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EFFECTS OF *CORYNEBACTERIUM PARVUM* ON MURINE MYELOID LEUKAEMIA

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Summary.—The effects of *C. parvum* on RFM/UN myeloid leukaemia were studied. Mice inoculated with 7·0 mg but not 0·7 mg *C. parvum* i.p. survived significantly longer than untreated leukaemic mice ($P < 0.001$). Administration of silica abrogated the effects of *C. parvum*, whilst polyvinyl pyridine-N-oxide prevented the inhibitory effects of silica. These studies demonstrate that a single large dose of *C. parvum*, either before or after leukaemic-cell passage, can significantly prolong the survival of RFM mice bearing myeloid leukaemia. The effects of silica and PVNO on *C. parvum* suggest a critical role for macrophages in *C. parvum* effects on myeloid leukaemia.

C. Parvum is a non-specific stimulator of the immune system which has been shown to exert antitumour activity in both animal (Currie & Bagshawe, 1970; Scott, 1974*a*; Fisher *et al.*, 1975; Suit *et al.*, 1977; Houchens *et al.*, 1976) and human tumours (Scott, 1974*b*; Israël *et al.*, 1975; Fisher *et al.*, 1976).

In this communication we report our initial studies of the effects of *C. parvum* on murine myeloid leukaemia (Preisler *et al.*, 1977). The effects of this immune stimulator are dose-dependent and demonstrable by the prolongation of survival of leukaemic mice. The administration of silica prevents the beneficial effects of *C. parvum* on this leukaemia, whilst polyvinyl pyridine N-oxide (PVNO) abrogates the inhibitory effects of silica.

MATERIALS AND METHODS

A. *Mice and tumour.*—Inbred RFM/UN female mice were used in these experiments. The mice were bred in our own animal facility. The pathophysiology of this murine leukaemia has been previously described (Preisler *et al.*, 1977). Mice were inoculated i.v. with spleen-cell suspensions from terminal leukaemic RFM mice, with the appropriate number of cells suspended in 0·2 ml saline. There were 5 mice in each experimental group, and food and water were allowed *ad lib.* At death, internal organs were inspected and livers and spleens weighed and fixed in formalin for histopathological studies. Each experiment was performed at least 3 times.

B. *C. parvum.*—The *C. parvum* which was kindly furnished by Burroughs Wellcome Company Research, Triangle Park, N.C., U.S.A., contained 7 mg dry weight of formalin-killed organisms per millilitre, suspended in 0·9% NaCl solution. Within each experiment varying amounts of *C. parvum* were always injected by the same route and in the same total volume.

C. *Silica, polyvinyl pyridine N-oxide (PVNO).*—In some experiments mice received either silica (2·5 mg in 1 ml PBS) i.v. on the day of tumour transplantation (*i.e.* 2 days before inoculation of *C. parvum* where applicable) and/or *PVNO* (4 mg in 0·1 ml PBS) s.c. one day before tumour transplantation.

RESULTS

Effects of administration of C. parvum before or after passage of disease

Mice were inoculated i.p. with varying

doses of *C. parvum* 2 days before or after inoculation with 10^4 leukaemic spleen cells. No increase in life span was detected with the lowest doses of *C. parvum* (0·07 or 0·35 mg). With increasing doses there was a corresponding increase in life-span, with the 7 mg dose providing the greatest increase (Table I). At this

TABLE I.—*Effects of differing doses of* C. parvum *on the survival of leukaemic mice*

C. parvum		Median survival time		
Dose (mg)	Day	Days	Range	%ILS*
Untreated controls		16		—
7·0	−2	19	(17–20)	19
3·5	−2	19	(17–21)	19
0·7	−2	18	(17–18)	12·5
0·35	−2	18	(17–18)	12·5
0·07	−2	16·5	(16–18)	3
0·07	+2	17	(16–18	6
3·5	+2	20	(18–20)	25
0·7	+2	18	(17–21)	12·5
0·35	+2	16	(16–18)	0
7·0	+2	24	(19–26	50

*Increase in life span.

dose level, *C. parvum* was effective in prolonging survival whether administered before or after the passage of the leukaemia.

