Dual Roles for Class II Major Histocompatibility Complex Molecules in Staphylococcal Enterotoxin-Induced Cytokine Production and In Vivo Toxicity

DOUGLAS GROSSMAN,¹ JAMES G. LAMPHEAR,¹ JOSEPH A. MOLLICK,¹
MARSHA J. BETLEY,² AND ROBERT R. RICH^{1,3*}

*Departments of Microbiology and Immunology¹ and Medicine,³ Baylor College of Medicine,
Houston, Texas 77030, and Department of Bacteriology, University of
Wisconsin—Madison, Madison, Wisconsin 53706²*

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The staphylococcal enterotoxins (SE) specifically bind to class II major histocompatibility complex (MHC) proteins, resulting in activation of monocytes and T cells. The SE cause weight loss in mice, which is dependent on T-cell stimulation and tumor necrosis factor alpha (TNF- α) production. Here we use a mutant of staphylococcal enterotoxin A that binds class II MHC molecules and activates monocytes but not T cells to evaluate the relative contributions of monocyte- and T-cell-stimulatory activities to in vivo toxicity. The mutant toxin did not cause weight loss in B10.BR mice but did stimulate monocyte TNF- α production in vitro, as did the wild-type toxin. Addition of a supernatant from toxin-activated T cells enhanced monocyte-stimulatory activity of both mutant and wild-type toxins fivefold. The effect of the supernatant could be mimicked by recombinant gamma interferon (IFN- γ) and was inhibited by antibody to IFN- γ . These results suggest that toxin-induced monocyte TNF- α production is upregulated by IFN- γ , which likely represents the T-cell requirement in SE-mediated weight loss. Our studies thus implicate two distinct class II MHC-dependent signaling pathways for SE, the first involving direct signal transduction through class II MHC molecules mediated by either mutant or wild-type toxin and the second requiring T-cell stimulation by toxin-class II MHC complexes with consequent production of IFN- γ . We suggest that both pathways are required for optimal monocyte TNF- α production in vitro and SE-induced toxicity in vivo.

The staphylococcal enterotoxins (SE) (SEA, SEB, etc.) are produced by certain strains of *Staphylococcus aureus* and have long been recognized as potent toxins in humans; they are commonly associated with food poisoning (43) and cause toxic shock if introduced into the bloodstream (40, 48). The toxic shock syndrome toxin (TSST), also an *S. aureus* product, is the causative agent of tampon-associated toxic shock syndrome (41). Although the SE and TSST have been purified (5) and characterized at the genetic level (23), our understanding of how they cause disease remains incomplete.

Recent appreciation of these staphylococcal toxins as superantigens (47) has provided us with new insights into possible mechanisms of pathogenesis. The SE and TSST specifically bind class II major histocompatibility complex (MHC) molecules to form a complex that activates T lymphocytes (19), inducing proliferation and secretion of lymphokines such as interleukin-2 and gamma interferon (IFN- γ) (9, 28). Marrack and colleagues (32) described a mouse model for SE-induced toxicity in which the animals undergo significant and rapid weight loss upon exposure to toxin. Those investigators argued that SEB-induced weight loss is mediated by T cells, as nude mice and animals containing few SEB-reactive T cells were less susceptible to the effects of that toxin (32).

The capacity of the SE and TSST to interact with class II MHC molecules presents the possibility that a mechanism other than T-cell stimulation may operate in vivo to mediate disease. Binding of these toxins to class II MHC molecules delivers a stimulatory signal to monocytes, resulting in

protein tyrosine phosphorylation (42) and production of various cytokines such as interleukin-1 and tumor necrosis factor alpha (TNF- α) (11, 12, 14, 24, 35, 36, 45). In addition to monocytes, mast cells also express class II MHC molecules (2), and their exposure to SEB has been shown to result in release of inflammatory mediators (26, 39). These observations raise the issue of whether class II MHC molecules serve an additional role, other than presentation of the toxin to T cells, in SE-mediated toxicity in vivo.

