height of antibody production. 3. It has been tentatively concluded that the action 6-MP is on the primary antibody response, rather than on the anamnestic response.

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Sensitivity of Mice to Endotoxin after Vaccination with BCG (*Bacillus Calmette-Guérin*)* (24282)

EMANUEL SUTER,† GRACE E. ULLMAN AND ROBERT G. HOFFMAN

Department of Microbiology, College of Medicine, and Statistical Laboratory, University of Florida, Gainesville

The establishment of tolerance to endotoxins derived from gram negative bacteria upon repeated injection is attributed to increased activity of the reticulo-endothelial system (RES). Tolerance is effective against many manifestations of endotoxin activity, such as fever, leukopenia, shock, and Shwartzman reactions(1) and may last in mice for 4 months(2). Single or repeated injections of endotoxin result in increased clearance of particles or large molecules from the blood stream by the RES(3). Similarly, infection with virulent tubercle bacilli or vaccination with BCG were found to result in increased clearance by the RES(4). In view of these findings it appeared possible that BCG vaccination might induce tolerance to endotoxin. This view is supported by the fact that a single injection of endotoxin increases resistance to tuberculous infection(5) indicating some relationship of the host's reaction to these 2 biologically active agents. Experiments were therefore undertaken to study the reaction of mice infected with tubercle bacilli to endotoxin derived from gram negative bacteria.

Methods and materials. Swiss albino mice of the Webster strain were used. Lipopolysaccharide was prepared according to the technic described by Westphal and Lüderitz from a strain of *E. coli B*(6). Preparations were also obtained from the Difco Laboratories, Detroit, Mich. (Lipopolysaccharide *E. coli* 026:B6) and from Wander S. A., Berne, Switzerland (Pyrexal Wander).‡ The lipopolysaccharide was suspended in physiological solution of sodium chloride and was injected intraperitoneally. For vaccination 0.2 ml of a BCG§ culture grown for 7 to 10 days in the liquid tween-albumin medium was injected intravenously. In some experiments cord factor derived from virulent tubercle bacilli‖ was

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‖ Cord factor was kindly supplied by Dr. Hubert Bloch, Dept. of Microbiology, Univ. of Pittsburgh Medical School, Pittsburgh, Pa.

used for the preparatory injection. Cord factor was dissolved in mineral oil (25 μg/ml) and 0.2 ml (*i.e.* 5 μg) was injected intraperitoneally twice at a 48-hour interval. In each experiment LD_{50}'s were determined simultaneously in control and experimental animals by injecting intraperitoneally groups of 3 to 5 mice each with dilutions of lipopolysaccharide containing 1,000, 100, 10 and 1 μg per dose (0.5 ml) respectively. Deaths were recorded up to 72 hours. All animals were usually tested 7 to 10 days after the preparatory injection with BCG or cord factor.

For evaluation of the results the square root of dosage units was used, because the square root transformation yielded a closer approximation to a straight line upon probit transformation of the ordinate, than did the logarithmic transformation. A probit equation using the maximum likelihood method was then fitted to the groups to obtain an LD_{50} and a standard deviation(7).

Results. The results are summarized in Fig. 1, in which each bar represents the square root of the LD_{50} in μg, the bracket indicating 2 standard deviation limits. 1) *Effect of vaccination with BCG on sensitivity to endotoxin*: (Chart A) The LD_{50} for endotoxin was 357 μg in control mice and 7 μg in mice which had been vaccinated 7 to 10 days previously. The course of the shock induced by endotoxin in vaccinated animals appeared to be more acute than in controls, *i.e.* death usually occurred within 6 to 10 hours in the vaccinated group and within 12 to 48 hours in the controls. When mice were tested with endotoxin at various intervals of time after vaccination, some increase of susceptibility to endotoxin became manifest within one to 3 days after injection of BCG. A rather sudden change of LD_{50} appeared between the 5th and 7th day after vaccination (Fig. 1, chart A). The state of hyperreactivity was still detectable 70 days after vaccination. 2) *Effect of cord factor derived from tubercle bacilli on sensitivity to endotoxin.* In an attempt to investigate whether any single component of tubercle bacilli could induce a similar state of hyperreactivity to endotoxin, the cord factor, isolated by Bloch from tubercle

