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Effects of Whole Body Irradiation in Mice Treated with *C. parvum**

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Abstract—*We have investigated the effect of whole body irradiation (WBI) upon the survival of* Corynebacterium parvum (C. parvum)*-treated CBA mice. Applied to mice* 7 days *after* C. parvum *WBI with* 350–950 rad *of X-rays killed more* C. parvum*-treated than -untreated mice. The number of endogenous hematopoietic colonies determined either at* 9 days *of irradiation or at the time of death of mice was greater in* C. parvum*-treated mice than in mice irradiated only. Using the exogenous spleen colony assay, it was found that CFUs from the spleen of mice treated with* C. parvum *were more sensitive to irradiation than the CFUs from the spleen of normal mice, Leucopenia caused by irradiation was equally severe in both groups of mice. However, a decrease in the number of erythrocytes was more pronounced in mice treated with* C parvum *and irradiation than in those given irradiation alone. Possible reasons for the increased radiosensitivity of* C. parvum*-treated mice as well as the relevance of these findings to tumor immunotherapy are discussed.*

INTRODUCTION

ANAEROBIC coryneforms are known for their immunostimulatory and antitumor properties [1]. They also increase the hematopoietic activity in experimental animals [2–4] and in humans [5]. Israel and Edelstein [5] suggested that this increased hematopoietic activity could account for the improved hematopoietic tolerance to chemotherapy which they observed in cancer patients treated with *Corynebacterium parvum* (*C. parvum*). However, more recent studies [6, 7] have not confirmed their findings. There are even observations of increased hematopoietic cell sensitivity, in mice treated with *C. parvum*, to toxic effects of cell cycle specific, but not of cycle nonspecific, chemotherapeutic agents [8].

In our studies on antitumor effects of anaerobic corynebacteria we have observed that treatment with *C. granulosum* protects mice against enhancement of artificially-induced pulmonary metastases caused by the whole body irradiation (WBI) [9]. In fact, *C. granulosum*-induced antitumor resistance was entirely nonsensitive to irradiation [9]. However, mice treated with both bacteria and irradiation appeared to be more anemic and overall less healthy than mice exposed to WBI alone. Here we describe experiments showing that *C. parvum*-treated mice are more sensitive to lethal effects of WBI.

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MATERIALS AND METHODS

Experiments were performed with 10–12 week old CBA mice of both sexes from our conventional mouse breeding colony. All mice used in each experiment were of the same sex. Mice were given single intraperitoneal (i.p.) injections of 0.25 mg *C. parvum* (lots BA3935A and CA582B, obtained from the Wellcome Research Laboratories, Beckenham, England) suspended in 0.5 ml solution A (8.0 g NaCl, 0.4 g KCl, 1.0 g glucose and 0.35 g $NaHCO_3$ in 1l. water). Seven days later they were whole-body irradiated with single doses of X-irradiation ranging from 350 to 950 rad using "Philips" X-ray machine and conditions as described earlier [10]. To determine hematopoietic activity, the animals were killed 9 days following the irradiation and hematopoietic spleen colonies (CFUs) counted [10].

In groups of mice that were left for checking the effect of irradiation on survival, the number of CFUs was determined at the time of death of animals. Furthermore, exogenous spleen colony assay was employed to determine the radiosensitivity of CFUs derived from the spleen of normal and *C. parvum*-treated animals. We have also determined the number of leucocytes and erythrocytes in blood samples drawn from the tail veins. The results were statistically evaluated by Student's *t*-test; differences between groups were considered significant if the *P* value of comparison was 0.05 or smaller.

RESULTS

To test whether *C. parvum* alters sensitivity of mice to lethal effects of WBI, mice were given *C. parvum* and 7 days later they were irradiated with doses of X-rays ranging from 350 to 950 rad. Results in Fig. 1 show that

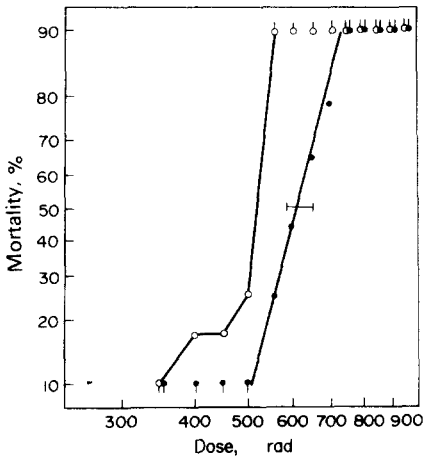

Fig. 1. Mortality of normal (●) and C. parvum *(○)-treated CBA mice exposed to WBI. A quarter of a miligram of* C. parvum *was injected i.p. 7* days *prior to irradiation. Groups contained* 5–31 *mice each.*

more animals died, at any radiation dose if they had been treated with *C. parvum*. In normal mice 100% mortality was achieved with 800 rad, while all *C. parvum*-treated mice died after 550 rad. Table 1 shows the mean survival times of normal and *C. parvum*-treated mice exposed to the WBI. In general, there was no marked difference in the survival time between untreated and *C. parvum*-treated mice although in certain groups *C. parvum*-treated mice appear to die earlier.

