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Mice (BALB/cJ, C3H/HeN, and C3H/HeJ) primed with actinomycin D became highly susceptible to lethal intoxication with staphylococcal enterotoxin B (SEB). The mice underwent toxicosis and toxic shock and died. Actinomycin D-primed C3H/HeN and C3H/HeJ mice showed equal sensitivity to SEB, suggesting that bacterial lipopolysaccharide derived from gram-negative bacteria in the gut may not be an important cofactor in intoxication. In a time course study of the illness, prominent pathological changes characterized by blood congestion and thickening of alveolar septa were seen in the lung, while blood congestion, inflammation, epithelial cell flattening, and villous blunting were seen in the small intestine. In lymphoid tissues, such as the spleen, congestion, inflammation, and lymphoid cell depletion were the major reactions. The pathological features of the mice had many similarities to those of rhesus monkeys intoxicated with intravenous SEB. The actinomycin D-primed C3H/HeJ mice are thus an ideal mouse model for studying SEB toxicosis and toxic shock.
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Increased Susceptibility to Staphylococcal Enterotoxin B Intoxication in Mice Primed with Actinomycin D

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Mice (BALB/cJ, C3H HeJ, and C3H/HeJ) primed with actinomycin D became highly susceptible to lethal intoxication with staphylococcal enterotoxin B (SEB). The mice underwent toxicosis and toxic shock and died. Actinomycin D-primed C3H/HeJ mice showed equal sensitivity to SEB, suggesting that bacterial lipopolysaccharide derived from gram-negative bacteria in the gut may not be an important cofactor in intoxication. In a time course study of the illness, prominent pathological changes characterized by blood congestion and thickening of alveolar septa were seen in the lung, while blood congestion, inflammation, epithelial cell flattening, and villois blunting were seen in the small intestine. In lymphoid tissues, such as the spleen, congestion, inflammation, and lymphoid cell depletion were the major reactions. The pathological features of the mice had many similarities to those of rhesus monkeys intoxicated with intravenous SEB. The actinomycin D-primed C3H/HeJ mice are thus an ideal mouse model for studying SEB toxicosis and toxic shock.

Staphylococcal enterotoxin B (SEB) is a superantigen (8, 14). It causes food poisoning when it is ingested and toxic shock and death when it enters the blood circulation and systemic tissues (3, 7, 21, 28). SEB-induced lethal toxic shock appears to be due to functional failures and pathological changes in several organs and organ systems (3, 7, 28). Although cytokines have been suggested to be the causative factors of the toxicosis and toxic shock, the mechanism of pathogenesis remains obscure (4, 9, 10, 11).

Monkeys and chimpanzees are the animal species most sensitive, next to humans, to SEB-induced food poisoning and toxic shock (2, 3). The clinical symptoms and pathological reactions in the monkey and human toxicosis and toxic shock cases are similar (2, 3, 18). We have used monkeys as a toxic shock model to investigate pathogenesis and to test vaccines (25). However, monkeys are expensive, genetically diverse, and limited in numbers and technical approaches. Recently, mice have been used to develop models of lethal intoxication by taking advantages of their relatively low cost and the availability of inbred strains and monoclonal antibody reagents to various cytokines and lymphocyte and leukocyte markers. However, for an unknown reason, mice are resistant to SEB-induced lethal toxic shock. They have to be manipulated if they are to be made susceptible to SEB-induced lethal intoxication.

Four methods of increasing SEB sensitivity have been reported: (i) priming with a large dose of β-galactosamine (15); (ii) further intoxication with a large dose of bacterial lipopolysaccharide (LPS) (23, 24); (iii) use of transgenic mice with certain T-cell receptor Vβ chains (16); and (iv) use of severe combined immunodeficiency mice with transplants of human fetal liver and thymus cells (1). Although all these mice have been proposed as mouse models of SEB intoxication, detailed pathological examinations of these mice have not been reported. Therefore, the extent of the similarity between the illness in these mice and the illness in monkeys and humans is still unknown.

For an ideal mouse model of SEB toxicosis and toxic shock, pathological reactions similar to those of monkeys or humans are a prerequisite. Actinomycin D (ACT-D) has been used to enhance the sensitivity of L929 cells to tumor necrosis factor in vitro assays (26). It also has an additive effect with LPS and tumor necrosis factor in inducing lethal toxic shock in mice (6). Tumor necrosis factor is thought to be the initiator of the cascade of reactions that leads to toxic shock and death in mice given LPS (5). Because there are similarities in the clinical symptoms of LPS- and SEB-induced toxic shock (3, 24), we have combined ACT-D with SEB and administered the combination to mice in the hope of developing a SEB toxic shock model. The results show that ACT-D indeed enhances SEB in inducing toxic shock and death in mice. The ACT-D-primed mice intoxicated with SEB have many pathological features and clinical signs similar to those of rhesus monkeys challenged intravenously with SEB. The ACT-D-primed mice appear to be a good mouse model of SEB-induced toxic shock.

