THE TOXICITY OF STAPHYLOCOCCAL ENTEROTOXIN B IN MICE IS MEDIATED BY T CELLS

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A group of toxins produced by *Staphylococcus aureus* has long been known to be responsible for a number of human diseases, including food poisoning and toxic shock (1, 2). It is also known that these toxins are powerful stimulators of human and mouse T cells (3–7). Recently, several groups have shown that some, and perhaps all, of these toxins bind to class II MHC proteins in man and, somewhat less well, in mouse (8–10). We and others have also shown that the ability of human and mouse T cells to respond to *S. aureus* toxins requires that the toxins first be bound to class II molecules on presenting cells, and that the ability of T cells to respond to the combination of toxin and MHC depends upon the structure of the TCR, in particular upon the Vβ segment of the receptor. T cells bearing human Vβ8 or mouse Vβ11, for example, respond very well to *S. aureus* enterotoxin E, whereas responses to the toxic shock-stimulating toxin are driven mainly by human T cells bearing Vβ2 or mouse T cells bearing Vβ15 or -10 as part of their receptors (6, 7, 11, 12).

It is not known why these toxins are pathogenic. Since they bind to class II molecules on macrophages and other class II$^+$ cells, their toxic effects could be mediated by stimulation of such cells to produce mediators with toxic effects, such as cachectin/TNF, IL-1, or leukotrienes (13–18). Alternatively, the toxins may cause disease because they stimulate large numbers of T cells to divide and secrete certain lymphokines at high levels, such as IL-2 or TNF. Finally, the staphylococcal toxins may lead to pathogenesis by a T cell/class II independent and unknown mechanism.

In this work, we have studied the pathogenic effects of one staphylococcal toxin, enterotoxin B (SEB)[1], on mice. Administration of the toxin to mice causes weight loss and immunosuppression in a dose-dependent fashion. The degree of weight loss induced is related to the MHC haplotype of the mice challenged. Nude mice, which almost completely lack T cells, or mice constructed genetically such that they contain few T cells able to respond to SEB, suffer little or no loss in weight when SEB

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[1] *Abbreviations used in this paper:* CSA, cyclosporin A; HEL, hen egg lysozome; PPD, purified protein derivative; SEB, staphylococcal enterotoxin B.

is given to them. Mice containing normal numbers of T cells, but deficient in those able to respond to SEB, are not immunosuppressed by the toxin. These results suggest that some or all of the pathogenic effects of SEB in mice, and perhaps man, are caused by the potent ability of the toxin to stimulate T cells.

Materials and Methods

Mice. B10.BRSgSn, CBA/J, and (C57Bl/6 × BALB/c)F_1 nude and littermate animals were purchased from The Jackson Laboratory (Bar Harbor, ME). B10.BRβBR mice, carrying the TCR β chain locus derived from C57Ls, were derived as previously described (19). These and other animals were bred in the animal care facility at the National Jewish Center for Immunology and Respiratory Medicine.

Response to Antigen. Animals were primed in the base of the tail, as previously described (20), with 100 μg hen egg lysozyme (HEL) (Sigma Chemical Co., St. Louis, MO) in CFA. 7 d later, cells were harvested from the draining lymph nodes of these animals, and T cells were purified by passage over nylon wool (21). T cells were titrated for response into cultures containing 1,000-rad irradiated antigen-presenting syngeneic spleen cells and 1 mg/ml HEL or purified protein derivative (PPD) (Parke Davis Corp.). 4 d later, responses in these cultures were monitored using 3-(dimethylthiazol-2-yl)-2,5-diphenyl tetrajolium bromide (MTT) as previously described (22).

Analysis of Vβ Expression. T cells were prepared from samples of peripheral blood as previously described. Aliquots of the cells were incubated with biotinylated anti-α/β, anti-Vβ3, anti-Vβ6, anti-Vβ7, or anti-Vβ8 antibodies (23–26). After washing, reactive cells were stained with phycoerythrin coupled to avidin, and analyzed using an Epics C flow cytometer.