Further experiments investigated possible reasons for the increased radiation-induced mortality in *C. parvum*-treated mice. Above results suggested that the mice might have died from the hematopoietic failure. *C. parvum* is known to be a potent stimulator of the hematopoietic activity [4]; when given to CBA mice it greatly increased the spleen weight and cellularity; the increase occurred a few days after treatment and was evident throughout the observation period of 35 days (Table 2). Exposure of mice to 650 rad reduced the spleen weight and the number of nucleated cells in the spleen to very small values which were similar in both normal and *C. parvum*-treated mice (Table 3). This was determined 1–10 days following WBI. Thus, many more cells were destroyed by irradiation in *C. parvum*-treated animals.

When mice were killed 9 days following WBI, the spleens of *C. parvum*-treated mice contained about twice as many hematopoietic colonies as the spleens of normal mice (Table 4). Also, mice which died from irradiation damage had more colonies in their spleen if they received *C. parvum* prior to irradiation (Fig. 2). Several possibilities may account for the elevated number of spleen colonies in *C. parvum*-treated mice subjected to the WBI including: (a) the presence of more CFUs at the time of irradiation, (b) increased radioresistance of CFUs and (c) increased trapping of CFUs in the spleen of treated mice. In addition the spleen of treated animals may act as a more "fertile soil" for proliferation of CFUs. Other investigators [2] and ourselves [4] have previously reported that *C. parvum* causes a severalfold increase in the number of CFUs in the spleen. The results given in Table 5 indicate that the number of CFUs in the spleen of CBA mice treated with *C. parvum* 7 days earlier is increased by tenfold, confirming thus already published data on the same subject. Since *C. parvum*-treated mice contain more CFUs than normal mice, it is likely that more of them will be spared from irradiation.

The following experiment was performed to test whether CFUs in *C. parvum*-treated mice exhibit changes in resistance to irradiation. Mice were treated with *C. parvum* and 7 days later exposed to 300 or 600 rad of X-ray. They were then killed within 2 hr of irradiation, and the cell suspension of their spleens injected into normal animals irradiated to the whole body with 800 rad. Spleen cells from normal mice irradiated with 300 or 600 rad were also assayed for the presence of CFUs. The results are given in Table 6, and they show that CFUs derived from the spleen of *C. parvum*-treated mice are more radiosensitive than CFUs from the spleen of normal mice.

Table 1. *Survival of normal and* C. parvum-*treated CBA mice subjected to the WBI*

WBI (rad)	Survival of mice (days)				
	Normal mice		*C. parvum*-treated mice*		
	Mean ± S.E.	Range	Mean ± S.E.	Range	P (*t*-test)
550	10,21, > 100†		12.8 ± 0.5	10–12	
600	12.0 ± 0.4	8–13	11.0 ± 0.3	8–14	N.S.‡
650	13.9 ± 0.6	8–15	10.7 ± 0.4	8–13	< 0.01
700	9.6 ± 0.4	8–13	9.9 ± 0.2	8–12	N.S.
750	9.5 ± 0.3	8–10	9.6 ± 0.2	5–11	N.S.
800	8.0 ± 0.4	5–11	8.3 ± 0.7	4–12	N.S.
850	9.0 ± 0.4	5–10	8.7 ± 0.2	7–9	N.S.
900	7.8 ± 0.5	5–10	5.8 ± 0.6	5–11	< 0.02
950	8.7 ± 0.5	5–11	8.0 ± 0.2	6–10	N.S.

*0.25 mg *C. parvum* was given i.p. 7 days before WBI.
†Individual values.
‡Not significant. Groups contained 7–31 mice each.

Table 2. *Effect of* C. parvum *on the spleen weight and cellularity in CBA mice*

Days after treatment with *C. parvum* (0.25 mg given i.p.)	Spleen weight (mg) (mean ± S.E.)	Spleen cellularity (10^6) (mean ± S.E.)
No treatment	69 ± 2.1	127 ± 2.2
2	83 ± 2.2	164 ± 2.3
5	118 ± 2.9	205 ± 3.6
8	200 ± 3.2	405 ± 2.9
12	223 ± 2.5	461 ± 3.7
15	229 ± 3.4	306 ± 3.6
18	307 ± 3.1	321 ± 2.2
26	256 ± 3.2	340 ± 3.1
35	211 ± 2.1	262 ± 1.9

Groups contained 7–10 mice each.