It is likely that the toxic effects of the SE and TSST in vivo are due to excessive production of cytokines. This view is supported by the association of interleukin-2 and TNF- α with effects in humans that resemble toxin-mediated diseases, particularly toxic shock syndrome (4, 44). Whether the particular cytokines that mediate disease arise as a consequence of T-cell activation or of direct signaling through surface class II MHC molecules on monocytes or mast cells is unclear. The present study was undertaken to evaluate the roles of class II MHC signaling and T-cell stimulation in cytokine production and SE-mediated weight loss. Using an SEA mutant toxin that is not mitogenic for T cells but retains class II MHC binding and monocyte-stimulatory activities, we demonstrate a positive role for class II MHC signaling by SE in the absence of T cells and further suggest that T cells are required for in vivo toxicity in order to supply IFN- γ , which upregulates toxin-induced monocyte TNF- α production.

MATERIALS AND METHODS

Toxins. The wild-type and Cys-106→Ala mutant *sea* genes (17) were expressed in *S. aureus* to obviate contamination

* Corresponding author.

with lipopolysaccharide (LPS) of toxins derived from *Escherichia coli*. The *sea*-encoding alleles of pMV2 (wild type) and pDG13 (Cys-106 $\rightarrow$ Ala mutant) (17) were fused to staphylococcal promoters as follows. First, pMJB250 was constructed by insertion of the 1.4-kb *Bgl*I (converted to a blunt end with T4 polymerase)-*Hind*III fragment of pMJB3 (6) into the multiple-cloning region of pGEM-7Zf(+) (Promega, Madison, Wis.) by using the *Cla*I (converted to a blunt end with Klenow fragment) and *Hind*III sites. The 1.4-kb *Bgl*I-*Hind*III fragment of pMJB3 contains the complete *sea* structural gene and its promoter (6, 7). pMJB250 has a single *Hinc*II site (in the region corresponding to the SEA protein signal sequence) and a single *Hind*III site (0.3 kb downstream of *sea*) (7). The 1.0-kb *Hinc*II-*Hind*III fragment of pMJB250 was replaced with the 0.7-kb *Hinc*II-*Hind*III fragments of pMV2 and pDG13, resulting in pMJB273 and pMJB275, respectively. Unlike pMJB250, both pMJB250 derivatives contained *Pst*I sites, which had originated in either pMV2 or pDG13 (17). By using unique *Hind*III sites, pMJB273 and pMJB275 were each ligated to pC194 (20), and the ligation mixtures were transformed into *E. coli*. Both composite plasmids were then isolated from *E. coli* and transferred to enterotoxin-negative *S. aureus* RN4220 (27) by electroporation and selected with chloramphenicol. These strains were denoted MJB841 and MJB844 (producing SEA and the Cys-106 $\rightarrow$ Ala mutant form of SEA, respectively). Because only this particular mutant was used in these studies, the terms "wild-type toxin" and "mutant SEA toxin" are used for simplicity. The molecular biological and genetic techniques used in this study have been described elsewhere (22). Toxin production was confirmed by Ouchterlony gel diffusion with anti-SEA antiserum, and toxins were produced, purified, and stored as described previously (16).

Native SEB was prepared from *S. aureus* as described previously (16). LPS was derived from *E. coli* O111:B4 (Sigma Chemical Co., St. Louis, Mo.) and stored at a concentration of 5 mg/ml in water at -70°C .

Weight loss in mice. All experiments employed 8- to 12-week-old male B10.BR/SgSnJ mice (Jackson Laboratory, Bar Harbor, Maine). Weight loss experiments were performed generally as described by Marrack et al. (32). Briefly, groups of animals (three per cage) were segregated for 10 to 14 days to allow body weights to stabilize. Toxins were diluted to a concentration of 375 $\mu\text{g}/\text{ml}$ in 50 mM phosphate-buffered saline, pH 7.0. Animals were weighed to the nearest 0.1 g and injected intraperitoneally with 0.2 ml of toxin (75 μg). Mice were weighed every 24 h over the next 4 days. To eliminate experimental bias, syringes and cages were coded and weights were recorded on separate cards each day.

