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Treatment of Mice with Lung Metastasis from a Dermally Implanted Fibrosarcoma: Comparison of Intratumoral Trehalose-6,6′-Dimycolate (Cord Factor) and Surgery

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Abstract—*Intralesional administration of emulsified trehalose-6,6′-dimycolate was compared to surgery in the treatment of mice with growing intradermal implants of a syngeneic fibrosarcoma. Treatments were given about one month after tumor implantation when a majority of animals so treated had metastatic foci of tumor in the lungs. Mice treated with trehalose-6,6′-dimycolate or by surgery survived significantly longer than untreated mice. Some treated mice were alive and tumor-free six months after tumor inoculation. The differences in cure rates and prolongation of survival between the trehalose-6,6′-dimycolate treated group and the surgically treated group were not statistically significant. Several mice died soon after surgery; no deaths could be attributed to the acute effects of immunotherapy.*

INTRODUCTION

INOCULATION into animals of trehalose-6,6′-dimycolate (TDM), a mycobacterial glycolipid known also as cord factor [1], produces some of the biological effects of mycobacterial infection. These effects include the formation of granulomas [2–4], enhanced immune response to antigens [5–7], stimulation of macrophage activity [8], increased resistance against microbial infections [9–11] and antitumour activity [12–15]. We have reported that BCG cell walls were at least as effective as living BCG in the treatment of guinea pigs with established dermal tumors and microscopic lymph node and artificial visceral metastases [16]. The study reported here was undertaken to determine whether intralesional inoculation of emulsified TDM would be effective treatment for mice with visceral metastasis arising from tumor cells that were implanted in the skin rather than inoculated intravenously.

MATERIALS AND METHODS

TDM and emulsions

TDM from *Mycobacterium bovis* strain AN5 was obtained from Dr. E. Lederer, Laboratoire de Biochimie, C.N.R.S., 91190 Gif-sur-Yvette, France. TDM was dissolved in mineral oil (Drakeol 6VR) before being emulsified by ultrasonication in 0.85% NaCl solution containing Tween 80 [15]. Final concentrations of emulsified components were: TDM, 1 mg/ml; oil, 10%; and Tween 80, about 0.2%. Emulsions lacking TDM were prepared similarly.

Animals

Male C3H/HeN mice, 20–25 g, were obtained from Charles River Breeding Laboratories, Wilmington, MA.

Murine tumor and treatment

All experiments were done with a methylcholanthrene-induced fibrosarcoma, designated 1023. This tumor was maintained by serial subcutaneous passage in syngeneic C3H/HeN male mice. Tumor-cell suspensions were prepared by pronase–DNase digestion of minced tumor tissue [17]. Male syngeneic mice each received an intradermal (i.d.) injection of 10^6 viable 1023 tumor cells on the dorsal surface. At 31 or 32 days thereafter, when the average tumor diameter was about 20 mm, animals in one group each received a single treatment consisting of the intratumoral and peritumoral (i.p.t.) administration of em-

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Table 1. Treatment of mice with lung metastasis by intra- and peritumoral injection of emulsified TDM

Experiment	Treatment*	No. of mice per group	No. of non-survivors		No. of survivors		No. of mice with complete regression of dermal tumor	Median survival time (*P*)†
			With macroscopic lung metastasis	Without macroscopic lung metastasis	With macroscopic lung metastasis	Without macroscopic lung metastasis (*P*)†		
1	No treatment	24	18	6	0	0	0	76
	Emulsion	24	19	5	0	0	0	74
	TDM	24	18	2	0	4 (<0.05)	5	95 (<0.01)
	Surgery	13‡	7	0	1	5 (<0.01)		91 (<0.01)
2	No treatment	25	15	10	0	0	0	60
	Emulsion	25	24	1	0	0	0	66
	TDM	25	16	5	1	3 (NS)	7	98 (<0.001)
	Surgery	22§	14	1	0	7 (<0.01)		105 (<0.001)

*Treated 31 (exp. 1) or 32 (exp. 2) days after i.d. injection of 10^6 tumor cells.
†Statistical evaluation in comparison with control groups (no treatment or emulsion) based on a two-tailed test of Wilcoxon. NS, not statistically significant. There were no statistically significant differences in the cure rates or in prolongation of survival between the surgically and the TDM treated groups.
‡Two mice died on the day of surgery and were excluded from the experiment.
§Eight mice died on the day of surgery and were excluded from the experiment.