Measurements of Animal Weight. Mice were confined, three to a cage, for at least 1 wk before beginning an experiment to allow social relationships to stabilize in the group. We found that mouse weight varied considerably, even in unmanipulated animals, for a few days after mice were transferred to new groups; thus, the preexperimental period of 1 wk was used to avoid complications in our experiments due to this factor. Mouse weight varies somewhat at different times of day, therefore, in any given experiment, mice were always weighed daily at the same time, usually between 12:00 noon and 4:00 pm. Mice were weighed using a balance that allowed accurate determination to within 0.1 gm.

To determine the effects of toxins on weight, mice were weighed 24 h before, and at the time of, toxin administration. They were then weighed at 24-h intervals thereafter. All mice in a given cage were given the same dose of toxin, to avoid contamination with toxin between individuals, because of toxin acquisition by grooming, for example.

Weight changes are shown as percent weight gain or loss compared with the weight of the animal before toxin administration.

Cyclosporin Treatment. Cyclosporin A (CSA) was a gift of Sandoz Pharmaceutical Corp., East Hanover, NJ. Animals were given 25 mg/kg cyclosporin in 30–40 μl olive oil intraperitoneally every day. Controls received olive oil alone. This dose of CSA has been shown to affect T cell development and function in vivo in mice (27). In our hands, effects were comparable with those published; this dose of CSA completely prevented the appearance of mature thymocytes in vivo. Mice were treated with CSA for 1 wk before toxin administration, and throughout the duration of the experiment. Neither CSA nor olive oil treatment had any effect on the weight of mice in the absence of SEB.

Results

Effects of Staphylococcal Enterotoxin B on Normal Mice. Groups of three 12-wk-old B10.BR mice were given doses of SEB ranging from 0 to 100 μg, i.p. The animals were weighed daily. As shown in Fig. 1, the animals suffered a rapid dose-dependent weight loss that was most profound 1 or 2 d after toxin administration. Similar effects were seen in many duplicate experiments, with some variations in the effects of a

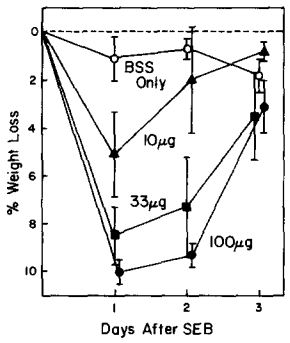

FIGURE 1. Dose dependence of weight loss after SEB administration. Groups of three B10.BR mice were caged separately for 1 wk and given the indicated dose of SEB intraperitoneally on day 0. Mice were weighed at this time and daily at the same time thereafter. Shown are the averaged percent weight losses by comparison with the weight of each mouse on day 0. Bars indicate the SEs of percentage weight loss within each group of mice. On day 0, the mice each weighed between 20 and 25 g. (●) 100 μg SEB; (■) 33 μg SEB; (▲) 10 μg SEB; (●) 0 μg SEB.

particular dose of toxin. In one experiment, for example, all mice given 100 μg of SEB actually continued to lose weight over 3 d and eventually died.

4 d after the mice described above were given toxin, they were killed, and the effects of the toxin on the mice were evaluated. Macroscopically, there was no effect on the gut, lungs, or liver, which all appeared normal in SEB-treated mice. Animals given high doses of toxin did contain less fat, however. For example, the fatty tissue surrounding the inguinal lymph nodes was significantly diminished in these mice. There was no significant change in the size of the lymph nodes and spleens of animals given toxin, nor was there a significant change in the numbers of T cells harvested from lymph nodes. The sizes and cellularity of the mouse thymuses were affected, however. As shown in Table I, the yield of thymocytes from SEB-treated animals was substantially less than that of normal mice of the same age. Again, the effects of SEB were dose dependent.

Analysis of Vβ expression on lymph node T cells from these animals showed that, although their total numbers were unaffected by the toxins, there had been a considerable effect of the toxins upon their composition. For example, the percentage of Vβ8-bearing cells rose from ~31% in normal B10.BR mice, to ~51% in mice given 100 μg SEB. Conversely, the percentage of Vβ6-bearing cells dropped from ~15% to <10% (Table I). These changes were predicted by the known Vβ specificity of SEB, since we and others have previously shown that this toxin stimulates T cells bearing Vβ3, -7, and -8, but not those bearing Vβ6 (6, 7). These results are best