Effects of C. parvum on established disease

Mice were inoculated with either of 2 doses of *C. parvum* 2 days after receiving 10^4 leukemic cells. Consistent with the previous experiment (Table I), the average life span of mice treated with 0·7 mg of *C. parvum* was only slightly increased, whilst the survival of mice given 7 mg was significantly prolonged (Table III). A life-table graph combining the results of these experiments is shown in Fig. 1. While the difference between the survival of mice inoculated with 0·7 mg of *C. parvum* and the control mice was not statistically significant ($P > 0·05$), the difference in survival between mice receiving 7 mg of *C. parvum* and the control

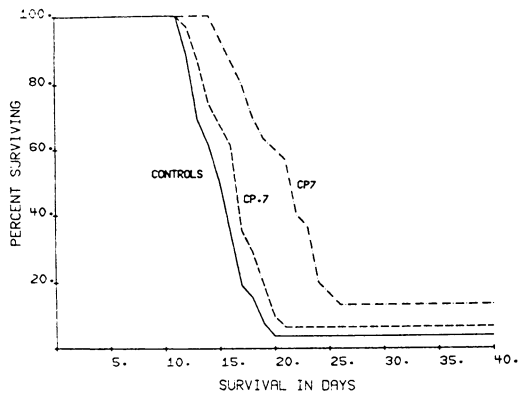

FIG. 1.—Cumulative survival for mice in Experiments 1–5 (Table III).

mice was highly significant ($P < 0·001$, Breslow test).

Histopathological studies

At the time of death, control leukaemic mice and those inoculated with *C. parvum* had massive hepatosplenomegaly, with diffuse infiltration by leukaemic cells. Leukaemic-cell thrombi of the pulmonary arteries appeared to be the immediate cause of death of both treated and control mice (Fig. 2). Mice which had been inoculated with *C. parvum* differed from control mice in that organ infiltration by leukaemic cells was less. There was evidence of significant tissue necrosis only in the spleens and livers of mice which had been inoculated with *C. parvum*. The histopathological findings in the mice receiving 0·7 and 7·0 mg *C. parvum* were indistinguishable at the time of death (Fig. 3).

Effects of silica and PVNO on leukaemic mice inoculated with C. parvum

In 3 of the experiments described in Table II, groups of mice received silica and/or PVNO in addition to or instead of *C. parvum*. Table III gives the median survival times and ranges for these mice. On an *a posteriori* contrast test, the least-significant-difference test was used

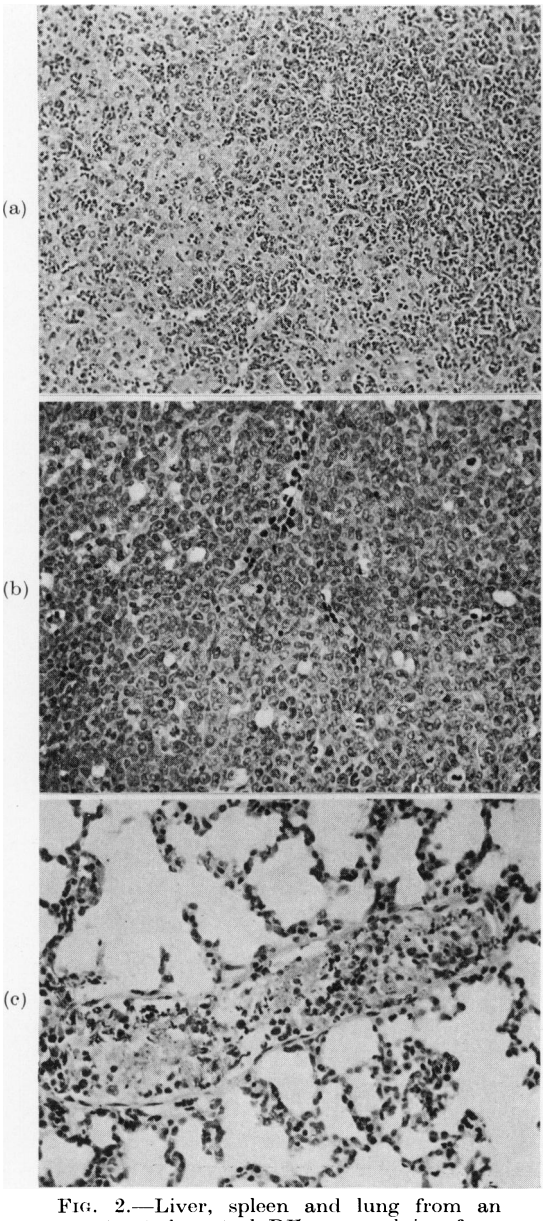

FIG. 2.—Liver, spleen and lung from an untreated control RF mouse dying from myeloid luekaemia. Haematoxylin and eosin. (a) Massive leukaemic infiltration of the liver. (b) Replacement of normal splenic architecture by leukaemic cells. (c) A pulmonary capillary thrombus composed of leukaemic cells, erythrocytes, fibrin, and necrotic cells.