ulsified TDM. The total volume of emulsified TDM injected into each animal was 1 ml and was distributed in several sites in and around the tumor in an attempt to saturate it with emulsion. Animals in a second group received similar injections of emulsion lacking TDM. The dermal tumors of animals in a third group were excised and a fourth group consisted of animals with tumors that were not treated. At the time of treatment the distribution of tumor sizes was approximately the same in each of the four groups. The time of treatment was based on the results of a preliminary experiment in which it was found that 30 days after i.d. inoculation of 10^6 tumor cells, the lungs of a majority of mice so treated contained cells capable of producing dermal tumors in normal recipients. Survivors were killed 6 months or more after intradermal implantation of tumor and were considered to be 'cured' if up until the time they were killed they appeared healthy, had no palpable tumor in skin or lymph nodes and at necropsy showed no evidence of visceral metastasis.

Statistical evaluation

Statistical significance of differences in cure rates or in survival times among treated and untreated animals was evaluated by the Wilcoxon nonparametric rank test [18].

RESULTS

In the first experiment animals were treatec i.p.t. 31 days after the i.d. injection of 10^6 tumor cells. At that time the tumors were 13–21 mm in diameter (median: 18 mm). Four of 24 animals treated with TDM were free o gross metastasis at 194 days (Fig. 1 and Table 1, exp. 1); 5 of 13 mice in which the dermal tumors were removed surgically were tumor-free. Untreated animals began dying on day 49 after i.d. inoculation of tumor cells and all were dead by day 94. Animals treated with emulsion alone began dying on day 40 and all were dead by day 116. The differences in cure rates and prolongation of survival between animals treated by emulsified TDM or by surgery and the control groups (no treatment or emulsion alone) were significant ($P<0.01$ based on a two-tailed test; see Table 1). The differences in cure rates and prolongation o survival between the TDM treated group and the surgically treated group were not statistically significant. In the second experiment animals were treated i.p.t. 32 days after i.d inoculation of 10^6 tumor cells. The range o the tumor sizes was 15–30 mm (median 23 mm). As in the first experiment, surgical treatment or i.p.t. administration of emulsified TDM resulted in significant prolongation o survival in comparison with the control groups (Fig. 2 and Table 1, exp. 2). In both

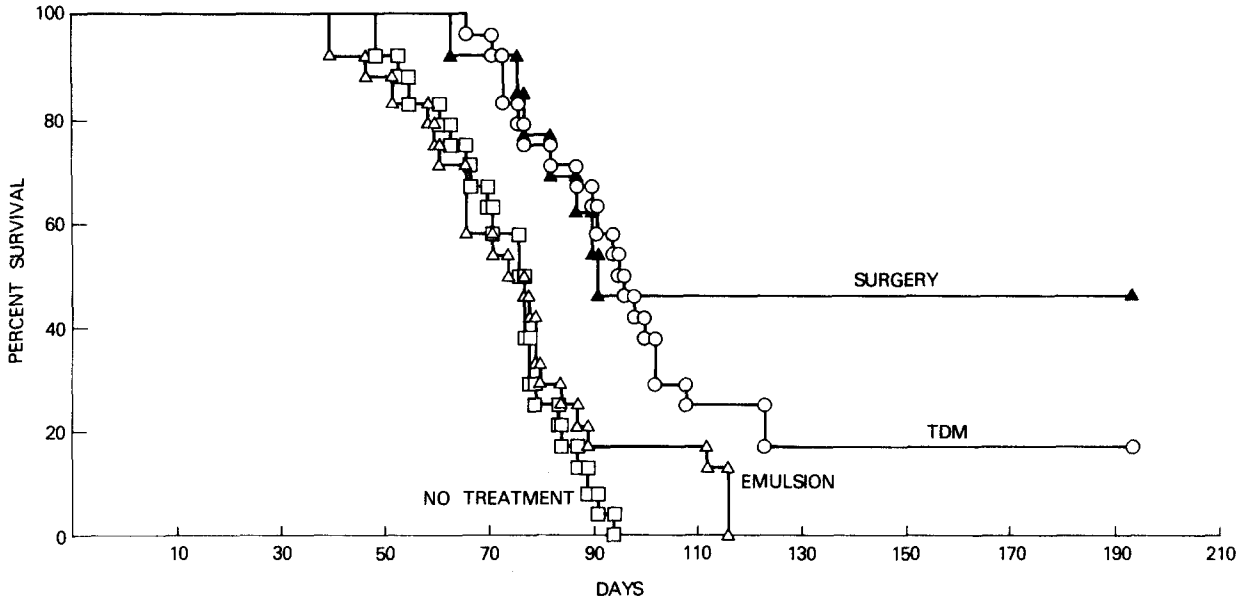

Fig. 1. Percentage of survival of mice with 31-day-old *dermal tumors and lung metastasis after i.p.t. administration of emulsion alone* (△) *or emulsified TDM* (○); *dermal-tumor excision* (▲); *untreated control.*(□). *For additional details see Table* 1.

experiments animals treated i.p.t. by emulsified TDM showed at least partial regression of the dermal tumors; in some animals the dermal tumor regressed completely (Table 1).