TABLE I

The Effects of SEB on Thymocytes and Lymph Node T Cells

SEB dose	Thymocytes/ mouse × 10^7	Percent lymph node T cells bearing: Vβ3	Vβ6	Vβ7	Vβ8s
μg					
0	9.1 ± 1.8	6.4 ± 0.4	14.9 ± 0.6	4.5 ± 0.5	30.9 ± 0.7
10	6.6 ± 2.0	4.6 ± 0.3	12.1 ± 1.6	4.6 ± 0.6	40.9 ± 6.2
33	3.0 ± 0.4	5.0 ± 0.1	10.4 ± 0.6	4.8 ± 0.3	43.9 ± 1.8
100	1.7 ± 0.6	5.2 ± 0.2	9.7 ± 0.3	6.0 ± 0.4	51.3 ± 2.2

Results shown are the mean and SE of data from three mice.

interpreted as an increase in those T cells bearing receptors with which SEB could interact, and a concomitant decrease in nonreactive T cells.

It has previously been reported that administration of SEB suppresses in vivo immune responses in mice (28, 29). To confirm this, under the conditions used in these experiments, mice were given different amounts of SEB and then immunized with HEL in CFA. HEL was chosen because responses to it in H-2^k animals are not dominated by T cells bearing Vβs, which interact with SEB. Instead, anti-HEL responses in such animals are often those of Vβ11-bearing cells (30).

7 d after immunization with HEL, draining lymph nodes were removed from the mice, and the T cells in them harvested and titrated for response to HEL or PPD, the major antigen in the mycobacteria in CFA. 3 or 4 d later, the proliferative responses in these cultures were measured.

As shown in Table II, substantially fewer T cells were harvested from immunized animals that had been previously given SEB. These cells also responded less well to HEL or PPD than those from control animals that had not been pretreated with SEB. Overall, the total T cell response/mouse recovered from animals given high doses of SEB was <20% of that from control animals. Mice given lower doses of SEB had less reduced responses to antigen.

Although SEB administration had several demonstrable effects in these experiments, we chose to measure weight loss as an indication of toxicity in our further studies because the phenomenon was reproducible, easily applied as a time course, and sensitive to toxin dose.

The Mouse MHC Type Affects its Response to SEB. In an attempt to find out whether there was any relationship between the MHC molecules available for SEB presentation in animals, and the toxic effects of SEB, we studied the consequences of exposure to SEB on B10.BR (IA^{k+}, IE^{k+}) and B10.A(4R) (IA^k, IE^-) mice. First, the toxin was titrated into cultures of purified B10.BR or B10.A(4R) T cells and syngeneic APC. The toxin was about an order of magnitude more effective, dose for dose, in stimulating B10.BR cells than cells from B10.A(4R)s (Fig. 2 *A*). This was probably because the toxin binds more efficiently to IE^k than to IA^k, as has been suggested by others (6).

The effects of SEB on these two strains of mice in vivo were then measured. Groups

TABLE II

Immunosuppressive Effects of SEB

SEB dose	Lymph node T cells/mouse	Response to:* HEL/T cell	HEL/mouse	Response to: PPD/T cell	PPD/mouse
μg	$\times 10^6$				
0	14.0	100	100	100	100
62.5	8.3	53.0	31.4	49.8	29.6
125	7.0	50.3	25.2	44.4	22.2
250	5.0	48.1	17.2	44.3	15.8

* Mice were given the indicated dose of SEB, or an equivalent volume of balanced salt solution. 3 d later, they were immunized with HEL in CFA. 7 d after immunization, draining lymph node cells were harvested, and T cells were purified and titrated for response to HEL or PPD. Proliferative responses/input T cell or responses/mouse were calculated and normalized to those of the BSS-treated control animals.

