Staphylococcal Enterotoxin A-Induced Fever Is Associated with Increased Circulating Levels of Cytokines in Rabbits

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Rabbits were injected intravenously with 10 to 100 ng of staphylococcal enterotoxin A (SEA) per kg, and colonic temperatures were monitored. The febrile responses were compared with circulating levels of interferon (IFN), tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-2, and IL-6 just before the injection of SEA. Both colonic temperatures and circulating levels of IFN, TNF, and IL-2 started to rise at 1 to 2 h and reached their peak levels at 3 to 5 h after SEA injection. Both the fever and the increased circulating levels of IFN, TNF, and IL-2 produced by SEA were decreased by pretreatment with indomethacin (a cyclo-oxygenase inhibitor) (15 mg/kg, intraperitoneally), anisomycin (a protein synthesis inhibitor) (15 mg/kg, subcutaneously), or dexamethasone (an effective anti-inflammatory and immunosuppressive agent) (4 mg/kg, intravenously) in rabbits. Rabbits were injected intravenously with 30 ng of SEA per kg on four consecutive days, and colonic temperatures were monitored. Compared to rabbits that received the single injection of SEA, rabbits that received four consecutive injections of SEA showed a lesser increase in circulating levels of IFN, TNF, and IL-2 as well as colonic temperatures in response to an intravenous dose of SEA (30 ng/kg). The data suggest that the prevention of the febrile response elicited by SEA by indomethacin, anisomycin, or dexamethasone is due to prevention by these compounds of the increase in the circulating levels of IFN, TNF, and IL-2. The pyrogenic hyporesponsiveness to repeated injection of SEA is associated with decreased production of these circulating cytokines.

Staphylococcus aureus produces a family of enterotoxins that cause most common cases of acute food poisoning in humans and other primates (2, 26). The staphylococcal enterotoxins (SE) are classified into several distinct immunological types designated as SEE to SEE (38). SEA, SEB, and SEC have been shown to stimulate T-cell division in rabbits (13). SEA stimulates the production of pyrogenic cytokines, including interferon (IFN), tumor necrosis factor (TNF), and interleukin-1 (IL-1), IL-2, or IL-6 (7, 11, 12, 14, 19, 21). In addition, intravenous (i.v.) injection of very small doses of SEA or SEB into rabbits, cats, and monkeys produces lethargy, fever, shock, and death (6, 9, 16, 23, 31, 32). It has been postulated that SEB may exert its toxicity by damaging the capillary endothelium in the large intestine and thereby permitting absorption of bacterial lipopolysaccharide (LPS) into the circulation (41).

In order to explore possible roles played by several pyrogenic cytokines in the development of SEA fever, experiments were carried out to assess both the body temperatures and circulating levels of IFN, TNF, IL-1, IL-2, and IL-6 in control rabbits, in rabbits pretreated with antipyretic drugs such as indomethacin and anisomycin, and in rabbits pretreated with an effective anti-inflammatory and immunosuppressive agent such as dexamethasone. In addition, a rabbit model of SEA tolerance was used, in which SEA-induced febrile and circulating cytokine responses to a single injection of SEA were compared with those to SEA given on four consecutive days. The febrile responses were compared with the circulating levels of cytokines in these groups of animals.

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FIG. 1. Mean (±SEM) changes in colonic temperature (Tco) (A) and concentrations of IFN (B), TNF (C), and IL-2 (D) in serum in rabbits injected i.v. with either saline (○) (n = 5) or SEA at 10 ng/kg (●) (n = 4), 30 ng/kg (▲) (n = 4), or 100 ng/kg (▲) (n = 4). *, P < 0.05 (significantly different from the control values by one-way analysis of variance).

