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, 1974a; Fisher et al., 1975; Suit et al., 1977; Houchens et al., 1976) and human tumours (Scott, 1974b; 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 10⁴ 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 dose level, *C. parvum* was effective in prolonging survival whether administered before or after the passage of the leukaemia.

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

| Dose (mg) | Day | Median survival time | %ILS* |
|-----------|-----|----------------------|-------|
| Untreated controls | 16 | 19 (17-20) | 19 |
| 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.

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...
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  |  |  |  |
|-------|---|---|---|
|       | # | 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 | 13 | 12-14 | 0 | 5 | 12 | 11-13 | — | — | 5 | 18 | 14-25 | 20 | — |
| CP 7 mg | 5 | 21 | 14-23 | 24 | 5 | 23 | 16-23 | 77 | 5 | 18 | 14-25 | — | — | 5 | 18 | 14-25 | 20 | — |
| Expt  | 4 | 5 | Total (5 expts) |
| 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#3 | 37 | — | — | — |

Mice were inoculated with 10⁴ 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* | 21 | 16-28 |
| 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† | 22 | 16-32† |
| 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, 1974a). 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 1978a). 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., 1978b).

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.

This study has been supported by USPHS Grant No. CA–17785.

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