Table 3. *Effect of WBI on the spleen weight and cellularity in normal and* C. parvum-*treated mice*

Days after 650 rad WBI	Spleen weight (mg)		Spleen cellularity ($\times 10^6$)	
	Normal mice (mean ± S.E.)	*C. parvum*-treated mice* (mean ± S.E.)	Normal mice (mean ± S.E.)	*C. parvum*-treated mice (mean ± S.E.)
1	33.4 ± 2.2†	35.6 ± 1.7	19.9 ± 1.3	13.6 ± 2.1
3	27.6 ± 1.7	27.6 ± 0.2	0.1 ± 0.1	0.15 ± 0.1
7	21.3 ± 0.7	17.8 ± 0.4	0.4 ± 0.1	0.3 ± 0.1
10	24.8 ± 0.9	23.0 ± 1.7	8.2 ± 0.3	5.1 ± 0.2

*0.25 mg *C. parvum* was given i.p. 7 days before WBI.
†Groups contained 7–8 mice each.

Table 4. Endogenous CFUs in normal and C. parvum*-treated CBA mice exposed to different doses of WBI*

Doses of WBI in rad*	Number of spleen colonies (mean ± S.E.)		P (*t*-test)
	Normal mice	*C. parvum*-treated mice	
650	4.6 ± 1.3	8.5 ± 1.3	<0.001
750	2.3 ± 0.6	5.2 ± 0.9	<0.001
850	1.3 ± 0.4	3.3 ± 1.1	<0.001
950	0.2 ± 0.1	1.0 ± 0.3	<0.001

*Irradiation was given 7 days after i.p. treatment with 0.25 mg *C. parvum*. Groups contained 13–18 mice each.

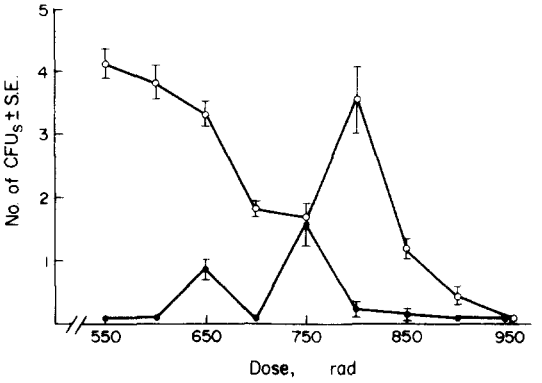

Fig. 2. Number of endogenous CFUs at the time of death of normal (●) and C. parvum *(○)-treated CBA mice exposed to WBI. A quarter of a* milligram *of* C. parvum *was injected i.p. 7 days prior to irradiation. Groups contained 5–31 mice each.*

To determine whether increased trapping and (or) more fertile microenvironment participated in the elevation of the number of spleen colonies in *C. parvum*-treated WBI mice, normal bone marrow cells were injected into *C. parvum*-treated animals irradiated with 800 rad. Injected cells produced an equal number of hematopoietic colonies in the spleen of both normal and *C. parvum*-treated recipients (Table 7) arguing thus against this possibility.

To determine whether *C. parvum* affects the differentiation of CFUs 800 rad WBI mice were injected with 2.5×10^4 bone marrow cells from normal or *C. parvum*-treated mice. Bone marrow cells from normal mice produced 8.8 ± 2.0 and those from *C. parvum*-treated mice 10.2 ± 0.5 colonies. Histology of these colonies revealed no changes in the percentage of colony types between the two groups (Table 8).

We have investigated also the effect of WBI on the number of peripheral blood leucocytes and erythrocytes in normal or *C. parvum*-treated mice. Figure 3A shows that *C. parvum* causes a drop in the white blood cell count which was detectable 2 days after *C. parvum* WBI with 650 rad caused a profound decrease in the number of leucocytes which did not show any tendency to improve during the 10 day observation period (Fig. 3B). Changes in the number of leucocytes were similar in both normal and *C. parvum*-treated mice. The number of erythrocytes was also affected by the *C. parvum* treatment (Fig. 4A); falling after 10 days, but recovering by 35 days following the treatment. When *C. parvum*-treated mice were exposed 7 days later to 650 rad WBI, the

Table 5. Number of CFUs in the spleen of CBA mice, normal or treated with C. parvum

Donors	Number of spleen colonies produced by 2.5×10^5 spleen cells	Calculated* total number of CFUs in the spleen ($\times 10^3$)
Normal mice	11.5 ± 2.0†	34 ± 2.1
C. parvum-treated mice	38.3 ± 1.6	365 ± 9.3

*On the basis of cellularity and CFU setting efficiency in the spleen (*f* value) of 17% (20).
†Mean ± S.E.
Groups contained 7–8 mice each.