FIG. 2. Mean (±SEM) changes in colonic temperature (Tco) (A) and levels of IFN (B), TNF (C), and IL-2 (D) in serum in rabbits treated with vehicle (s.c.) plus SEA (30 ng/kg, i.v.) (▲) (n = 4), in rabbits treated with anisomycin (Anis) (15 mg/kg, s.c.) plus vehicle (i.v.) (○) (n = 4), and in rabbits treated with anisomycin (15 mg/kg, s.c.) plus SEA (30 ng/kg, i.v.) (▲) (n = 4). *, P < 0.05 (significantly different from the control values by one-way analysis of variance).
TNF bioassay. TNF activity in serum samples was measured by an in vitro cytotoxicity assay with TNF-sensitive L5 cells (a kind gift from H. Fujisawa, Biomedical Research Center, Osaka University Medical School, Osaka, Japan) as previously described (20) with slight modifications. Briefly, 2.5 × 10^5 cells were plated in 96-well microplates (Nunc), followed by the addition of washed 7TD1 cells (2 × 10^3 cells/well) in AIM-V medium (GIBCO BRL). The cells were incubated at 37°C in a CO2 incubator. After 3 days of incubation, each well was pulsed with 0.5 µCi of [3H]thymidine (DuPont NEN) for 6 h. The cells were harvested on glass fiber filters with an automatic cell harvester (Cambridge). The radioactivity incorporated was assayed in a liquid scintillation counter (LS 5000TA; Beckman). The sensitivity of this assay is 1 pg/ml.

IL-1 bioassay. IL-1 was measured with the IL-1-dependent murine T-cell line D10N4M (a kind gift from C. C. Chao, Neuroimmunology and Host Defense Laboratory, Minneapolis Medical Research Foundation, Minneapolis, Minn.) as previously described (8, 33). Briefly, the D10N4M cells were maintained in RPMI 1640 (GIBCO BRL) containing 10% FBS, 2 µg/ml of gentamicin (Sigma), and 50 µg of gentamicin (Sigma) per ml and were fed every 3 days before being assayed. The serially diluted rabbit serum samples or recombinant human IL-1 (R&D) were incubated with 2 × 10^5 D10N4M cells per ml (50 µl). After 24 h of incubation in a CO2 incubator, each well was pulsed with 0.5 µCi of [3H]thymidine (DuPont NEN) for 5 h. The cells were harvested on glass fiber filters with an automatic cell harvester (Cambridge). The radioactivity incorporated was assayed in a liquid scintillation counter (LS 5000TA; Beckman).

IL-2 bioassay. IL-2 bioassay. IL-2 activity was measured with the IL-2-dependent cell line 7TD1 as previously described (46). This cell line was kindly provided by J. Van Snick (Ludwig Institute for Cancer Research, Brussels, Belgium). Briefly, 7TD1 cells were cultured in RPMI 1640 (GIBCO BRL) containing 10% FBS, 2 ng of recombinant human IL-2 (R&D) per ml, and 50 µM 2-mercaptoethanol (Serva). Serum samples in serial dilutions or recombinant human IL-2 was added to each well of microplates (Nunc), followed by the addition of washed 7TD1 cells (2 × 10^5 cells/well) in AIM-V medium (GIBCO BRL). The cells were incubated at 37°C in a CO2 incubator. After 3 days of incubation, each well was pulsed with 0.5 µCi of [3H]thymidine (DuPont NEN) for 6 h. The cells were harvested on glass fiber filters with an automatic cell harvester (Cambridge). The radioactivity incorporated was assayed in a liquid scintillation counter (LS 5000TA; Beckman). The sensitivity of this assay is 1 pg/ml.

Statistical analysis. Temperature responses were assessed as changes from the preinjection value. Results are expressed as the means ± standard errors of the means (SEM) for n experiments. Results were compared by one-way analysis of variance followed by Duncan’s test when appropriate. A P value of <0.05 was considered significant.

RESULTS

Cytokine production and fever. The i.v. administration of SEA (10, 30, and 100 ng/kg) caused fever in rabbits (Fig. 1A). Colonic temperatures started to rise at 1 h and reached the peak level at 5 h after SEA injection. Body temperatures returned to the preinjection level at 12 h. In parallel with the...
colonic temperature change, the concentrations of IFN (Fig. 1B), TNF (Fig. 1C), and IL-2 (Fig. 1D) in serum also started to rise at 1 h and reached the peak level at 3 to 5 h after SEA injection. The levels of these cytokines in serum returned to the preinjection values at 7 to 12 h. However, unlike the case for IFN, TNF, and IL-2, SEA injection did not affect the concentration of either IL-1 or IL-6 in serum (data not shown).