T-cell preparation and stimulation. T cells were purified from spleens by lysis of erythrocytes and adsorption of B cells onto goat anti-mouse immunoglobulin-coated magnetic beads (Advanced Magnetics, Cambridge, Mass.). Such preparations routinely contained $>85\%$ T cells (anti-CD3 antibody 145.2C11) (30) as assessed by flow cytometry (Epics Profile; Coulter Electronics, Inc., Hialeah, Fla.).

The culture medium consisted of Hanks' minimal essential medium (Whittaker Bioproducts, Inc., Walkersville, Md.) containing 10% heat-inactivated fetal calf serum and supplemented with minimal essential medium amino acids ($0.5\times$), minimal essential medium nonessential amino acids ($1.5\times$), L-glutamine (5 mM), sodium pyruvate (1 mM), dextrose (0.1%), 2-mercaptoethanol (5×10^{-5} M), sodium bicarbonate (0.15%), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (5 mM), and gentamicin sulfate (50 $\mu\text{g}/\text{ml}$). The supplements (all obtained from GIBCO Labora-

tories, Grand Island, N.Y.) and fetal calf serum were stored at -70°C , and fresh culture medium was prepared each day.

For T-cell proliferative responses, toxins were dissolved in culture medium, sterile filtered, and diluted threefold in 96-well round-bottom microtiter plates. Purified T cells (6×10^4 cells per well) and irradiated (2,000-rad) syngeneic spleen cells (2×10^4 cells per well) were added in a final volume of 0.2 ml of culture medium. Cells were incubated in a humidified atmosphere (5% CO_2) at 37°C for 3 days, with 1 μCi of [^3H]thymidine (2 Ci/mmol) (Du Pont Co., Wilmington, Del.) added per well for the final 24 h. Incorporation of [^3H]thymidine was assessed by liquid scintillation counting.

For T-cell cytokine production, purified T cells (3×10^5 cells per well) and irradiated (2,000-rad) syngeneic spleen cells (10^5 cells per well) were added to 96-well flat-bottom microtiter plates in a final volume of 0.2 ml of culture medium containing various concentrations of toxins. Cells were incubated in a humidified atmosphere (5% CO_2) at 37°C for 3 days. Supernatants were assayed for IFN- γ by enzyme-linked immunosorbent assay (ELISA) (Genzyme Corp., Boston, Mass.) according to the manufacturer's instructions. T-cell supernatants used for monocyte stimulation experiments were generated in the presence of 10 ng of wild-type SEA per ml, sterile filtered, and stored at -70°C for up to 6 weeks. Multiple experiments were carried out at different toxin concentrations and yielded qualitatively similar results.

Monocyte preparation and stimulation. Monocytes were recovered from the peritoneum by repeated lavage with ice-cold Hanks balanced salt solution containing 5% heat-inactivated fetal calf serum and 10 mM HEPES (wash solution). Cells were pelleted, washed once in wash solution at 4°C , and resuspended in culture medium. The typical yield was 10^6 cells per mouse. Cells were added (2.5×10^5 cells per well) in 0.15 ml of culture medium to 96-well flat-bottom microtiter plates and incubated in a humidified atmosphere (5% CO_2) at 37°C overnight. Nonadherent cells were removed by repeated gentle pipetting with wash solution. For examination of cytokine production, stimulants were added in 0.15 ml of culture medium. Plates were returned to the incubator for 20 h, and supernatants were assayed for TNF- α by ELISA (Genzyme Corp.). In some cases, the supernatants were stored at -70°C for 1 to 2 days prior to assay. Multiple experiments were carried out at different toxin concentrations and yielded qualitatively similar results.

RESULTS

SEA mutant toxin does not cause weight loss or stimulate T cells. Earlier work (16) in our laboratory suggested that the conserved disulfide loop in the SE is important for stimulating T cells but not for binding class II MHC molecules or stimulating monocytes. In subsequent studies (17), we generated an SEA mutant (Cys-106 $\rightarrow$ Ala) toxin that was not mitogenic for murine T cells yet exhibited class II MHC binding activity comparable to that of the wild-type toxin.