SEB prepared by the method of Schantz et al. was the same preparation used previously (12, 20, 25). It was relatively pure, showing an intense band of 29 kDa and two very faint bands of 10 and 17 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. BALB/cJ, C3H/HeJ, and C3H/HeN mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. They were primed with ACT-D mannitol (Sigma Chemical Co., St. Louis, Mo.) by intraperitoneal injection; this was followed by intravenous or intraperitoneal SEB challenge. Both ACT-D and SEB were dissolved in pyrogen-free normal saline. and 0.4 ml of the solutions was injected into each mouse. Mice were observed for signs of illness and sacrificed at the terminal stage or at an appropriate time point; their tissues were removed and processed for histopathological studies.
stages of toxicosis. Shortly appeared healthy and did not show such signs of illness. On the The results showed that C3H/HeJ and

only with low doses of mice) died. These results suggest that BALB/cJ mice primed sensitivity to

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studies of pathogenesis. Thus, to develop a mouse model. Monkey tissues and histological sections were from previous

studies in which Wallace Baze was the pathologist.

BALB/cJ mice are commonly used in immunological studies. Their T and B cells as well as macrophages have been extensively described. If activation of antigen-presenting cells. T cells, and other lymphoid cells is the main cause of SEB toxics, toxic shock, and death (10, 13, 14). BALB/cJ mice would provide an advantage over other animal species for studies of pathogenesis. Thus, to develop a mouse model, BALB/cJ mice were tested first. They were intraperitoneally primed with ACT-D, intravenously or intraperitoneally challenged with various doses of SEB, and observed for illness and ultimate outcome. Intraperitoneal and intravenous challenges with SEB showed essentially the same results. A representative result is summarized in Table 1. BALB/cJ mice were highly resistant to lethal toxic shock when given ACT-D alone. A dose of more than 500 μg per mouse was generally required. When BALB/cJ mice were primed with 10 μg of ACT-D per mouse and challenged with 100 μg of SEB, they became sick (see below), but they recovered. However, when BALB/cJ mice were treated with 20 μg of ACT-D per mouse, lethality began to be seen at a SEB dose of 20 μg per mouse (one of five mice died). When the doses of SEB were increased to 50 and 100 μg per mouse in these ACT-D-primed mice, 80% (four of five mice) died. These results suggest that BALB/cJ mice primed with ACT-D became susceptible to lethal SEB-induced toxic shock.

Although no deaths occurred when mice were challenged only with low doses of SEB or were primed with a low dose (10 μg per mouse) of ACT-D and then challenged with a low dose of SEB, the mice generally appeared sick in the first 24 h (Table 1). They were inactive, stopped drinking and eating, and gathered close to one another. In severe cases, the mice had ruffled fur and shook while walking, with a splitting abdomen. Mice primed only with ACT-D or with saline appeared healthy and did not show such signs of illness. On the other hand, the dying mice that had been intoxicated with a high dose of SEB or that had been primed with ACT-D and challenged with a lower dose of SEB generally showed four stages of toxicosis. Shortly (1 to 2 h) after SEB challenge, they became inactive and gathered close to each other. Later, they trembled with a splitting abdomen, isolated themselves, and displayed increased lethargy. Subsequently, at 24 to 72 h after SEB challenge, the mice went into coma. They collapsed on the floor, showed rapid and short breathing, and did not respond at all to the touch of a pencil, indicating a stage of toxic shock. A fatal outcome followed shortly (30 to 120 min) after the onset of this comatose stage. Mice that did not undergo the comatose stage began to recover 3 to 4 days later.

In rabbits intoxicated with SEB, significant levels of bacterial LPS have been detected in the blood circulation (17). It has been suggested that the LPS from endogenous, gram-negative bacteria in the gut may have a synergistic effect with SEB, causing intoxication (17, 24). To minimize or eliminate the influence of LPS, an experiment similar to the one described above with BALB/cJ mice was conducted in C3H/HeJ mice, which are resistant to LPS activation and LPS-induced toxic shock (19, 22, 27). The results are summarized in Table 2. C3H/HeJ mice were somewhat more sensitive than BALB/cJ mice to SEB after priming with ACT-D. When primed with 10 μg of ACT-D per mouse, C3H/HeJ mice were sensitive to SEB at 100 μg per mouse, while BALB/cJ mice at the same ACT-D and SEB doses were not sensitive (Table 1 and 2). Although priming with a higher dose of ACT-D (30 μg per mouse) further increased the lethality of SEB in C3H/HeJ mice (Table 2), death was quicker. However, what appeared to be increased sensitivity to SEB may in fact have been the direct toxic effect of ACT-D or an additive effect of the toxicity of SEB and the toxicity of a high dose of ACT-D.