In addition, to ascertain whether the SEA-induced fever is mediated by endotoxin, experiments were carried out to assess the effect of i.v. injection of SEA (300 ng/mouse) on the colonic temperature in the C3H/HeJ strain of endotoxin-resistant mice. It was found that C3H/HeJ mice still responded to SEA (1.15 ± 0.12°C, n = 10), suggesting that the SEA induction of fever was not related to endotoxin contamination.

**Antipyresis with anisomycin and indomethacin.** The increases in both the colonic temperatures (Fig. 2A) and the concentrations of IFN (Fig. 2B), TNF (Fig. 2C), and IL-2 (Fig. 2D) in serum were significantly attenuated by pretreatment of rabbits with anisomycin (15 mg/kg, subcutaneously [s.c.] 1 h before SEA administration (30 ng/kg, i.v.). Likewise, pretreatment of animals with indomethacin (15 mg/kg, intraperitoneally [i.p.] 1 h before SEA (30 ng/kg, i.v.) injection significantly attenuated the SEA-induced fever (Fig. 3A) and decreased the concentrations of IFN (Fig. 3B), TNF (Fig. 3C), and IL-2 (Fig. 3D) in serum. An s.c. dose of 15 mg of anisomycin per kg or an i.p. dose of 15 mg of indomethacin per kg produced an insignificant effect on either the colonic temperature or the concentration of IFN, TNF, or IL-2 in serum. However, an i.v. dose of SEA (30 ng/kg) produced a slight decrease, rather than an increase, in the colonic temperature in rabbits pretreated with anisomycin or indomethacin (Fig. 2 and 3).

**Antipyresis with dexamethasone.** An i.v. dose of dexamethasone (4 mg/kg; 1 h before SEA injection), although showing no effect on basal colonic temperature or concentrations of IFN, TNF, and IL-2 in serum, did attenuate the SEA-induced fever (Fig. 4A), as well as the increased concentrations of IFN (Fig. 4B), TNF (Fig. 4C), and IL-2 (Fig. 4D) in serum.

**Pyrogenic tolerance to SEA.** To test for the development of pyrogenic tolerance to SEA, rabbits were injected i.v. with 30 ng of SEA per kg for four consecutive days. In addition to measurement of colonic temperatures, the concentrations of IFN, TNF, and IL-2 in serum were determined at 0, 1, 3, and 5 h after SEA injection in rabbits treated with a single injection and in rabbits treated with four consecutive injections. As shown in Fig. 5, an i.v. dose of SEA produced lesser increases in serum IFN (Fig. 5B), TNF (Fig. 5C), and IL-2 (Fig. 5D) production as well as in colonic temperature (Fig. 5A) in rabbits that received four consecutive SEA injections than in rabbits that received a single injection of SEA.

**DISCUSSION**

The present experiments on tolerance to SEA were carried out by using the febrile response in rabbits. Daily injection of SEA rendered the animals tolerant to the SEA. The unresponsiveness has been suggested to reflect at least two different
mechanisms: physical elimination (depletion) (18, 47) and functional inactivation (anergy) (34, 35). Recently, the effect of the superantigen SEA on the balance between T-cell response and nonresponsiveness in T-cell receptor Vβ3 transgenic mice has been investigated by Sundstedt et al. (42). Their results suggest that SEA-induced hyporesponsiveness involves CD4\(^{-}\)-cell depletion and a failure to produce cytokines in the remaining CD4\(^{+}\)-T-cell compartment. In the present study, compared to rabbits that received a single injection of SEA, rabbits that received four consecutive injections of SEA had lesser increases of circulating levels of cytokines in response to an i.v. dose of SEA. Thus, it appears that the attenuated effect with repeated injection of SEA is related to T-cell depletion and/or a failure to produce cytokines in the remaining T-cell compartment. Recently, good evidence that the consecutive injection of LPS increases endogenous glucocorticoid levels has been presented (44). As glucocorticoids inhibit the production of cytokines and the induction of cyclo-oxygenase-2 (COX-2) or nitric oxide synthase (NOS), it is more likely that repeated injection of SEA causes an increase in endogenous glucocorticoid levels, which inhibits the production of proinflammatory cytokines and, hence, fever. Glucocorticoids suppress a wide range of proinflammatory functions of macrophages, including synthesis of TNF-\(\alpha\), IL-1\(\beta\), IL-6, and IL-8 (3, 15, 39). Indeed, in the present study, dexamethasone (a synthetic glucocorticoid) attenuated both the fever and the increased circulating levels of cytokines produced by SEA in rabbits.