Marrack and colleagues (32) have argued that T-cell stimulation is required for SEB-mediated toxicity in mice while suggesting that cytokine production from monocytes or other class II MHC-expressing cells may also play a role in pathogenesis. We extended this experimental system to SEA in order to use the disulfide loop mutant to evaluate the contribution of class II MHC-mediated toxin stimulation independent of T-cell stimulation. As had been described for SEB (32), the wild-type SEA toxin induced significant ($P <$

FIG. 1. Mutant SEA toxin does not cause weight loss in mice. Groups of mice were injected intraperitoneally with 75 μ g of wild-type or mutant SEA toxin as described in the text. Purified bovine serum albumin (Pierce Chemical Co., Rockford, Ill.) was used as a negative control. Mice were weighed prior to injection (day 0) and every 24 h thereafter. Percent body weight change was calculated as follows: $(\text{weight}_{\text{day } x} - (\text{weight}_{\text{day } 0})/(\text{weight}_{\text{day } 0})$. Error bars indicate the standard error of the mean for three animals tested in each group. Similar results were obtained in four independent experiments. The weight change induced at 24 h by the wild-type toxin was judged significant ($P < 0.0001$) compared with that induced by the mutant toxin and control protein.

0.0001) weight loss in 24 h and the animals recovered over the next few days (Fig. 1). By contrast, animals injected with the mutant SEA toxin behaved like those given the control protein and did not lose weight. The inability of the mutant SEA toxin to cause weight loss correlated with its inability to induce T-cell proliferation (Fig. 2A) or cytokine (IFN- γ) production (Fig. 2B) in vitro. These results are consistent with a requirement for T-cell stimulation in SEA-mediated weight loss.

Wild-type and mutant toxins stimulate monocyte TNF- α production. The inability of the mutant toxin to cause weight loss suggested that direct stimulation of class II MHC-

FIG. 3. Mutant SEA toxin stimulates monocyte TNF- α production. Purified monocytes were stimulated with wild-type and mutant SEA toxins for 20 h as described in the text. TNF- α content in the supernatants was determined as described in the text. The experiment shown is representative of three performed.

bearing cells may not contribute to in vivo toxicity. Yet recent studies (33, 49) with SE and earlier work (44) with TNF- α , demonstrating a role for this cytokine in the pathogenesis of toxic shock, suggested an alternative conclusion. Despite its inability to stimulate T cells, we would expect the mutant SEA toxin to elicit TNF- α production from monocytes through its ability to bind class II MHC (17). In fact, the mutant (as well as the wild-type) SEA toxin stimulated significant TNF- α production from purified monocytes (Fig. 3). The capacity of the mutant SEA toxin in particular to stimulate monocyte TNF- α production demonstrated a positive role for SE-mediated class II MHC signaling independent of direct T-cell stimulation, yet its inability to cause weight loss suggested that the levels of TNF- α produced may have been insufficient to mediate toxicity in vivo.

It was important to exclude the possibility that some or all of this monocyte-stimulatory activity was due to LPS contaminating the toxin preparations. We took advantage of the

FIG. 2. Mutant SEA toxin does not stimulate T cells in vitro. Purified T cells were stimulated with wild-type or mutant SEA toxin for 3 days as described in the text. Proliferative responses (A) and IFN- γ production (B) were measured as described in the text. Error bars indicate the standard error of the mean for triplicate (A) and the standard deviation for duplicate (B) determinations. We consistently found that concentrations of up to 1,000 ng of the mutant toxin per ml were unable to stimulate detectable levels of IFN- γ .