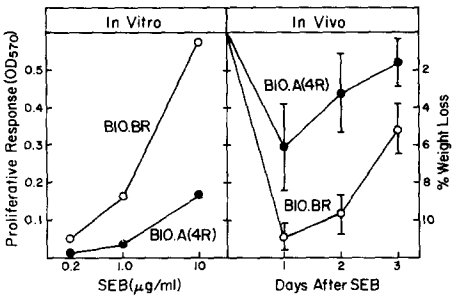

FIGURE 2. The response to SEB in vitro and in vivo is similarly MHC dependent. (*A*) (In vitro) T cells were prepared from B10.BR (●) or B10.A(4R) (●) animals and titrated into cultures containing saturating irradiated APC and 1 μg/ml SEB. Proliferation was assayed 3 d later using MTT (22). Data shown are the averages of triplicate cultures. (*B*) (In vivo) Groups of three B10.BR (○) or B10.A(4R) (●) mice were housed, treated with SEB, and weighed as described in the legend to Fig. 1.

of three mice of either strain were given balanced salts solution or 50 μg SEB intraperitoneally, and weighed daily. As shown in Fig. 2 *B*, weight loss of B10.A(4R) animals was small, and the mice recovered rapidly. B10.BR animals, on the other hand, suffered a more profound and more prolonged weight loss.

These results suggested that the toxic effects of SEB were related to the ability of the toxin to bind MHC, either because binding to class II caused cells bearing such MHC proteins to secrete toxic products, such as IL-1, or because binding to class II triggered some other toxic event in the animals, for example, massive T cell stimulation.

T Cell-deficient Animals Are Less Susceptible to the Toxic Effects of SEB. Mice were made T cell deficient in two ways. In the first, animals were treated daily with a dose of CSA in olive oil known to suppress lymphokine secretion. These animals and controls were then given SEB. Cyclosporin-treated animals suffered no weight loss as a consequence of the CSA. Control and CSA-treated animals did not lose weight after administration of balanced salts solution. Control mice given SEB lost weight as expected. Weight loss of SEB-treated mice was smaller and less prolonged (Fig. 3).

These results suggested that T cell release of lymphokine in response to SEB was partially or completely responsible for weight loss in mice induced by SEB. Since we had no independent measurement of the effectiveness of the CSA treatment on lymphokine release in vivo, we could not be sure that the residual effects of SEB in CSA-treated animals were due to incomplete suppression of the T cell responses in these animals by the dose of CSA we used.

In a second approach to this problem, nude (C57Bl/6 × BALB/c)F_1 mice, and their littermate controls, were treated with SEB. As shown in Fig. 4, T cell-deficient

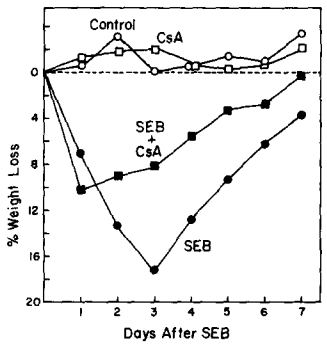

FIGURE 3. Cyclosporin partially prevents weight loss due to SEB administration. Groups of three B10.BR mice were housed, treated with SEB, and weighed as described in the legend to Fig. 1. Two groups received 25 mg/kg SEB in olive oil daily (*squares*), and two control groups received olive oil alone (*circles*). On day 0, two groups received 50 μg SEB/mouse intraperitoneally (*closed symbols*) and two received an equal volume of balanced salts solution (*open symbols*). The mice were weighed at this time and daily thereafter and the results calculated as described in the legend to Fig. 1. SEs on the values ranged from 0.6 to 4.6%.

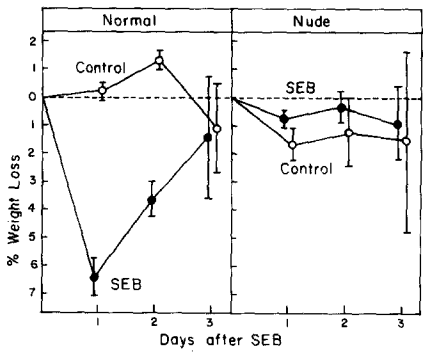

FIGURE 4. SEB does not induce weight loss in nude mice. (C57Bl/6 × BALB/c)F_1 nude and wild-type littermates were purchased from The Jackson Laboratory. On day 0, mice were given 125 μg SEB or balanced salts solution intraperitoneally. Animals were housed, weighed, and the results were calculated as described in the legend to Fig. 1. (●) SEB-treated mice; (○) balanced salt solution-treated mice.

nude animals suffered no significant weight loss after SEB injection, whereas their T cell-positive littermates did. The effects of SEB in these F_1s were not as powerful as in B10.BR mice, probably because of the difference in MHC haplotype.