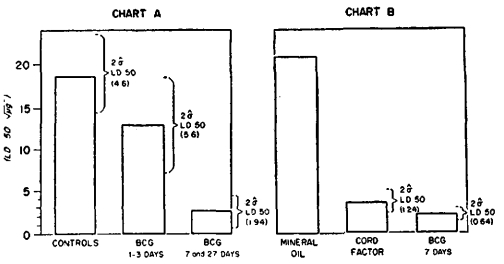

FIG. 1. Mean of square roots of LD_{50} of endotoxin in μg in mice pretreated with BCG, cord factor or mineral oil. The brackets indicate 2 standard deviation limits. *Chart A:* Comparison of LD_{50} in control mice with that in mice vaccinated with BCG 1 to 3 and 7 to 27 days previously. *Chart B:* Comparison of effect of pretreatment with cord factor, BCG and mineral oil.

bacilli was selected. This component, a trehalose dimycolate, is known to cause death of mice after repeated injections of small doses (8). Cord factor dissolved in mineral oil was injected twice and the mice were challenged with endotoxin 7 days later. The LD_{50}'s were as follows (Fig. 1, chart B): In mice which had received cord factor 15 μg, in controls injected with mineral oil 441 μg and in BCG vaccinated animals 6 μg.

Discussion. Contrary to expectation, the results clearly indicate that mice infected with an attenuated strain of tubercle bacilli, namely BCG, became highly sensitive to lethal doses of lipopolysaccharide derived from gram negative bacteria. This hyperreactivity appeared within 5 to 7 days and lasted for at least 70 days. In preliminary experiments in which mice were infected with a virulent strain of tubercle bacilli, a similar degree of hyperreactivity to endotoxin was found. Compared with the effect of BCG vaccination, no correlation existed between severity of infection and degree of hyperreactivity to endotoxin. It is known from earlier experiments that certain acute and subacute bacterial infections induce a state of increased sensitivity to bacterial endotoxins. That is, a single injection of bacterial culture filtrate or preparations of endotoxin causes a generalized Shwartzman reaction in rabbits or guinea pigs which have an active infection with cholera bacilli, streptococci or *Coxiella burneti*(9). In these experiments, the animals were tested one or 2 days after infection. When guinea

pigs were injected with a large dose of tubercle bacilli (5 to 10 mg) and challenged intraperitoneally with either suspensions of heat killed *E. coli* or with culture filtrates, the animals showed a much more severe response than control animals leading to shock and death(10). The high degree of reactivity to these materials was interpreted as depending upon the persistence of nodular lesions within the omentum due to deposit of large masses of BCG. The mechanism of this hyperreactivity is unknown and usually described as a generalized Shwartzman phenomenon. Sufficient data are not available from our experiments to decide whether this high degree of sensitivity coincides with or is dependent upon actual multiplication of tubercle bacilli. It had been shown that BCG multiplies in tissues of mice for approximately 2 to 3 weeks, and that the population of bacilli declines gradually thereafter(11). In our experiments the state of hyperreactivity was demonstrable beyond this time, and probably not related to continued growth of bacilli in the tissues.

The fact that cord factor, a trehalose dimycolate derived from virulent tubercle bacilli, induced a similar state of hyperreactivity as did BCG, would indicate that this reactivity was not related to delayed hypersensitivity to tuberculin, because cord factor does not induce tuberculin hypersensitivity of this type. Recently it has been shown that tuberculin shock in guinea pigs may be due to a generalized Shwartzman reaction rather than to an immunological reaction of the delayed type. These conclusions were based on the fact that tuberculin sensitive guinea pigs resisted high doses of tuberculin when they were rendered tolerant by repeated injections of lipopolysaccharide from *E. coli*(12). Experiments are in progress to investigate the mechanism of the phenomenon here described and to explore its possible role in pathogenesis of the tuberculous infection.

Summary. 1. It is shown that a single intravenous injection of BCG in mice induces a state of hyperreactivity to bacterial endotoxin. The LD_{50} for *E. coli* lipopolysaccharide is decreased from 357 μg in controls to 6 or 7 μg in BCG vaccinated animals. This change appears around the 5th day after vaccination. 2. Trehalose dimycolate, a component derived from virulent tubercle bacilli induces a similar state of hyperreactivity to endotoxin upon single or repeated injections.

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