse i.p. Ten mice/group were used.
[b] Mean ± S.E.
[c] $p < 0.05$ (Dunette test) compared to untreated controls.
[d] $p < 0.05$ (Mann-Whitney *U* test) compared to untreated controls.

Table 5

Effect of combination treatment with B. pertussis and CY on growth of CaD_2 tumors

Weekly treatment beginning at the following times[a] Day 3	Day 6	Tumor size (sq mm) on Day 20 Experiment 1	Tumor size (sq mm) on Day 20 Experiment 2	Mean survival time (days) Experiment 1	Mean survival time (days) Experiment 2
No treatment		195 ± 14[b]	287 ± 20	32	29
CY		56 ± 6[c]		39[d]	
	CY	118 ± 13[c]	81 ± 10[c]	38	35
B. pertussis		81 ± 14[c]	162 ± 53[c]	34	37
	B. pertussis	113 ± 16[c]		36	
B. pertussis	CY	30 ± 5[c, e]	24 ± 5[c]	41[d]	52[d, f]
CY	*B. pertussis*	31 ± 5[c]		41[d]	

[a] All mice received 5×10^4 CaD_2 cells s.c. on Day 0. CY, 45 mg/kg i.p.; *B. pertussis*, 8×10^9 KO/mouse.
[b] Mean ± S.E.
[c] $p < 0.05$ (Newmann-Kuels test) compared to untreated mice.
[d] $p < 0.05$ (Mann-Whitney *U* test) compared to untreated mice.
[e] $p < 0.05$ (Newmann-Kuels test) compared to either single agent alone.
[f] $p < 0.05$ (Mann-Whitney *U* test) compared to either single agent alone.

Table 6

Effect of BCG or C. parvum combined with CY on growth of CaD_2 tumors

Weekly treatment beginning at the following times[a] Day 3	Day 6	Tumor size (sq mm) Experiment 1 on Day 20	Tumor size (sq mm) Experiment 2 on Day 22	Mean survival time (days) Experiment 1	Mean survival time (days) Experiment 2
No treatment		200 ± 18[b]	232 ± 23	27	33
CY		50 ± 8[c]	100 ± 15[c]	38	43
	CY	101 ± 12[c]	89 ± 12[c]	34	46
BCG		91 ± 11[c]	114 ± 22[c]	31	37
	BCG	147 ± 18[c]	131 ± 13[c]	34	34
C. parvum		69 ± 6[c]		31	
	C. parvum	62 ± 8[c]		37	
CY	BCG	37 ± 7[c]	15 ± 5[c, d]	45	56
BCG	CY	35 ± 5[c, d]	39 ± 10[c, d]	39	54
CY	*C. parvum*	18 ± 4[c]	11 ± 4[c, d]	49[e]	53
C. parvum	CY	14 ± 4[c, f]		44	

[a] All mice received 5×10^4 CaD_2 cells s.c. on Day 0. CY, 45 mg/kg i.p.; BCG (lyophilized Tice), 2×10^7 colony-forming units/mouse i.p.; *C. parvum*, 1400 μg/mouse i.p.
[b] Mean ± S.E.
[c] $p < 0.05$ (Newmann-Kuels test) compared to untreated mice.
[d] $p < 0.05$ (Newmann-Kuels test) compared to CY or immune stimulant alone.
[e] $p < 0.05$ (Mann-Whitney *U* test) compared to CY on Day 3.
[f] $p < 0.05$ (Newmann-Kuels test) compared to CY on Day 6.

ment with either agent alone. However, combination treatment with the agents given in the reverse order was not more effective than CY treatment alone. Combination treatment with *C. parvum* and CY, with immune stimulant given prior to chemotherapy, was more effective than treatment with CY alone, but it was not more effective than treatment with *C. parvum* alone. As with BCG-CY, combination treatment with these agents in the reverse order was not more effective than treatment with CY alone. Only combination treatment with *C. parvum* and CY (CY given first) significantly prolonged survival of mice. In the second experiment, combination treatment with BCG and CY, with immune stimulant given before or after chemotherapy, and combination treatment with *C. parvum* and CY, with immune stimulant given after chemotherapy, were all significantly more effective than single agent treatment with immune stimulant or CY. In neither experiment was there a significant difference between the effects of BCG and *C. parvum* in combination treatment. Although the survival times of mice given the combined treatments appeared prolonged compared to that of untreated mice, the differences were not statistically significant.

LMS-CY. Although no dose of LMS studied inhibited tumor growth when administered alone, we selected a dose of 12 mg/kg of this agent to examine the antitumor effect of LMS when combined with CY (Table 7). In this experiment, each agent was given as a single i.p. injection. Mice that were treated with LMS alone had tumor sizes similar to those of untreated mice, whereas those treated with CY had significantly smaller tumors than did untreated mice. Combination treatment using CY and either LMS or *C. parvum* resulted in significantly better tumor control than that obtained by CY treatment alone, but CY combined with *C. parvum* was significantly more effective than CY combined with LMS. Only combined treatment with CY and *C. parvum* significantly improved survival of tumor-bearing mice over that of mice given no treatment.

Direct Comparison of Immune Stimulants Combined with CY. In the final 2 experiments, which were performed as a part of those reported in Table 4, we compared the antitumor effects of LMS, *B. pertussis*, *C. Parvum*, and BCG in combination with CY as treatment for the CaD_2 tumor (Table 8). To facilitate comparison of these experiments with others reported in this paper, we have reported these data after 38 to 42 days of growth when mean tumor size in CY-treated mice was near 200 sq mm. Combination treatment with BCG and CY (in the first experiment) or with *C. parvum* and CY (in both experiments) resulted in significantly smaller tumors compared to mice given only CY treatment. Treatment with *B. pertussis* or LMS combined with CY was not more tumor inhibitory than treatment with CY alone. No combination treatment improved survival of tumor-bearing mice in comparison to mice given only CY treatment.