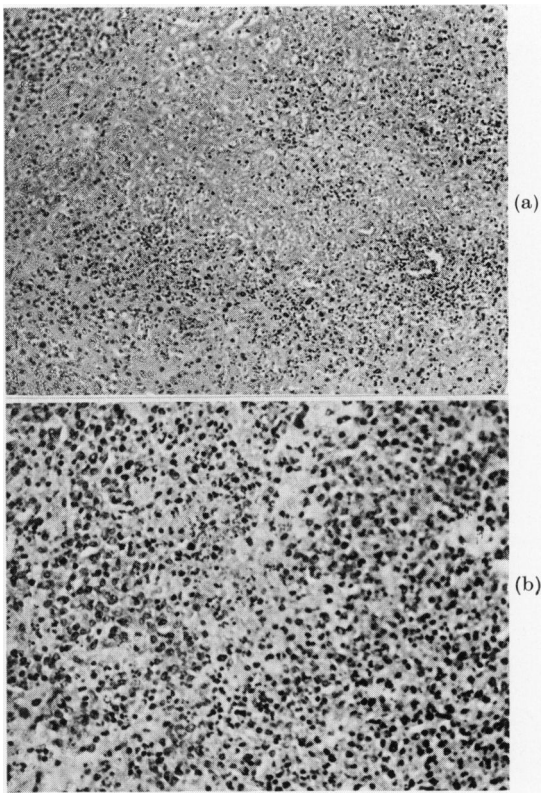

FIG. 3.—Liver and spleen from a mouse treated with 0·7 mg *C. parvum* 2 days after inoculation with 10^4 leukaemic spleen cells i.v. (a) Extensive necrosis of liver tissue and sparse infiltration by leukaemic cells. (b) Numerous necrotic cells, normal spleen cells and rare leukaemic cells.

to determine which experimental groups were significantly different from each other.

The administration of silica, PVNO, or PVNO + silica had no effect on the survival of mice, compared to the control group. By contrast, survival was significantly prolonged in mice which received *C. parvum* or PVNO + *C. parvum*. The administration of silica to mice which received *C. parvum* abrogated the increase in survival produced by *C. parvum*. The administration of PVNO to mice which received silica and *C. parvum* abrogated the inhibitory effects of silica and restored the beneficial effects of *C. parvum*. The observation that mice which received *C. parvum* together with PVNO survived the longest is of interest.

TABLE II.—*Effects of 2 doses of* C. parvum (CP) *on the survival of leukaemic mice*

Expt	1				2				3			
	#	MST	Range	% ILS	#	MST	Range	% ILS	#	MST	Range	% ILS
Control	5	21	11–19	—	5	13	12–14	—	5	15	14–16	—
CP 0·7 mg	5	18	16–20	6	5	13	12–14	0	5	12	11–13	—
CP 7 mg	5	21	14–23	24	5	23	16–23	77	5	18	14–25	20

Expt	4				5				Total (5 expts)			
	#	MST	Range	% ILS	#	MST	Range	% ILS	#	MST	Range	% ILS
Control	5	14	12–14	—	5	16	15–*[1]	—	25	15	11–19*[1]	—
CP 0·7 mg	10	16	15–19	14	5	18	16–*[2]	19	30	16	11–20*[2]	7
CP 7 mg	10	20·5	16–*[3]	46	5	21	15–*[1]	31	30	20·5	14–25*[4]	37

Mice were inoculated with 10^4 leukaemic cells. Two days later they received 0·7 mg or 7·0 mg *C. parvum* i.p.

* Asterisk with a number indicates survivors at Day 90.

\# Number of mice.

MST Median survival time (days).

ILS Increase in life span.

TABLE III.—*Effect of silica and PVNO on anti leukaemia efficacy of* C. parvum

	I		II		III	
	MST	Range	MST	Range	MST	Range
1. Control	17	11–19	15	14–16	15	14–16
2. CP	21	14–23	18	14–25	21	16–25
3. Si	16		13	11–13	18	16–18*
4. Si+CP	15	10–20	16	14–17	13	11–21
5. P	16	15–17	12	12–13	17	17–19
6. P+Si	17	15–19	14	13–15	32	18–32†
7. P+CP	24	15–32	20	16–73*	19	13–28
8. P+Si+CP	22	21–24	17	16–18	26	20–26†

*One mouse survived > 90 days.