TABLE 1. Differential sensitivities of SE and LPS to heat treatment^a

Stimulus (ng/ml)	Heat treatment ^b	TNF- α production
None		<25
LPS (200)	No	680 ($\pm$ 36) ^c
LPS (200)	Yes	700 ($\pm$ 55)
SEA (200)	No	218 ($\pm$ 9)
SEA (200)	Yes	<25
SEA (400)	No	453 ($\pm$ 48)
SEA (400)	Yes	101 ($\pm$ 0)
SEA mutant (200)	No	145 ($\pm$ 15)
SEA mutant (200)	Yes	<25
SEA mutant (400)	No	254 ($\pm$ 23)
SEA mutant (400)	Yes	<25
SEB (500)	No	281 ($\pm$ 26)
SEB (500)	Yes	<25
SEB (1,000)	No	400 ($\pm$ 162)
SEB (1,000)	Yes	<25

^a Purified monocytes were stimulated with untreated or heat-treated toxins or LPS at indicated final concentrations, and TNF- α production (in picograms per milliliter) was measured as described in the text.

^b Heat treatment consisted of incubating samples at 95°C for 1 h.

^c Standard deviation from the mean of duplicate determinations.

differential heat stabilities of the SE and LPS to distinguish between these two stimuli. As shown in Table 1, while LPS was completely resistant to heat treatment, monocyte-stimulatory activities of both mutant and wild-type SEA toxins, as well as SEB, were abrogated under these conditions. These experiments indicate that the capacity of SE to stimulate monocyte TNF- α production is not due to contam-

FIG. 4. T-cell-derived supernatant enhances monocyte TNF- α production. Monocytes were incubated in culture medium with or without 200 ng of wild-type or mutant SEA toxin per ml alone (open bars) or in the presence of a 1/5 final dilution of T-cell supernatant (hatched bars) that was prepared as described in the text. The supernatant used here contained approximately 150 U of IFN- γ per ml as determined by ELISA (Genzyme Corp.). Error bars indicate the standard deviation for duplicate determinations. Qualitatively similar results were obtained with toxin concentrations of 100 ng/ml.

TABLE 2. IFN- γ mimics properties of T-cell supernatant^a

Toxin (ng/ml)	Addition (U/ml) ^b	TNF- α production ^c
None	None	<25
None	T-cell supernatant	78
None	IFN- γ (50)	<25
None	IFN- γ (100)	27
SEA (200)	None	614
SEA (200)	T-cell supernatant	1,582
SEA (200)	IFN- γ (50)	1,263
SEA (200)	IFN- γ (100)	1,222
SEA (400)	None	783
SEA (400)	T-cell supernatant	1,945
SEA (400)	IFN- γ (50)	1,252
SEA (400)	IFN- γ (100)	1,405
SEA mutant (200)	None	208
SEA mutant (200)	T-cell supernatant	850
SEA mutant (200)	IFN- γ (50)	812
SEA mutant (200)	IFN- γ (100)	818
SEA mutant (400)	None	294
SEA mutant (400)	T-cell supernatant	1,202
SEA mutant (400)	IFN- γ (50)	862
SEA mutant (400)	IFN- γ (100)	1,058

^a Adherent monocytes were stimulated for 20 h with wild-type or mutant SEA toxins in the absence or presence of IFN- γ or a T-cell-derived supernatant as indicated. The experiment shown is representative of three performed.

^b T-cell supernatant was prepared as described in the text and added at a final dilution of 1/3. The preparation used here contained approximately 150 U of IFN- γ per ml as determined by ELISA (Genzyme Corp.). Recombinant murine IFN- γ (Genzyme Corp.) was added at a final concentration of 100 U/ml.

^c Supernatants were assayed for TNF- α content as described in the text. Values are expressed in picograms per milliliter.

inating LPS but is rather a direct consequence of the toxin-class II MHC interaction.

T-cell factors upregulate toxin-mediated monocyte TNF- α production. We thus postulated that the basis for the T-cell requirement in SE-mediated toxicity is enhancement of TNF- α production by monocytes. To test this hypothesis, purified monocytes were stimulated with wild-type and mutant toxins in the absence or presence of a supernatant from T cells stimulated with wild-type SEA. As shown in Fig. 4, the T-cell supernatant alone did not induce significant monocyte TNF- α production. However, the combination of T-cell supernatant and wild-type SEA toxin resulted in a four- to fivefold enhancement of TNF- α production over that induced by the wild-type toxin alone (Fig. 4). Similarly, addition of T-cell supernatant significantly increased monocyte TNF- α production induced by the mutant toxin. By contrast, addition of supernatant from T cells incubated with mutant toxin had no effect on toxin-induced monocyte TNF- α production (data not shown). These results suggest that SEA stimulates T cells to produce a factor that causes upregulation of toxin-mediated TNF- α production by monocytes.

