Adjuvant Disease—the Paradox of Prevention and Induction with Complete Freund's Adjuvant (36900)

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(Introduced by W. S. Jeter)

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Adjuvant disease may be induced in rats by injection of killed mycobacteria in mineral oil (1, 2), and is sometimes used as an experimental model for the study of rheumatoid arthritis in man (3, 4). Although important differences between the two disorders are known (3, 5), striking similarities also exist (3, 5).

Adjuvant disease may be suppressed in many ways. Some effective nonspecific treatments are: removal of draining lymph nodes at critical times (6); thoracic duct drainage (7), injection of anti-lymphocytic serum (8); whole body irradiation (9, 10), and treatment with immunosuppressive drugs (11–17). Some preventive procedures which may be nonspecific are injections (simultaneous with induction or preinduction) of: living malarial parasites (18); killed Nocardia (19); certain bacterial lipoproteins (20); pine pollen waxes (19); and a variety of plasma proteins (19, 21–24). Zahiri *et al.* (25) report that mineral oil alone will protect against adjuvant disease.

Protection which is apparently more specific is afforded by injections of mycobacteria or wax D prior to induction (9, 25–27) and up to 5 days postinduction (28). These protective mycobacterial substances were suspended in saline for injection. Wood and Pearson (20) noted in 1962 that wax D was protective in saline, whereas in mineral oil it was arthritogenic. The purpose of this study was to determine whether pretreatment with killed mycobacteria in mineral oil can prevent adjuvant disease induction by the usual procedure.

Materials and Methods. Rats. Eight groups of 8, 9-wk-old, 170–200 g, female, randombred, Holtzman rats were used. The animals were housed two to a cage and fed Purina Rodent Lab chow *ad libitum.*

Pretreatment. On Day 0, animals in groups 1 through 6 were treated with 0.1 ml of sterile paraffin oil (white, USP, viscos 340-355, M C & B, Cincinnati, O) containing 12.5, 25, 50, 100, 200, or 400 μg of heat-killed *Mycobacterium butyricum* (Difco) respectively. Group 7 received 0.1 ml of sterile paraffin oil alone. Group 8 was injected with 0.1 ml of sterile saline. All injections were given subcutaneously, 3 in. distal to the base of the tail.

Induction. Fourteen days after pretreatment, as described, each test animal was given a subcutaneous injection containing 0.5 mg of heat-killed *M. butyricum* in 0.1 ml sterile paraffin oil, 1 in. distal to the base of the tail.

Evaluation of arthritis. A visual scoring system was employed three times weekly throughout the experiment to ascertain the extent of disease in each animal. The scoring system was a modification of that used by other investigators (17, 21, 29, 30). Some of the test animals appeared to have more difficulty walking than visible lesions would justify. Therefore, assessment of walking ability along with visible lesions allowed a more accurate but simple assessment of disease. The scoring system was:

A. Each paw was considered in zones: toes, foot, and ankle.

These zones were graded:	Score
No lesions	1

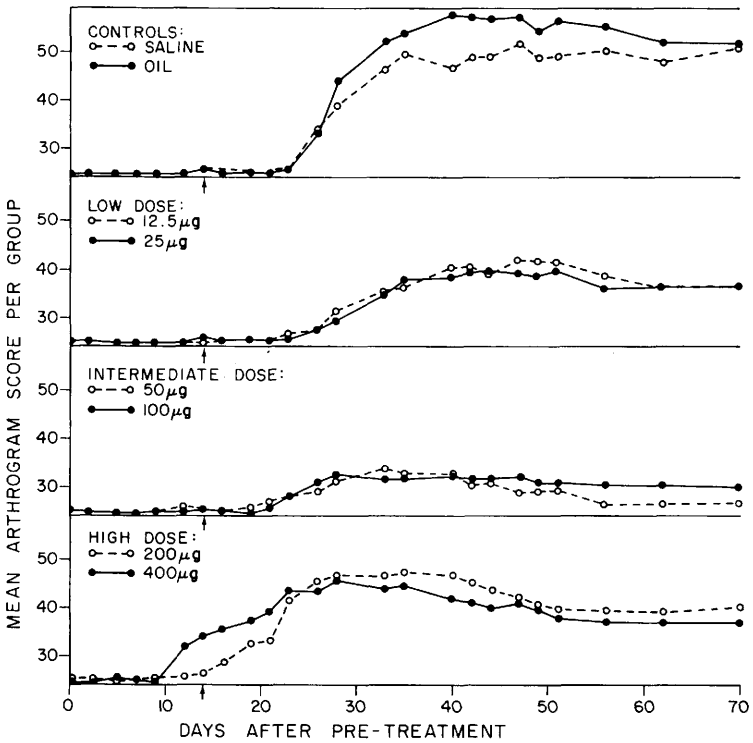

FIG. 1. Influence of mycobacterium in oil pretreatment dose on adjuvant disease. Induction with 0.5 mg killed organism in mineral oil on Day 14; 8 rats/group. All injections given subcutaneously in the tail.

