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NOTES

Failure of Trehalose-6,6'-Dimycolate (P_3 or Cord Factor) to Enhance Endotoxin Lethality in Mice

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The increased endotoxin lethality in mice pretreated with BCG was not observed in mice pretreated with trehalose-6,6'-dimycolate instead of BCG.

Biological responses to mycobacterial infection can be duplicated by treatment with the mycobacterial glycolipid trehalose-6,6'-dimycolate (TDM) (cord factor or P3). These responses include: (i) granulomatous reactions (2, 3, 5, 9), (ii) enhanced reactivity to antigens (6, 8, 12), (iii) stimulation of macrophages as measured by morphological, enzymatic, or functional changes (E. Yarkoni and A. Bekierkunst, manuscript in preparation), (iv) increased resistance against microbial infections (16), and (v) antitumor effects in vivo (4, 11). BCG is being widely used to treat experimental and clinical cancer (1); therefore the antitumor effects of TDM are of special interest. A major goal of BCG studies is to define and isolate a component(s) of mycobacterial organisms with maximal antitumor effects and minimal toxicity. The development of a nonviable vaccine containing mycobacterial cell walls in an oil/water emulsion with effective antitumor properties was an advance towards this goal (18). One toxic effect of viable BCG retained in nonviable preparations including the cell wall emulsions was potentiation of endotoxin lethality (15). This problem is especially important in infection-prone cancer patients. Indeed, the mortality of gram-negative sepsis in cancer patients (75%) was three times higher than that of noncancer patients (7). It has been previously suggested that potentiation of endotoxin lethality was related to TDM and in fact that TDM was directly toxic (15). These inferences, however, were from studies in which TDM was administered in oil. In contrast to these reports, we have found that TDM in an oil/water emulsion retained potent antitumor effects (E. Yarkoni, M. S. Meltzer, and H. J. Rapp, manuscript in preparation) yet was not directly toxic when injected intravenously (i.v.). Therefore, we examined the effect of this TDM preparation on the mean lethal dose (LD_{50}) of endotoxin.

General-purpose male Swiss mice received i.v. injections of one of the following: (i) 0.2 ml of viable BCG (Phipps substrain, TMC 1029, Trudeau Mycobacterial Collection, Saranac Lake, N.Y.) at 2×10^7 colony-forming units/ml of Middlebrook 7H9 broth; (ii) 0.2 ml of TDM in an oil/water emulsion prepared by ultrasonication of 150 μg of TDM (p3 from *Mycobacterium tuberculosis*, Aoyoma B strain, obtained from Hamilton Biochemical Research Laboratory, Hamilton, Mont.; National Institutes of Health contract no. 26376-C-0017CC) in 0.75% mineral oil (Drakeol 6VR) and 0.2% Tween 80 in saline; or (iii) 0.2 ml of an oil/water emulsion without TDM. Eight days after i.v. injection, treated mice and untreated controls received an i.v. injection of lipopolysaccharide B from *Salmonella typhosa* 0901 (Difco Laboratories, Detroit, Mich.); mice were observed for 5 days. Almost all deaths occurred within 1 day after endotoxin injection.

Mice pretreated with BCG i.v. showed significant potentiation of the lethal toxicity of a subsequent i.v. injection of endotoxin compared with nonpretreated controls (Table 1). Increased lethality to endotoxin induced by BCG pretreatment has been previously reported to be maximal by 6 to 9 days (15) or 10 to 15 days (13) after BCG treatment. The 8-day time point used in this report was within this range. Indeed, the LD_{50} of endotoxin in BCG-pretreated mice was 21 μg compared with the LD_{50} of 453 μg in control mice at 8 days. In contrast to BCG, endotoxin lethality in mice pretreated with the TDM emulsion or emulsion control was not statistically different by the Wilcoxon nonparametric rank test (14). The failure of the TDM emulsion to enhance endotoxin lethality after 8 days contrasts with observations in which several biological properties of the TDM emulsion were maximal at this time: (i) pulmonary granulomas (5), (ii) stimulation of perito-

TABLE 1. *Endotoxin lethality of mice pretreated with BCG or trehalose-6,6'-dimycolate*

Pretreatment	No. of deaths/no. treated					
	10[a]	30	90	270	810	LD_{50}[b] (μg)
No treatment			0/18	1/18	18/18	453
Emulsion			0/18	2/18	18/18	438
Trehalose-6,6'-dimycolate	0/18	1/18	2/18	4/18	18/18	355
BCG	6/18	10/18	17/18	18/18		21

[a] Micrograms of endotoxin injected.
[b] Calculated by the method of Reed and Muench (10).

TABLE 2. *Survival of mice treated with emulsions of trehalose-6,6'-dimycolate intravenously*

Material	Oil concn (%)	No. of deaths[a]/ no. treated
Emulsion control[b]	1	0/5
Trehalose-6,6'-dimycolate[c]	1	0/5
Emulsion control	10	0/5
Trehalose-6,6'-dimycolate	10	4/5

[a] Mice observed for 7 days.
[b] Emulsions contained mineral oil in 1% Tween 80 in saline. Volume injected was 0.1 ml intravenously.
[c] Amount injected was 100 μg.

neal macrophages (E. Yarkoni et al., manuscript in preparation), (iii) increased nonspecific resistance to bacterial infection (16), and (iv) tumor suppression (17). In this report, lungs of both BCG- and TDM emulsion-treated mice showed granulomatous reactions not present in the emulsion control.

The failure of 150 μg of TDM in an oil/water emulsion given i.v. to potentiate endotoxin lethality contrasts with previous findings of Suter and Kirsanow, who demonstrated that intraperitoneal pretreatment of mice with 5 μg of TDM in undiluted mineral oil increased endotoxin lethality (15). Although the routes of administration were different, this contrast focuses attention on the important role of the oil vehicle in modulating the biological effects of TDM. Indeed, i.v. injection of 100 μg of TDM in an emulsion containing 10% oil killed four of five mice; 100 μg of TDM in an emulsion with 1% oil was not toxic (Table 2.) Thus, by titration of the relative concentrations of TDM and oil, preparations with maximal antitumor effects can be obtained with minimal toxicity.

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