In contrast to C3H/HeJ mice, C3H/HeN mice are sensitive to LPS activation and toxic shock (19, 22, 27); an additive effect of SEB and LPS should have been seen in the C3H/HeN mice after SEB intoxication if LPS is essential in SEB-induced toxic shock. To further study the possibility that the lethal toxicosis of C3H/HeJ mice is mainly due to the effect of SEB rather than to the additive effect of SEB and endogenous LPS, a comparative study of C3H/HeJ and C3H/HeN mice was conducted. The results showed that C3H/HeJ and C3H/HeN mice had essentially the same sensitivity to SEB whether or not they were primed with ACT-D (data not shown). Thus, LPS from the gut may not be an essential cofactor in SEB-induced lethal intoxication in mice.
Pathological information derived from monkeys and humans in SEB toxic shock cases is mainly from postmortem studies. There is no information concerning the time course of pathological changes under SEB toxicosis. Our ACT-D-primed mice allowed us to conduct a time course study. For this purpose, C3H/HeJ mice were intraperitoneally primed with ACT-D (10 μg per mouse) and then intraperitoneally challenged with SEB (100 μg per mouse). At timed intervals, mice were observed for signs of illness, and groups of four to five mice were sacrificed for pathologic studies. Mice that went into shock in 4 h after SEB challenge. In the lung, congestion in the venules and capillaries and “pavementing” with polymorphonuclear leukocytes (PMNs) to the vessel wall were noticeable as early as 4 h after SEB challenge. This reaction was followed (at 8 h) by thickening of the alveolar septa due to increased cellularity and “pavementing” with polymorphonuclear leukocytes (PMNs) in the lamina propria.

In the intestine, the prominent features were blood congestion and PMN pavementing and infiltration, which were consistently seen at all times after SEB challenge. These features were later (at 16 h) followed by blood congestion, epithelial flattening with villous blunting, lymphocyte necrosis in the Peyer's patches, which persisted to the comatose stage (36 to 72 h). The first reactions in the spleen were mild blood congestion and the appearance of many PMNs in the red pulp at 4 h after SEB challenge. At 8 h after challenge, the PMNs increased in numbers, and mitotic lymphocytes appeared in both the red pulp and the white pulp. Subsequently (at 16 h), many necrotic lymphocytes and macrophages with phagocytosed materials appeared in both the red pulp and the white pulp. Subsequently (at 16 h), many necrotic lymphocytes and macrophages with phagocytosed materials appeared in both the red pulp and the white pulp. Subsequently (at 16 h), many necrotic lymphocytes and macrophages with phagocytosed materials appeared in both the red pulp and the white pulp.

The histopathological reactions in the ACT-D-primed and SEB-challenged mice were compared with those of rhesus monkeys challenged with intravenous SEB. A comparison of the histopathologic findings is summarized in Table 4. Although there were differences, similarities were many. In both the mice and the monkeys, similar pictures of blood congestion and hemorrhage, PMN pavementing, and inflammatory cell infiltration were prominent in the lung and intestine. Also, in the lung, thickening of the alveolar septa due to increased cellularity was seen in both the monkeys and the mice. Intraalveolar edema was only seen in the monkeys, whereas hydrothorax was seen in mice. Both the mice and the monkeys
FIG. 1. Pathological reactions in the lung, small intestine, and spleen of C3H/HeJ mice primed with ACT-D (10 μg per mouse) and challenged with SEB (100 μg per mouse). (A) Thickening of the alveolar septa (8 h after SEB challenge; hematoxylin and eosin stain; magnification, ×50). (B) Epithelium flattening with villose blunting of the ilium (24 h after SEB challenge; hematoxylin and eosin stain; magnification, ×100). (C) Necrotic lymphocytes and macrophages with phagocytosed materials in the spleen (16 h after SEB challenge; hematoxylin and eosin stain; magnification, ×100).
had parenchymal cell degeneration in various tissues. Mice generally had more prominent macrophage phagocytosis and lymphocyte necrosis. Although focal hepatocyte necrosis could be easily found in mice, it was only occasionally seen in monkeys. Ileal epithelial flattening with villous blunting was prominent in both the monkeys and the mice. In summary, we have shown in the present study that mice (BALB/cJ, C3H/HeJ, and C3H/HeN) primed with ACT-D become very susceptible to SEB-induced lethal intoxication. The mice underwent toxicosis and toxic shock and died. This increased sensitivity to SEB is not due to the influence of LPS derived from the gram-negative bacteria in the gut. Prominent pathological changes were seen in the lungs, intestine, and lymphoid tissues such as the spleen. These pathological changes in the mice had many similarities with those in rhesus monkeys challenged with SEB intravenously. The ACT-D-primed mice, particularly the LPS-resistant C3H/HeJ mice, are thus a good small animal model for studying the pathogenesis mechanism of SEB toxicosis and toxic shock. The mouse model provides an advantage in that it allows one to perform studies of the time course of pathological changes, which are difficult to perform in monkeys and impossible to perform in humans.

Yiran Qiao is a National Research Council Fellow.

REFERENCES