Less than one-half of area involved	2
One-half, or more, of area involved	3
B. Each ear was observed for nodules:	
No nodules	1
One or more small nodules, < 2 mm diam	2
One or more large nodules, ≥ 2 mm diam	3
C. Walking ability was assessed:	
Normal walking	10
Limping	20
Moving across table but no weight borne on hind legs	30
Unable to move across table	40

All of these values were then added to obtain a final arthrogram score ranging from 24 for no visible signs of disease to 82 for most severe disease.

Results. The scoring system used in this study allows for statistical analysis of disease migration patterns. No statistically significant differences could be found among scores obtained by several evaluators.

The pretreatments employed significantly altered the progression of disease following induction (Fig. 1). Statistical analysis of the results for the eight pretreatment dosages justified rearrangement of the animals into four groups:

Controls	Saline and mineral oil
Low dose	*M. butyricum*, 12.5 and 25 μg
Intermediate dose	50 and 100 μg
High dose	200 and 400 μg

Analysis of variance revealed differences among the four groups which were significant at the 99.9% level.

The controls showed no signs of disease until Day 12, postinduction. Their mean scores rose to a peak of 50–57 and remained

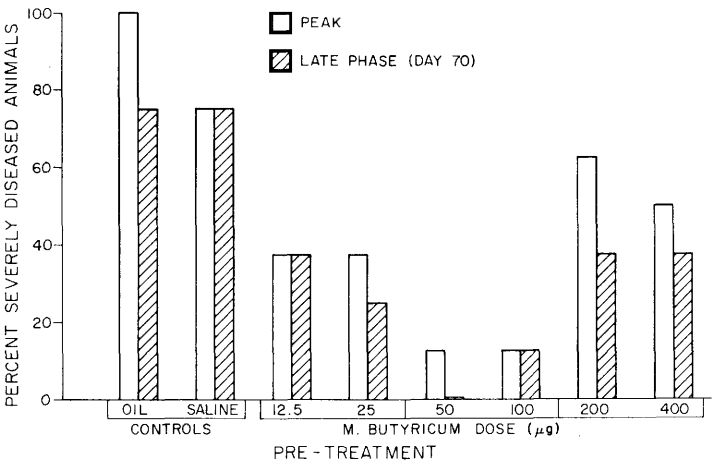

FIG. 2. Percentage of rats developing severe disease vs pretreatment. Severe disease score ≥ 40; 8 animals/group.

at approximately 50 throughout the 71 day experiment. Animals pretreated with mineral oil appeared to develop more severe disease than the saline controls. However, the difference was statistically significant only on Day 40, or 26 days postinduction.

Signs of disease were seen in the low dose group simultaneously with appearance in the controls. In contrast to the controls, however, average scores of the low dose group rose to a moderate peak of approximately 40, and remained above 35 during the observation period.

The intermediate dose group received the best protection. An accelerated response to induction was seen, reaching an early and low mean peak score of 33, and, particularly in the case of the 50 μg dose, dropped to a virtually disease-free state.

The high dose group showed an accelerated response, with the 400 μg dose animals developing disease before the induction injections. An early average peak score of 46–47 developed, but soon receded to moderate levels similar to the low dose group.

The percentage of animals developing severe disease was calculated (Fig. 2). An arthrogram score of 40, or greater, was considered to be severe disease.

Seventy-five percent of the saline-treated controls developed severe arthritis and, at the peak of disease, 100% of the mineral oil-treated controls were severely affected. Both at the peak of disease and later in its course, all of the pretreatments with mycobacteria in oil reduced the percentage of severely affected animals when compared with controls. The intermediate dose protected the greatest percentage (87–100%) of animals.

Discussion. All the mycobacterial pretreatments offered a measure of protection against adjuvant disease induced 2 wk later. The high doses apparently were arthritogenic, sensitizing (reflected by accelerated response), and somewhat protective.

No arthritogenesis was seen in the intermediate dose groups but perhaps the inducing injection was too soon to allow a late response to develop. These animals were apparently sensitized and received optimal protection.

Neither arthritogencity at the time of challenge, nor accelerated disease development appeared in the low dose groups. However, some protection was provided.

Contrary to the findings of Zahiri *et al.* (25), our pretreatment with mineral oil alone aggravated adjuvant disease in rats. Perhaps the 2 wk interval between pretreatment and induction caused variance from his results which were obtained after a 4 wk interval. In addition, we used Holtzman rats and Zahiri employed Sprague-Dawley animals.

The protection demonstrated by this exper-

iment could be explained by low-dose induction of tolerance, dose-dependent stimulation of an "enhancing antibody" which could coat and protect target tissues, or activation of suppressor T lymphocytes (31). We have no evidence which clarifies the protective mechanism(s).

Summary. Subcutaneous pretreatment of rats with heat-killed *Mycobacterium butyricum* in mineral oil protected against the induction of adjuvant disease 2 wk later. A dose of 50 to 100 μg protected optimally while higher and lower doses were less protective. Mineral oil pretreatment alone appeared to aggravate the disease.

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