SEB Is Less Toxic in Mice Deficient in SEB-reactive T Cells. The experiments described above strongly suggested that weight loss caused by SEB was due to T cell reactions. To prove this definitively, we decided to breed a collection of mice that would contain varying numbers of SEB-reactive T cells. This enterotoxin stimulates mouse T cells bearing Vβ3, -7, -8.1, -8.2, and -8.3. Vβ8-bearing cells are absent in mice carrying the C57BR-derived Vβ locus, which lacks the genes for these Vβs (31). Vβ3-bearing T cells are suppressed in mice expressing the mouse superantigens, Mls-2^a and/or Mls-3^a, and a permissive MHC haplotype (24, 32, 33). Vβ7-bearing T cells are deleted in mice expressing a self superantigen, which is, or maps closely to, Mls-1^a, and a permissive MHC haplotype (Drs. D. Woodland and E. Palmer, personal communication). IE^k is functional for presentation of these superantigens. Mice containing the C57BR-derived Vβ locus do contain a functional gene for Vβ17a. T cells bearing Vβ17a do respond well to SEB (J. Callahan, unpublished results). Vβ17a$^+$ cells are almost completely absent from the mice used in these experiments, however, as a result of clonal deletion caused by expression of IE^k (34).

(B10.BRβBR × CBA/J)F_1 mice were therefore created, and male F_1s backcrossed to B10.BRβBR. All these animals express IE^k. B10.BRβBR animals are homozygous for the C57BR-derived, Vβ8$^-$, Vβ locus. CBA/J animals express Mls-1^a and Mls-2^a, and/or Mls-3^a; these superantigens are dominant in F_1s, and will cause the deletion of most T cells bearing Vβ3, -6, -7, and -8.1 (24, 32, 33, 35, 36). Among the backcrossed animals, we therefore expected that about half the animals would be homozygous for the Vβ8 deletion, and about half would be heterozygous for Mls-1^a or its associated superantigen expression, and therefore delete Vβ7 (and Vβ6)-bearing cells. At the time we started this breeding, it was not known which of the Vβ3-deleting superantigens were expressed in CBA/J mice. We therefore expected that either half or three-quarters of the backcrossed animals would express one, the other, or both of the superantigens Mls-2^a and Mls-3^a. Since none of these Vβ-deleting loci are genetically linked, we expected that about one-eighth of the animals would contain few SEB-reactive T cells, and about one-eighth would contain a reasonable number of these cells.

Animals were typed for Vβ expression using samples of their peripheral blood.

The frequencies with which T cells bearing different Vβs were deleted were very much as expected. Of ~100 mice typed, 46% were homozygous for the B10.BRβBR Vβ-deleted chromosome. 54% of the mice eliminated Vβ7-bearing T cells, probably due to Mls-1^a expression. 38% of the animals eliminated Vβ3$^+$ cells. This last result indicated that CBA/J animals carry either Mls-2^a or Mls-3^a, but not both.

Animals expressing low and high frequencies of SEB-reactive T cells were given SEB intraperitoneally, and weighed daily thereafter (Fig. 5). Mice containing few reactive cells were barely affected by the toxin, whereas their littermates containing more T cells that would be stimulated by SEB rapidly lost weight. Preliminary experiments indicated that none of these animals suffered changes in thymus size due to toxin administration, perhaps because the numbers of reactive T cells in the mice were below the threshold required for this effect.

Discussion

It is apparent that SEB has a number of pathological effects in susceptible mice, causing rapid weight loss, thymus depletion, and immunosuppression. The data in this paper demonstrate that the weight loss at least is dependent upon T cell activation caused by the toxin, since animals containing few SEB-reactive T cells, either because of genetic defects or because of cyclosporin-mediated immunosuppression, lose little or no weight after challenge with SEB. This idea is supported by the fact that SEB administration has no effect on the weight of neonatal mice, which contain very few mature T cells (7, and personal observations).

The consequences of SEB-mediated T cell activation on thymus size and T cell responsiveness could not, of course, be measured in thymus-deficient or cyclosporin-treated animals. The toxin did not, however, affect thymus size in B10.BRβBR × CBA/J backcrossed animals, mice that contained ~13% fewer SEB-reactive T cells than their B10.BR relatives in which SEB did cause thymus depletion. This result suggests that massive T cell activation mediated by SEB may be required for significant thymus depletion.