Table 6. Fraction of spleen CFUs from normal or C. parvum*-treated CBA mice surviving irradiation with* 300 *and* 600 *rad X-rays*

Donors*	Survival fraction following 300 rad		600 rad	
Normal mice	0.0165 ± 0.0055†		0.0076 ± 0.0013	
		<0.05‡		<0.01‡
C. parvum-treated mice§	0.0060 ± 0.0015		0.00207 ± 0.00034	

*Mice were whole body irradiated with 300 or 600 rad X-rays and within 2 hr thereafter killed for the spleen cell preparation. Different numbers of spleen cells were injected into mice irradiated with 800 rad X-rays, and the number of spleen colonies determined 8 days later.
†Mean ± S.E.
‡*P* (*t*-test).
§0.25 mg *C. parvum* was given i.p. 7 days before irradiation.
Groups contained 8–9 mice each.

Table 7. Number of spleen colonies in 800 rad *irradiated normal or* C. parvum*-treated CBA mice produced by bone marrow cells from normal donors*

Recipients	Number of spleen colonies with injection of 2.5×10^4 cells	10^5 cells
Normal mice	6.4 ± 0.5*	12.4 ± 0.7
C. parvum-treated mice	6.7 ± 0.8	12.7 ± 1.2

*Mean ± S.E.
Groups contained 7 mice each.

Table 8. Number and percentage of types of exogenous spleen colonies (CFUs) analysed in 800 rad *irradiated CBA mice*

Types of analysed colonies	Number and percentage (in parentheses) of colonies produced by bone marrow from Normal mice	*C. parvum*-treated mice
Erythroid	88 (66)	87 (58)
Myeloid	30 (23)	33 (22)
Megacariocytic	0	9 (6)
Undifferentiated	9 (4)	11 (7)
Mixed	6 (7)	13 (8)
Total	133 (100)	153 (100)

Mice were injected with 2.5×10^4 bone marrow cells from normal mice or mice treated with *C. parvum* 7 days earlier.

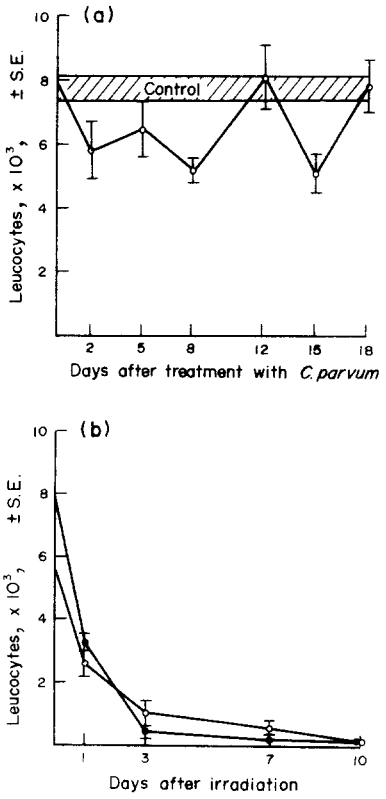

Fig. 3.(a). Number of peripheral blood leucocytes in CBA mice injected i.p. with 0.25 mg C. parvum (○). *(b) Number of peripheral blood leucocytes in normal* (●) *and* C. parvum (○)*-treated CBA mice exposed to* 650 rad *WBI.* C. Parvum [*as in (a)*] *was given* 7 days *before irradiation. Groups contained* 7–8 *mice each.*

