Granuloma Formation in Lungs of Mice After Intravenous Administration of Emulsified Trehalose-6,6'-Dimycololate (Cord Factor): Reaction Intensity Depends on Size Distribution of the Oil Droplets

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The intensity of the granulomatous reaction evoked in lungs of mice by trehalose-6,6'-dimycololate administered intravenously in oil-water emulsion depended on the size distribution of the oil droplets. Emulsions containing the greatest number of the largest oil droplets were the most granulomagenic.

Trehalose-6,6'-dimycololate (TDM), a glycolipid extracted from mycobacteria, induced a granulomatous reaction in the lungs of mice after intravenous (i.v.) injection (1, 4, 8). The cellular composition of the granulomas induced by TDM was indistinguishable from that caused by living BCG cells. In both, the granulomas were composed of epithelioid cells, macrophages, and lymphocytes (4).

Studies with a syngeneic, transplantable guinea pig hepatoma suggested a relationship between the granulomatous response and the antitumor activity elicited by intrasplenic injection of living BCG (11, 12). Development of lung tumors by urethan was suppressed in mice with lung granulomas induced by emulsified TDM or by BCG (6). The study reported here was undertaken to facilitate future investigations of the possibility that the antitumor activity of mycobacterial vaccines requires granulomas formation.

Mineral oil-water emulsions of TDM (P3 from Mycobacterium tuberculosis, Aoyama B strain, obtained from Hamilton Biochemical Research Laboratory, Hamilton, Mont.; National Institutes of Health contract no. 263-76-C-0531CC) were prepared by ultrasonic treatment or by grinding with a tissue grinder. The final concentrations of TDM, oil, and Tween 80 in these emulsions were 2 µg/ml, 1%, and 0.2%, respectively. Preliminary experiments showed that emulsions of this composition were suitable for studies of the granulomatous response in lungs of mice. The ground and ultrasonic emulsions were each divided into two portions. One portion of each emulsion was injected i.v. into male NIH Swiss mice (in a group of six animals). The second portions were re-emulsified (for another 2 min) by the other method before testing for granulomagenic activity. Emulsions were injected i.v. in 0.2-ml volumes. Mice were killed 4 days after TDM administration (preliminary study showed that at that time maximal numbers of granulomas were found), and their lungs were fixed in Bouin fluid and stained with hematoxylin and eosin. The number of granulomas per 100-mm² areas of lung sections was determined for each mouse. The results of this experiment showed that a strong cellular reaction was induced by the emulsion that was prepared by grinding (Fig. 1). An emulsion produced by grinding and then treated ultrasonically contained smaller-sized oil droplets than the ground emulsion and produced only moderate reaction. Neither the size distribution of oil droplets nor the granulomagenic activity of an emulsion that was prepared initially by ultrasonic treatment was significantly altered by grinding. About 30% of the oil droplets in the emulsion produced by grinding alone were larger than 1 µm in diameter; some were as large as 40 µm. Only about 1% of the oil droplets in ultrasonically prepared (or re-emulsified) emulsions was larger than 1 µm, and none was larger than 11 µm.

Results of the present study indicate that size distribution of the oil droplets in an emulsion was an important factor determining the intensity of the granulomatous response in mouse lungs. Studies of the granulomatous response induced by ultrasonically prepared TDM emulsion showed that the size of the oil droplets and the intensity of the granulomatous reaction were affected by two other factors (see Fig. 2): (i) amount of oil—the average size of oil droplets increased with increasing oil concentration; (ii) amount of Tween 80—the average size of oil droplets decreased with increasing Tween concentration. These results may mean that most of the small oil droplets passed through the lungs and infiltrated into other tissues, whereas
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emulsions injected i.v. contained 2 µg of TDM, 1% oil, and 0.2% Tween in 0.2-ml volumes. Symbols: 

eulsion prepared by grinding; emulsion prepared by grinding and re-emulsified by ultrasonic treatment; emulsion prepared ultrasonically; and emulsion prepared ultrasonically and re-emulsified by grinding.

FIG. 1. Influence of the method of preparation on lung granuloma production by emulsified TDM. Emulsions injected i.v. contained 2 µg of TDM, 1% oil, and 0.2% Tween in 0.2-ml volumes. Symbols: , emulsion prepared by grinding; , emulsion prepared by grinding and re-emulsified by ultrasonic treatment; , emulsion prepared ultrasonically; and , emulsion prepared ultrasonically and re-emulsified by grinding.

FIG. 2. Influence of oil and Tween concentrations on production of lung granulomas by emulsified TDM. (a) Emulsions injected contained 10 µg of TDM, the indicated concentration of oil, and 1% Tween in 0.2-ml volumes. (b) Emulsions injected 2 µg of TDM, 1% oil, and the indicated concentration of Tween in 0.2-ml volumes.

the larger oil droplets were trapped in the lungs and induced the cellular reaction. Indeed, 7 to 28 days after i.v. administration of emulsified TDM, granulomas were found in liver and spleen tissues (unpublished data). They also suggest that the ultrasonically prepared emulsions may be useful for systemic adjuvant immunotherapy of leukemias, when dissemination of TDM into other tissues (e.g., liver and spleen) may be desired, rather than pulmonary deposition. A variety of mycobacterial vaccines (3, 6, 7, 15, 17a, 19; H. J. Rapp, E. Yarkoni, L. Ruco, and J. T. Hunter, Cancer Immunol. Immunother., in press) in oil-water emulsion are being studied for their possible application to cancer immunotherapy. Our findings may be useful in those studies.

The granulomagenic activity and the stability of ultrasonically prepared emulsions were not changed by heat at 70°C for 20 min or by freezing at -20°C for at least 1 week; during this period, the emulsion may be thawed several times. On the other hand, ground emulsions partially separated into oil and emulsion under these conditions.

Bekierkunst et al. showed that the granulomatous reactions induced by TDM were accompanied by adjuvant, antibacterial, and antitumor activities (2-7, 9, 17, 18). These observations were confirmed and extended by other laboratories (10, 13-16, 17a; Rapp et al., Cancer Immunol. Immunother., in press). The study documented in this report may facilitate future investigations of the possibility of immunotherapeutic treatment of lung tumors by emulsified TDM, as has been already suggested by Bekierkunst et al. (6). Experiments attempting to correlate the intensity of the granulomatous activity with the antitumor effect of emulsified TDM in lungs of mice are now in progress.

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LITERATURE CITED


9. Bekierkunst, A., E. Yarkoni, E. Flechner, S. Morecki,