It appears, therefore, that much or all of the measurable pathological effects of SEB in mice are indirect consequences of T cell activation caused by the toxin. At present, it is not clear how T cell activation has such effects. It is likely that the SEB-activated T cells secrete large amounts of a number of different lymphokines, including IL-2 and cachectin, both of which have been shown to have toxic effects similar to those described for SEB in mouse and man (16, 17, 37, 38).

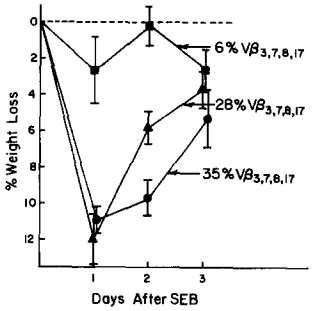

FIGURE 5. Weight loss induced by SEB is proportional to the numbers of SEB-reactive T cells in the mouse. B10.BRβBR × (B10.BRβBR × CBA/J)F_1 animals were bred. Their percentages of peripheral blood T cells bearing Vβ3, -6, -7, and -8 were evaluated (19). Mice were caged in groups of three according to the percent of SEB-reactive T cells they expressed. Animals were given 50 μg of SEB on day 0, and the effects of the toxin on their weight were monitored as described in the legend to Fig. 1. (■, ▲) B10.BRβBR × (B10.BRβBR × CBA/J)F_1 mice containing 6.3 ± 0.4 and 27.6 ± 1.6% SEB-reactive T cells, respectively. (●) B10.BR mice containing 35% SEB-reactive T cells.

The staphylococcal toxins are potent stimulants for human T cells, as well as for those of mice (3–7). In addition, the toxins have, of course, a number of pathological effects in man, including diarrhea, vomiting, and shock (1, 2). In light of the conclusions of this paper, it is worth asking whether some or all of these effects may be due to T cell activation and consequent lymphokine secretion. Since massive T cell activation in man, after anti-CD3 administration, or infusion of large quantities of a T cell-derived lymphokine and stimulator, IL-2, has been reported to cause shock, in a fashion similar to some of the staphylococcal toxins, we would like to suggest that this is so.

In the past, the preferred animal models for studies of staphylococcal toxins have been rabbits, monkeys, or primates. Relatively little work, except on the staphylococcal exfoliating toxins, has been done in mice (39). Probably, this is due to the fact that high doses of the toxins, by comparison with those that cause pathology in man, are needed to cause disease in mice. In fact, some strains of mice are almost unaffected by SEB (personal observations). It is an educated guess that the difference in dose-response curves to SEB between man and mouse is primarily due to the fact that SEB binds to human class II proteins with much higher affinity than it binds to mouse class II (Drs. J. Fraser and A. Herman, personal communication). Since binding to class II is a prerequisite for T cell activation, this could easily account for the difference in susceptibility of the two species. In this context, it is worth mentioning that *aureus* is not the species of *Staphylococcus* found on mice (40). *Staphylococcus xylosus*, the species found on mouse, may secrete toxins with higher affinity for mouse MHC, and consequently, a lower threshold for pathological effects.

Summary

Staphylococcal enterotoxin B (SEB) has been shown in the past to be a potent T cell stimulant in mouse or man. The toxin acts as a superantigen that is, it binds to class II MHC proteins and, as such a complex, stimulates T cells bearing particular Vβs as part of their receptors. The toxin also has several pathological effects, causing, in mice, rapid weight loss, thymus atrophy, immunosuppression, and, at high doses, death. The data in this paper show that at least one of these effects, weight loss, is T cell mediated. Staphylococcal entertoxin-mediated weight loss is MHC dependent, and is almost absent in animals expressing MHC class II molecules, which, complexed with SEB, are poor T cell stimulants. Also, mice that lack T cell function, genetically or because of cyclosporin A treatment, lose no or less weight than controls in response to SEB. Finally, animals bred such that they express few T cells bearing Vβs with which SEB can interact lose much less weight in response to the toxin than littermate controls that have higher numbers of reactive T cells. It is therefore suggested that the pathological effects of the staphylococcal, T cell–stimulating toxins in mouse and man may be partially or wholly the consequence of massive T cell stimulation.

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