IFN- γ is the active component of T-cell supernatant. The significant levels of IFN- γ present in SEA-activated T-cell supernatants (Fig. 2B) and its known stimulatory effects on murine monocytes (1, 8, 18) led us to consider that this cytokine might be the predominant factor in T-cell supernatants upregulating monocyte TNF- α production. As shown in Table 2, recombinant IFN- γ alone did not stimulate detectable monocyte TNF- α production. In support of our

FIG. 5. Antibody to IFN- γ blocks T-cell-mediated enhancement of TNF- α production. Monocytes were stimulated with 100 ng of wild-type or mutant SEA toxin per ml alone (open bars) or in the presence of a 1/5 final dilution of T-cell supernatant (hatched bars), supernatant plus anti-CD3 (solid bars), or supernatant plus anti-IFN- γ (shaded bars). The supernatant used here contained approximately 150 U of IFN- γ per ml as determined by ELISA (Genzyme Corp.). The anti-CD3 (30) and anti-IFN- γ (Genzyme Corp.) were both hamster anti-mouse immunoglobulin G monoclonal antibodies and were used at a final concentration of 20 μ g/ml. Antibodies and T-cell supernatant were coincubated for 1 h prior to use in the experiment. TNF- α was not detectable in supernatants from monocytes incubated in culture medium alone (data not shown). Error bars indicate the standard deviation for duplicate determinations. Qualitatively similar results were obtained with toxin concentrations of 200 ng/ml.

hypothesis, however, it was able to substitute for the T-cell supernatant in enhancing monocyte TNF- α production in response to wild-type SEA (Table 2). Similarly, recombinant IFN- γ upregulated mutant toxin-induced production of TNF- α . Moreover, addition of neutralizing antibody against IFN- γ , but not control antibody, significantly reduced supernatant-enhanced TNF- α production in response to both wild-type and mutant toxins (Fig. 5). These results suggest that T-cell production of IFN- γ is critical for maximal TNF- α production by monocytes *in vitro* and may represent the T-cell requirement for toxin-induced weight loss in mice.

DISCUSSION

Binding of SE to class II MHC molecules *in vivo* can potentially initiate two distinct stimulation pathways (Fig. 6). The first pathway involves signal transduction through class II MHC molecules on monocytes, resulting in production of TNF- α . The second pathway leads to activation of T cells by these surface SE-class II MHC complexes with consequent production of IFN- γ . The goal of these studies was to assess the relative contributions of these two modes of SE-mediated stimulation to the production of cytokines that mediate *in vivo* toxicity.

We demonstrate here that both pathways are required for optimal monocyte TNF- α production *in vitro* and propose that both are important for SE-mediated toxicity *in vivo*. Failure of the mutant SEA toxin to stimulate T cells uncouples these two pathways, limiting stimulation to the monocyte compartment. Although the TNF- α produced by toxin-stimulated monocytes can be detected *in vitro*, it is probably not sufficient to mediate toxicity *in vivo*. In the absence of T-cell stimulation, there is suboptimal monocyte production

FIG. 6. Dual class II MHC-dependent signaling pathways for SE. These studies suggest that two class II MHC-dependent signaling pathways for SE are required for optimal monocyte production of TNF- α *in vitro* and induction of toxicity *in vivo* (left panel). For the disulfide loop mutant toxin, only the first pathway is operative in the absence of T-cell stimulation (right panel), and the resulting small amount of TNF- α produced likely accounts for its inability to cause weight loss. S-S, intrachain disulfide loop.

of TNF- α , which likely accounts for the inability of the mutant toxin to cause weight loss. Monocyte TNF- α production is significantly enhanced by T-cell-derived IFN- γ , which is likely the basis for the T-cell requirement (32) in SE-mediated toxicity in the mouse.

