Doxycycline Is Anti-Inflammatory and Inhibits Staphylococcal Exotoxin-Induced Cytokines and Chemokines

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Proinflammatory cytokines mediate the toxic effect of superantigenic staphylococcal exotoxins (SE). Doxycycline inhibited SE-stimulated T-cell proliferation and production of cytokines and chemokines by human peripheral blood mononuclear cells. These results suggest that the antibiotic doxycycline has anti-inflammatory effects and is therapeutically useful for mitigating the pathogenic effects of SE.

Staphylococcal toxic shock syndrome toxin 1 (TSST-1) and the structurally related exotoxins are bacterial exotoxins that bind directly to major histocompatibility complex class II molecules on antigen-presenting cells (1, 5, 8, 18, 23) and activate T cells expressing specific Vβ elements (7). These toxins are called superantigens because of their ability to polyclonally stimulate large populations of T cells (1, 4, 7, 14). Thus, staphylococcal exotoxins (SE) are potent activators of the immune system and cause a variety of diseases in humans, including food poisoning, toxic shock, and autoimmune diseases (1, 2, 6, 21). Their interactions with cells of the immune system result in massive production of proinflammatory cytokines and chemokines (1, 4, 15, 17). The cytokines tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and gamma interferon (IFN-γ) are key mediators in superantigen-induced toxic shock (1, 21). Both TNF-α and IL-1 have potent immunostimulating activities and act synergistically with IFN-γ to enhance immune reactions and promote tissue injury (16). Consequently, these cytokines are pathogenic at high concentrations in vivo and are responsible for fever and toxic shock induced by SE (13, 14, 18, 19).

Doxycycline is a broad-spectrum antibiotic widely used for infections caused by both gram-negative and gram-positive microorganisms. It acts as a bacteriostatic agent and is highly effective against many microorganisms, including Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, and Yersinia pestis. Doxycycline belongs to the tetracycline antibiotic family, the members of which have been shown to have other biological actions independent of their antimicrobial effects (10). Doxycycline inhibits phorbol-12-myristate-13-acetate-mediated matrix metalloproteinase 8 (MMP-8) and MMP-9 in human endothelial cells (11). Doxycycline also decreases elastin degradation and reduces MMP activity in a model of aneurismal disease (3). More recently, doxycycline was shown to inhibit the production of IL-1β in lipopolysaccharide-treated corneal epithelial cultures to an extent comparable to that achieved by corticosteroids (25). In vivo, doxycycline protected mice from lethal endotoxemia by downregulating cytokine and nitrate secretion in blood (20). This study was undertaken to determine the modulatory effect of doxycycline on staphylococcal superantigen-induced T-cell activation and cytokine production from human peripheral blood mononuclear cells (PBMC).

Purified SEB and TSST-1 were obtained from Toxin Technology (Sarasota, Fla.). The endotoxin content of these preparations was <1 ng of endotoxin/mg of protein, as determined by the Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, Md.). Human recombinant TNF-α (hTNF-α), antibodies against hTNF-α, peroxizide-conjugated anti-rabbit immunoglobulin G, and peroxidase-conjugated anti-goat immunoglobulin G were obtained from Boehringer Mannheim (Indianapolis, Ind.). Recombinant monocytic chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), MIP-1B, and antibodies against hIL-1β, hIL-6, hMIP-1α, and MIP-1β were purchased from R&D Systems (Minneapolis, Minn.). Human rIL-1β was kindly provided by J. Oppenheim (National Cancer Institute, Frederick, Md.). Human recombinant IFN-γ (rIFN-γ) and rIL-6 were obtained from Collaborative Research (Boston, Mass.). Antibodies against hIFN-γ and MCP-1 were obtained from Pharmingen (San Diego, Calif.). Doxycycline was purchased from Sigma (St. Louis, Mo.) and dissolved in phosphate-buffered saline, pH 7.4. All other reagents were also from Sigma.

Human PBMC were isolated by Ficoll-Hypaque density gradient centrifugation of heparinized blood from normal human donors. PBMC (10⁶/ml) were cultured at 37°C in 24-well plates containing RPMI 1640 medium and 10% heat-inactivated fetal bovine serum. Cells were incubated with either SEB (200 ng/ml) or TSST-1 (200 ng/ml) for 16 h, and the supernatants were harvested and analyzed for IL-1β, TNF-α, IL-6, IFN-γ, MCP-1, MIP-1α, and MIP-1β. Cytokines and chemokines were measured by an enzyme-linked immunosorbent assay with cytokine- or chemokine-specific antibodies in accordance with the manufacturer’s instructions (15, 17). Human recombinant cytokines and chemokines (20 to 1,000 pg/ml) were used as standards for calibration on each plate. The detection limit of each assay was 20 pg/ml. The cytokine and chemokine data were expressed as the mean reading ± the standard deviation (SD) of duplicate samples. Doxycycline, when present, was added simultaneously with the stimulating agent. Cytotoxicity

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**Subject Terms**
- staphylococcal exotoxins
- cytokines
- chemokines
- superantigens
- doxycycline
- antibiotic
was measured by the release of lactate dehydrogenase (LDH) from the cytosol into culture supernatant. LDH was quantitated by using a colorimetric cytotoxicity assay kit (Boehringer Mannheim) as instructed by the manufacturer. The maximum amount of releasable LDH (100%) was obtained by lysing cells with 1% Triton X-100. T-cell proliferation was assayed with PBMC (10^5/well), which were plated in triplicate with SEB or TSST-1 (200 ng/ml), with or without doxycycline, for 48 h at 37°C in 96-well microtiter plates. Cells were pulsed with 1 µCi of [3H]thymidine (New England Nuclear, Boston, Mass.) per well during the last 5 h of culture as described previously (15). Cells were harvested onto glass fiber filters, and incorporated [3H]thymidine was measured by liquid scintillation. All data were analyzed for significant differences by Student’s t test with Stata (Stata Corp., College Station, Tex.). Differences between doxycycline-treated and untreated control groups were considered significant if \( P < 0.05 \).

On the basis of the report that doxycycline blocked lipopolysaccharide-induced IL-1 in epithelial cells and prevented lethal endotoxemia in vivo (20, 25), we tested the hypothesis that this antibiotic might have direct effects on SE-induced cytokines. As shown in Fig. 1, doxycycline dose dependently inhibited the production of the cytokines IL-1β, IL-6, TNF-α, and IFN-γ and the chemokines MCP-1, MIP-1α, and MIP-1β by PBMC incubated with SEB. Similar dose-dependent reduction of cytokines and chemokines by doxycycline was also observed for TSST-1-stimulated PBMC (data not shown). The inhibitory effect of doxycycline on SEB- or TSST-1-mediated cytokines and chemokines obtained with PBMC from seven normal donors is summarized in Fig. 2. Production of MCP-1 and IFN-γ was completely blocked by 50 μM doxycycline. This concent-

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)
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REFERENCES


FIG. 3. Inhibition of T-cell proliferation in PBMC stimulated with 200 ng of SEB or TSST-1 per ml by various concentrations of doxycycline. Values are the mean count ± the standard error of the mean of triplicate cultures and represent five experiments. Results are statistically significantly different (P < 0.02) between SE and SE-plus-doxycycline samples.