In the present study, the increase in the circulating levels of proinflammatory cytokines was associated with an increase in core body temperature. The increase in the circulating levels of cytokines as well as the associated febrile response caused by SEA was attenuated by indomethacin (an inhibitor of COX), anisomycin (a protein synthesis inhibitor), or dexamethasone (a potent inhibitor of the transcription of inducible NOS) (44). Arachidonate metabolites, mostly prostaglandins (28, 45), and NOS (22) are thought to be involved in the development of pyrogenic fever. The production of COX or NOS is induced by LPS or cytokines such as TNF-\(\alpha\), IFN-\(\gamma\), IL-1, or IL-6 (1, 10, 17, 24, 43). COX exists in at least two isoforms. COX-1 is present constitutively in various types of cells, including endothelial cells (30), whereas the expression of COX-2 is induced by inflammatory stimuli, including cytokines and LPS (10, 25, 29). Unlike indomethacin, a protein synthesis inhibitor such as anisomycin or dexamethasone (a potent inhibitor of NOS) prevented the rise in the circulating levels of IFN, TNF, and IL-2. In addition, the inducible NOS was shown to be inhibited by a protein synthesis inhibitor, cycloheximide (27). Given the present findings, the prevention of the increase in body temperature caused by indomethacin, anisomycin, or dexamethasone in rabbits treated with SEA is likely to be the result of the prevention by indomethacin, anisomycin, or dexamethasone of the rise in the circulating levels of these proinflammatory cytokines. Indeed, by preventing the formation of these proinflammatory cytokines, indomethacin, anisomycin, or dexamethasone...
some would presumably also prevent the expression of COX-2 or the inducible isoenzyme of NOS.

Endotoxin (LPS) is the major active agent in the pathogenesis of septic shock (48). A shocklike state can be induced by a single injection of LPS into animals. This toxic syndrome is mediated by macrophage-derived inflammatory cytokines. Not only endotoxins but also bacterial exotoxins from certain gram-

positive bacteria cause toxic shock in humans (36, 38). LPS caused a marked production of TNF-α, IL-8, IL-1α, IL-1β, and IL-6 (4). In contrast, SEA induced a marked production of TNF-α, TNF-β, IFN-γ, and IL-2. Recently, Stiles et al. (40) demonstrated that mice injected with SEA plus LPS had greatly elevated concentrations of TNF-α, IL-1α, IL-6, and IFN-γ in serum. However, in the present study we found that rabbits injected with SEA had a significant increase in the levels of IFN, TNF, and IL-2 in serum but had no change of either IL-1 or IL-6 in serum. Previous results (37) showed that injection of TNF-α or IL-1 produced a concomitant rise in the serum IL-6 level. This makes it difficult to explain why the serum IL-6 concentrations were unaffected by SEA injection in rabbits in the present study.

In summary, the present results showed that i.v. injection of SEA produced fever and a rise in the circulating levels of IFN, TNF, and IL-2. Both the fever and the augmented circulating levels of IFN, TNF, and IL-2 induced by SEA were attenuated by indomethacin, anisomycin, dexamethasone, or repeated injections. These results suggest that the SEA-induced fever is mediated by LPS or PSK-activated human plastic-adherent peripheral blood mononuclear cells. Cell. Immunol. 144:58–366.


kin-6 from human monocytes by antithymocyte globulin: requirement for de