Table 7

Effect of LMS or C. parvum combined with CY on growth of CaD_2 tumors

Single treatment beginning at the following times[a]		No. of mice	Tumor size (sq mm) on Day 19	Mean survival time (days)
Day 3	Day 6			
No treatment		9	128 ± 11[b]	40
CY		10	90 ± 13[c]	36
	LMS	9	138 ± 12	36
CY	LMS	15	59 ± 9[c, d]	37
CY	*C. parvum*	15	23 ± 6[c, d, e]	45[f]

[a] All mice received 5 × 10^4 CaD_2 cells s.c. on Day 0. CY, 45 mg/kg i.p.; LMS, 12 mg/kg i.p.; *C. parvum*, 1400 μg/mouse i.p.
[b] Mean ± S.E.
[c] $p < 0.05$ (Newmann-Kuels test) compared to untreated or LMS-treated mice.
[d] $p < 0.05$ (Newmann-Kuels test) compared to mice treated with CY alone.
[e] $p < 0.05$ (Newmann-Kuels test) compared to mice treated with LMS and CY.
[f] $p < 0.05$ (Mann-Whitney *U* test) compared to untreated mice or mice treated with CY alone.

Table 8

Direct comparison of immune stimulants used in combination with CY to treat CaD_2 tumors

Weekly treatment beginning at the following times[a]		Tumor size (sq mm)		Mean survival time (days)	
Day 3	Day 6	Experiment 1 on Day 38	Experiment 2 on Day 42	Experiment 1	Experiment 2
CY		198 ± 92[b]	142 ± 67	51	63
CY	LMS	205 ± 42	171 ± 42	42	58
CY	*B. pertussis*	157 ± 28	185 ± 80	47	77
CY	BCG	63 ± 22[c]	50 ± 23	55	34
CY	*C. parvum*	100 ± 25[c]	22 ± 9[c]	51	80

[a] All mice received 5 × 10^4 CaD_2 cells s.c. on day 0. CY, 45 mg/kg i.p.; LMS, 12 mg/kg i.p.; *B. pertussis*, 8 × 10^9 KO/mouse i.p.; BCG, 2 × 10^7 colony-forming units/mouse i.p.; *C. parvum*, 1400 μg/mouse i.p. Ten mice/group were used.
[b] Mean ± S.E.
[c] $p < 0.05$ (Dunette test) compared to mice treated with CY alone.

DISCUSSION

The antitumor efficacy of various immune stimulants (*C. parvum*, *B. pertussis*, LMS, and several strains of BCG) alone and in conjunction with CY was studied in the CaD_2 mammary adenocarcinoma system using schedules developed previously with *C. parvum* and CY (5, 6). When the immune stimulants were given systemically (i.v. or i.p.) as the sole treatment, LMS was consistently ineffective, but each of the other agents had significant effects on tumor size and survival under certain circumstances. *C. parvum* had the most consistent effects, and it caused the greatest tumor inhibition in any given experiment. However, interagent differences were not statistically significant. It is possible that *B. pertussis* or BCG would have had greater or more consistent therapeutic effects under other schedules, since they were tested in a protocol optimized for therapeutic effects of *C. parvum*. Repeated injections of the agents caused slight but not significant improvement in therapeutic effects.

When treatment consisted of CY (45 mg/kg i.p.) and an immune stimulant, given 3 days apart, each stimulant was able to make a significant contribution to the therapeutic effect. As in the single-agent protocol, differences between agents were usually not statistically significant. *C. parvum* and BCG were consistently effective, while *B. pertussis* and LMS were inconsistent in their effects when combined with CY. The reason for the inconsistency of some agents and not others is not known.

We have noted in several experiments that significant inhibition of tumor growth was not always associated with significant prolongation of survival. Conversely, in a few instances, treatment that did not significantly alter tumor size prolonged survival significantly. It seems fair to conclude that death is not a simple function of tumor size in this model, that the effects of

the treatments are complex, and that some treatments may alter the 2 end points independently.

The antitumor efficacy of different immune stimulants administered alone or in combination with chemotherapy in a single tumor system has been little studied. Fisher *et al.* (4) compared the effects of a number of immune stimulants, including *C. parvum*, BCG, and LMS, with and without combination with CY in a C3H mammary carcinoma system. In their experiments, BCG had slight antitumor activity when administered alone, but it failed to significantly improve the effect of CY on tumor growth. LMS had no effect on tumor growth when given alone, and it failed to improve the antitumor effects of CY. The results were in contrast to those obtained with *C. parvum* which had antitumor activity when given alone and strongly improved the effect of CY chemotherapy.

We also found that *C. parvum* was superior to other immune stimulants in augmentation of the effect of CY chemotherapy; however, certain of our results differed from those of Fisher *et al.* (4). For example, we found that, under certain conditions of dose and schedule, *B. pertussis* and certain strains of BCG were as effective as *C. parvum* in inhibiting tumor growth when these agents were administered as sole treatment for the CaD_2 tumor. Similarly, we found that, when BCG was combined with CY, it was equivalent to *C. parvum* in improving the effect of CY on tumor growth. BCG was, however, inferior to *C. parvum* in prolonging survival of tumor-bearing mice over those receiving only chemotherapy.

We conclude that in the CaD_2 tumor system *C. parvum*, BCG, and *B. pertussis* administered systemically significantly inhibited tumor growth. Optimal effects on tumor growth were obtained when these agents were administered in conjunction with CY. *C. parvum* was superior to the other immune stimulants studied in augmenting the effects of CY drug therapy, both in reducing tumor growth rates and in prolonging survival of tumor-bearing mice.

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