Shwartzman Reaction in Germfree Rabbits

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The local Shwartzman reaction occurred in germfree rabbits which had no natural antibody to endotoxin and none or only a very small amount of immunoglobulin G. From the results it was concluded that the presence of natural antibody to endotoxin is not a prerequisite of the production of the local Shwartzman reaction by bacterial endotoxin.

The Shwartzman reaction has long been considered to be a nonspecific reaction that is unrelated to any immunological mechanism (2). Stetson (12), however, has advanced the hypothesis that the biological activity of endotoxins, including the Shwartzman reaction, might be based on the existence in normal animals of a delayed-type hypersensitivity which is spontaneously induced by the endotoxin derived from the endogenous bacterial flora. This idea has prevailed among workers in this field. I therefore bred germfree rabbits and attempted to induce the Shwartzman reaction to determine the influence of intestinal flora on the reaction.

Both male and female rabbits of a native white Japanese strain were used. Hysterectomies were performed aseptically, on pregnant rabbits, and the babies were delivered into a sterile isolators and reared in germfree environments. The conventionally raised control rabbits were born naturally but were immediately removed from their mothers and placed in a room controlled to match the conditions inside the isolators. All of the newborn rabbits were hand fed a diet of goat milk fortified with vitamin supplements until week 4 of life. By week 5, they were weaned to a solid diet of Lobund Laboratory L-478 prepared by Nihon CLEA Co., Tokyo, Japan (9). Milk, solid diet, and water were sterilized at 121°C for 20 min in an autoclave. After being weaned, each animal was housed in a stainless-steel cage (30 by 45 by 30 cm), and both food and distilled water were given ad libitum. A sterility test on the food, the tools, and the rabbits in the isolators was performed weekly by the technique of Wagner (14).

Endotoxin was extracted from Escherichia coli O55 by the method of Boivin et al. (1). The endotoxin was solubilized in saline, passed through a 0.22-μm (pore size) membrane filter (Millipore Corp., Bedford, Mass.), distributed in sealed glass ampoules, and transferred to the germfree isolators. The skin-preparatory dose used was 0.2 ml of various dilutions of the endotoxin and was injected intradermally into the right upper quadrant of the abdomen. The reaction was induced 24 h later by the injection of 2 ml of endotoxin into the marginal ear vein. The rabbits were bled by cardiac puncture, and the sera were stored at −80°C. The hemagglutination test for endotoxin-sensitized erythrocytes was used to detect antibody to endotoxin (6). Hemolytic antibody to sheep erythrocytes and complement activity were determined by the method of Mayer (7, 8). Immunoelectrophoresis was performed by the method of Grabar and Williams (4). Goat antiserum against whole rabbit serum (Cappel Laboratories, Cochransville, Pa.) was used as antibody.

The Shwartzman reaction occurred in all of the germfree rabbits (Table 1). In conventional rabbits, the reaction occurred in 8 of 11 (73%) animals. Shwartzman had shown the positive reaction in rabbits bred under conventional conditions to be 78% (11). The reason for the difference in percentages of positive reactions in germfree and conventional rabbits cannot be determined because the number of germfree rabbits used did not afford a sufficient basis for such a discussion. In conventional rabbits, the titers (mean ± standard deviation) of natural antibody to endotoxin and hemolytic antibody to sheep erythrocytes were 4.81 ± 0.83 and 5.09 ± 0.68 log2, respectively. These antibodies, however, could not be found in the sera of germfree rabbits, and the amount of gamma globulin was very small or nonexistent (Fig. 1). It is therefore conceivable that the endotoxin had very little effect on germfree rabbits, although even germfree animals are not completely free of antigen. Since we have not yet found any reports of a method for detecting natural hypersensitivity to endotoxin in vitro, however, we can say

![Table 1: Local Shwartzman reaction with E. coli endotoxin in germfree and conventional rabbits](image)

The rabbits used in this experiment were 100 to 130 days old.

Degree of hemorrhagic necrosis: +++, diameter more than 15 mm; ++, diameter 10 to 15 mm; +, diameter less than 10 mm; −, no reaction.
from the results of this experiment that the presence of natural antibody to endotoxin is not a prerequisite for the Shwartzman reaction.

Polymorphonuclear leukocytes play an essential role in the preparation of the skin for the Shwartzman reaction (13). In these experiments, the leukocyte count in germfree rabbits was 1,700/mm³, which is one-third to one-fourth that found in conventional rabbits (Fig. 2). At 24 h after a preparatory injection of endotoxin, however, the number of leukocytes increased to 7,000/mm³, and migrations of leukocytes were observed in the skin lesions, as determined from the increase in the blood. After provocative injection, more leukocytes existed in the lesions. Polymorphonuclear leukocytes made up almost the entire increase in the leukocytes.

Complement factor in blood was also essential for the appearance of the reaction (3). Complement existed in the blood of the germfree rabbits at a level comparable to that in the conventional rabbits. It was therefore thought that the appearance of the Shwartzman reaction in germfree rabbits was due to the existence of complement as well as to the rapid increase of leukocytes.

It was noticed that, in germfree rabbits, preparations of the skin with endotoxin produced only a faint erythema with no induration or visible reaction, whereas in conventional rabbits the same preparation yielded an edematous erythema almost always accompanied by an induration of varying intensity. After the germfree rabbits were exposed to a conventional environment, however, edema was produced in endotoxin-injected skin (data not shown).

Stetson has suggested that the Shwartzman reaction is a delayed-type hypersensitivity to endotoxin (12). Watson and Kim have classified the actions of endotoxin as (i) primary, intrinsic toxicity and (ii) secondary toxicity related to the bacterial cell wall antigen, which can react with the natural hypersensitivity of the host (15). These workers also thought

that the Shwartzman reaction is a delayed-type hypersensitivity. In an experiment on the lethal effect of endotoxin on mice, Schaedler and Dubos showed that E. coli-free mice which had low sensitivity to the endotoxin acquired a high sensitivity to it when they were infected with E. coli (10). Jensen et al. have also reported that germfree mice had lower sensitivity to endotoxin than conventional mice and that the sensitivity of the germfree mice increased when they were returned to a conventional environment (5). These authors therefore suggested that animals acquire the hypersensitivity to the endotoxin by being exposed to the intestinal bacteria for a long time. Since they did not prove the existence of endotoxin hypersensitivity in vitro, however, it is uncertain as to whether such hypersensitivity actually exists or whether it is a specific or a nonspecific immunity. Although the results of these experiments suggest that the presence of natural antibody to endotoxin is not a prerequisite for the Shwartzman reaction, further experiments are necessary to resolve the question of how changes in the host exposed to intestinal flora for a long time relate to endotoxin reactions.

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LITERATURE CITED

![FIG. 1. Immmuenoelectrophoretic analysis of sera from germfree and conventional rabbits. C: Conventional rabbit serum; GF, germfree rabbit serum; Anti-C, goat antiserum against conventional rabbit serum. The albumin lines are shown on the right, and the gamma globulin lines are shown on the left. The albumin lines were observed in the sera of both conventional and germfree rabbits, but the gamma globulin lines were not clearly identifiable in the sera of germfree rabbits.](http://iai.asm.org/)

![FIG. 2. Changes in the numbers of polymorphonuclear leukocytes (□) and mononuclear cells (■) in total leukocytes in germfree (A) and conventional (B) rabbits during the Shwartzman reaction. The arrows indicate preparatory (0-h) and provocative (24-h) injections of endotoxin. The results are expressed as the means ± standard deviations of three to five experiments.](http://iai.asm.org/)