†Two mice survived > 90 days.

Mice received the various treatments as described in the Methods section. Statistical analysis demonstrated that mice in Groups 2 and 8 lived significantly longer than mice in Groups 1, 3, 4, 5, 6 ($P < 0·05$) and mice in Group 7 lived longer than mice in Groups 2 and 8 ($P < 0·05$).

DISCUSSION

The studies demonstrate that the administration of a single large dose of *C. parvum* can significantly prolong survival in RFM mice bearing myeloid leukaemia. *C. parvum* given 2 days before transplantation of the leukaemia produced a slight (> 20%) prolongation of survival. When administered 2 days after the passage of the leukaemia, no significant effect was seen with 0·7 mg *C. parvum*, but 7·0 mg consistently produced an increase in life span. Dose-dependent therapeutic effects of *C. parvum* have been described in P388 leukaemia (Houchens *et al.*, 1976), which is the only murine leukaemia in which *C. parvum* alone has been found to be effective (Houchens *et al.*, 1976; Mathé *et al.*, 1969; Scott, 1974*a*). The dose levels of *C. parvum* in the previously reported studies were much lower than the 250–300 mg/kg levels in our studies. This difference in dose levels may in part explain the apparent lack of effectiveness of *C. parvum* in the tested systems. Also, L1210 and AKR leukaemias are lymphoid malignancies, passaged i.p. in the reported experiments, which may for these reasons have responded differently to *C. parvum* from our myeloid leukaemia.

C. parvum has been shown to be a general stimulant of the reticuloendothelial system and of macrophages (Woodruff and Dunbar 1973). Silica has been shown to decrease macrophage func-

tion because of its effects upon lysosomal membranes. Silica-induced inhibition of macrophage function can be prevented by the prior administration of the macrophage stabilizer PVNO, an observation similar to that reported by Lotzova & Cudkowicz (1974) with respect to marrow grafts. These observations, taken together with those now reported, lead to the conclusion that the increased survival of mice with myeloid leukaemia is dependent upon stimulation of intact macrophage function. This has been shown in several other tumour systems (McBride *et al.*, 1975; Keller, 1977; Jones & Castro, 1977). One cannot conclude, however, that phagocytic macrophages are the immediate effectors of antitumour resistance, since the activity of natural killer cells has also been shown to be augmented by *C. parvum* and inhibited by silica (Oehler & Herberman 1978*a*). Because natural killer cells are not phagocytic, it has been suggested that the effects of *C. parvum* and silica on these cells are indirect, and mediated by the effects of these agents on the reticuloendothelial system (Oehler *et al.*, 1978*b*).

Histopathologic studies of leukaemic mice treated with *C. parvum* showed hepatosplenomegaly, with necrotic areas in the liver and spleen, as previously described by Lampert *et al.* (1977), possibly caused by intravascular coagulation resulting from antigen–antibody interaction. In preliminary studies we found that the administration of *C. parvum* to normal mice did not cause death. The terminal event in the leukaemic mice appeared to be leukaemic-cell pulmonary emboli and/or thrombi, the apparent immediate cause of death of mice with this myeloid leukaemia (Preisler *et al.*, 1977). Hence, despite toxicity related to the administration of *C. parvum*, the mice ultimately died from leukaemia.

The studies described here suggest that the administration of *C. parvum* might be of therapeutic benefit for patients with acute myelocytic leukaemia. An initial study of *C. parvum* in the treatment of leukaemia in man failed to demonstrate any therapeutic benefit (Pavlovsky *et al.*, 1978). On the basis of our dose–response experience in the mouse studies presented here, one would expect to see therapeutic benefit only with *C. parvum* doses which are far in excess of any doses hitherto administered to man. Clearly the hepatosplenic toxicity seen at the high doses administered to our mice would seem to make such an attempt unwise. Because of the possible relationship between the presence of *C. parvum* bacilli in the bloodstream and the toxic manifestations, we are currently attempting to circumvent this problem by encapsulating the bacilli within phospholipid vesicles (Papahadjopoulos and Preisler, unpublished observation). Since i.v. administered phospholipid vesicles are rapidly cleared by the reticuloendothelial system, we will attempt to deliver the encapsulated *C. parvum* directly to the phagocytic cells of reticuloendothelial system while avoiding direct exposure of other tissues to the bacterium.

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