DISCUSSION

The study reported here demonstrated that surgery or i.p.t. administration of a mycobacterial component resulted in a prolongation of survival of animals with lung metastasis. I.p.t. treatment with emulsified TDM was sometimes able to cause complete regression of large (2 cm in diameter) tumors; other tumors treated with TDM showed partial regression. To our knowledge this is the first report of successful immunotherapeutic treatment of mice with such a large tumor burden. The data do not allow a conclusion as to whether or not surgery or TDM treatment led to the destruction of pulmonary metastases present at the time of therapy. However, it has been reported that TDM enhanced the immune response to antigens [5–7] and this activity

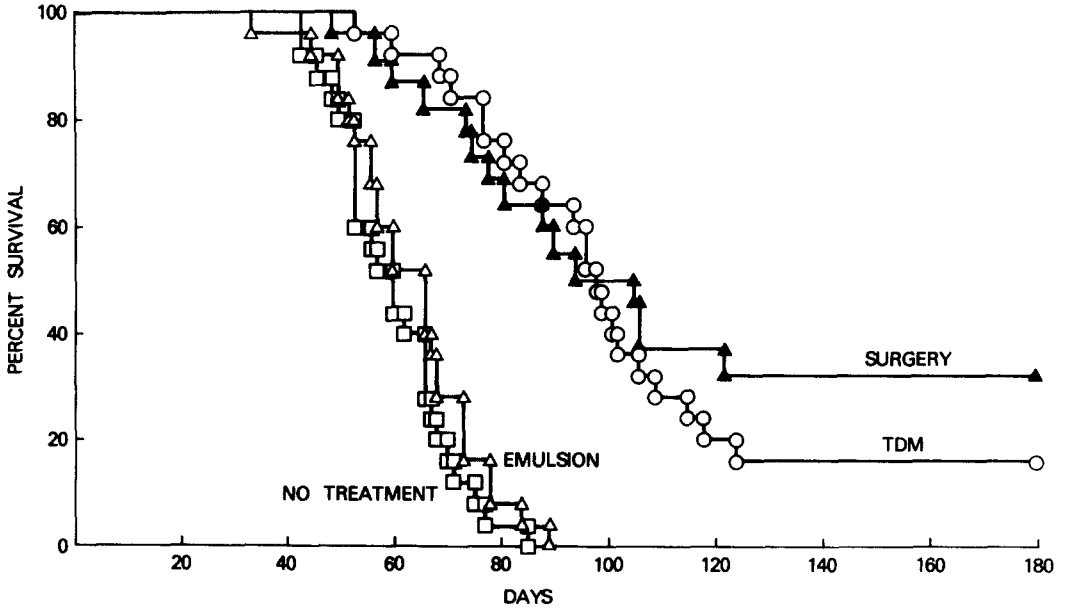

Fig. 2. Percentage survival of mice with 32-day-old *dermal tumors and lung metastasis after i.p.t. administration of emulsion alone* (△) *or emulsified TDM* (○); *dermal-tumor excision* (▲); *untreated control* (□). *For additional details see Table* 1.

may lead to destruction of lung metastasis. Most of the mice in which complete tumor (No. 1023) regression occurred, after intralesional inoculation of emulsified TDM, resisted the growth of 10^6 tumor cells in a challenge inoculum [15]. Induction of chemotaxis [19] and stimulation of macrophages by TDM [8] may contribute to the local antitumor activity of TDM. Direct cytotoxicity by emulsified TDM was not seen *in vitro* [20]. The contribution of the granulomatous activity of TDM to the antitumor activity in C3H/HeN mice is questionable [20].

Another significant feature of the immunotherapeutic treatment with emulsified TDM revealed in this study was that no deaths of treated mice could be attributed to the acute effects of immunotherapy, whereas several mice died as an immediate result of surgery.

The murine fibrosarcoma used in this study may provide a model for testing the efficacy of agents for the treatment of animals with advanced malignant disease.

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