The differential capacities of the wild-type and mutant SEA toxins to induce weight loss might be explained by greater instability of the mutant toxin *in vivo*. This seems unlikely, however, in light of our previous studies (17) demonstrating that the mutant and wild-type toxins are similarly resistant to proteolysis by trypsin. Moreover, in related studies (5a) we have found that the mutant toxin is as resistant to degradation as the wild-type toxin upon prolonged exposure to monkey gastric fluid at 37°C.

LPS is a potent stimulator of monocyte TNF- α production (31, 46), and one report (38) has suggested a role for class II MHC molecules in LPS-mediated monocyte stimulation. Yet the possibility that contaminating LPS is stimulating monocyte cytokine production was not excluded in earlier studies (3, 11, 12, 14, 24, 34–36) describing induction of monokines by bacterial superantigens. This seemed unlikely in the present experiments, given that the toxins used for these studies were not derived from gram-negative organisms, and we had previously (16) shown that SEA-induced monocyte TNF- α production could be inhibited by anti-class II MHC antibody. Nevertheless, we were able to dissociate SE and LPS monocyte-stimulatory activities by differential sensitivities to heat.

Distinguishing the relative contributions of T cells and class II MHC-expressing cells to the *in vivo* effects of SE has been difficult, given the interdependence of their T-cell-stimulatory and class II MHC binding activities. The availability of an SEA mutant toxin that binds class II MHC molecules and stimulates monocytes but not T cells allowed us to evaluate the role of direct class II MHC-mediated

stimulation, independent of T-cell stimulation. Our studies confirm the recent findings of Kappler et al. (25), who used mutants of SEB that were also nonmitogenic but retained class II MHC binding activity; those investigators concluded that class II MHC binding was not sufficient to cause weight loss in mice but left open the possibility that direct stimulation of monocytes through class II MHC may contribute to SE-induced toxicity. Here we extend that work by demonstrating that class II MHC binding activity is sufficient to mediate monocyte TNF- α production in the absence of T cells and suggest that it is monocytes rather than T cells that produce the cytokines directly mediating SE-induced toxicity in the mouse. Our data further provide an explanation for the reported T-cell requirement for toxicity (32) and monokine production (13, 15), as T-cell-derived IFN- γ significantly enhanced production of TNF- α by monocytes.

The capacity of IFN- γ to upregulate surface expression of class II MHC on a variety of cell types (29) is a potential explanation for its positive influence on SE-mediated monocyte TNF- α production. Russell et al. (37) have argued for a positive feedback loop involving toxin stimulation of T-cell IFN- γ , which leads to an increase in surface class II MHC on accessory cells, providing more receptors for the toxin and thus further contributing to T-cell stimulation. It is not known whether the IFN- γ -associated effect on monocyte TNF- α production demonstrated here results directly from increased levels of surface class II MHC, although the kinetics of IFN- γ -mediated effects on class II MHC expression (29) are consistent with the time course of TNF- α production. An alternative is that IFN- γ enhances transcription of the TNF- α gene (10) after binding to the IFN- γ receptor, independent of its effect on class II MHC expression. It is likely that both mechanisms make important contributions to the process.

Finally, it is interesting to note that upon determining the amino acid sequence of SEA, Huang et al. (21) proposed that Cys-106 and adjacent residues are responsible for emetic activity as this part of the molecule is highly conserved in all the SE. In related studies, we have found that the Cys-106 $\rightarrow$ Ala SEA mutant used here did not cause emesis in rhesus monkeys (5a). This result is consistent with the present findings in mice, suggesting that toxin-mediated signaling through class II MHC on monocytes and mast cells is not sufficient to mediate toxicity in vivo. While weight loss in the mouse is clearly dependent on stimulation of both monocytes and T cells, a role for either cell type in SE-induced food poisoning remains to be established.

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