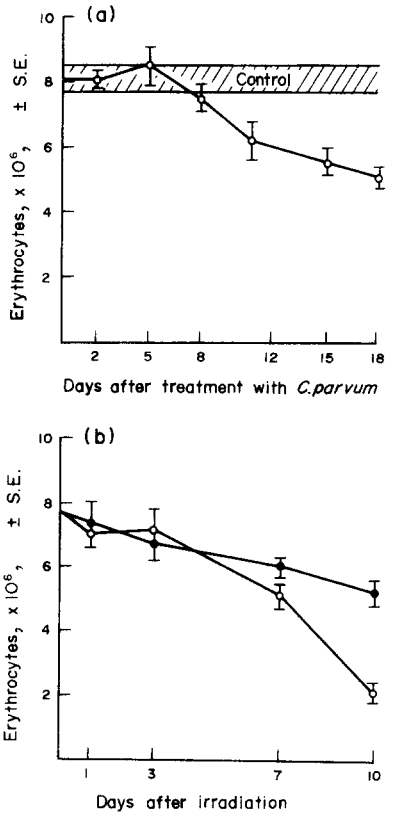

Fig. 4.(a). Number of peripheral blood erythrocytes in CBA mice injected i.p. with 0.25 mg C. parvum (○). *(b) Number of peripheral blood erythrocytes in normal* (●) *and* C. parvum (○)*-treated CBA mice exposed to* 650 rad *WBI.* C. parvum [*as in (a)*] *was given* 7 days *before irradiation. Groups contained* 7–8 *mice each.*

decrease in the number of erythrocytes was marked, and more important than that in normal mice (Fig. 4B).

DISCUSSION

This work has shown that *C. parvum* increased in a high proportion the mortality of mice subjected to the whole body X-irradiation. Even doses of irradiation, such as 550 rad, which caused only a small percentage of mortality in normal mice were lethal for all *C. parvum*-treated mice. However, the onset of death of mice was, in general, similar in both treated and untreated groups; in only 2 of 9 groups did *C. parvum*-treated animals die earlier than normal mice (Table 1). Recently, Gordon *et al.* [11], reported that multiple treatments with BCG or *C. parvum* delayed the onset of death of mice subjected to chronic γ-irradiation, but shortened the survival time of acutely irradiated mice. In addition, they observed that BCG, but not *C. parvum*, treatment caused more mice to die from acute irradiation.

The onset of death of mice in our experiments implied that mice of both untreated and treated groups died from hematopoietic failure. *C. parvum* induces extensive proliferation of nucleated cells (Table 2) which are mainly macrophages [3] and hematopoietic cells [3, 4]. WBI with 650 rad destroyed 5 times more nucleated cells in the spleen of *C. parvum*-treated mice than in spleen of normal mice. This is in concordance with our earlier observations made in specific-pathogen free mice [9]; the difference, however, was that the effect of irradiation was more pronounced in conventional mice which we used in the present experiments. Maruyama *et al.* [12] have recently shown that *C. parvum* strongly stimulated proliferative activity of hematopoietic CFUs in the spleen of mice but it increased their radiosensitivity. Our data (Table 6) also indicate that CFUs derived from the spleen of mice treated with *C. parvum* 7 days before irradiation were more sensitive to irradiation than CFUs from the spleen of normal mice. Although irradiation destroyed significantly more cells in the spleen of *C. parvum*-treated mice it left in these mice more CFUs than in normal mice (Table 3). This increased number of hematopoietic cells, however, was insufficient to protect *C. parvum*-

treated mice from the irradiation-caused death.

Profound leucopenia was caused by irradiation in both *C. parvum*-treated and untreated mice, and could not be the reason for the increased mortality of the former. It is interesting to see, however, that the number of erythrocytes following WBI decreased more in mice treated with *C. parvum* than in mice which were only irradiated, and this anemia could be a reason for the increased mortality of the treated mice. The basis for the more pronounced anemia in *C. parvum*-treated and irradiated mice is not known. However, *C. parvum*, by itself, causes erythrocytes to decrease in the peripheral blood (Fig. 4), [13–15] due to an increased phagocytosis and destruction of erythrocytes by *C. parvum* stimulated reticuloendothelial system [15]. *C. parvum* antigens may attach to red cells and thus facilitate the phagocytic removal of erythrocytes [14, 15].

Furthermore, *C. parvum* treatment increases sensitivity of mice to histamine [16] and endotoxin [17], which was associated with a release of large amounts of lysosomal enzymes into circulation. Macrophages activated by *C. parvum* are rich in lysosomes and destruction of even a small proportion of these cells by irradiation could result in a release of toxic enzymes. Also, radiation can cause release of endotoxin from the gut which then affects more severely *C. parvum*-treated mice. Therefore, it is likely that the damage caused by the release of lysosomal enzymes and endotoxin may, together with severe anemia, be the major cause for the increased mortality of *C. parvum*-treated animals exposed to WBI.

Data presented in this paper suggest that *C. parvum* should be used with caution when combined with radiation procedures which are expected to cause a substantial damage to the lymphoreticular and hematopoietic tissue. Studies with experimental animals made so far have shown that combination of *C. parvum* with local irradiation, either single or fractionated, of solid tumours growing in the leg or the flank skin proved to be safe as far as the mortality of irradiated animals is concerned [18, 19].

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