Coordinate Suppression of Superantigen-Induced Cytokine Production and T-Cell Proliferation by a Small Nonpeptidic Inhibitor of Class II Major Histocompatibility Complex and CD4 Interaction

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Proinflammatory cytokines mediate the toxic effect of superantigenic staphylococcal exotoxins (SE). TJU103, a small nonpeptidic molecule that blocks the interaction between major histocompatibility complex class II and CD4 molecules inhibited SE-stimulated T-cell proliferation (by 92%) and production of tumor necrosis factor, interleukin 1β, interleukin 6, and gamma interferon (by 66, 56, 76, and 72%, respectively) by human peripheral blood mononuclear cells. These data suggest that TJU103 may be useful for mitigating the pathogenic effects of SE.

Staphylococcal exotoxins (SE) are among the most common etiological agents that cause vomiting, diarrhea, skin desquamation, fever, and toxic shock (1, 2, 13). Staphylococcal toxic shock syndrome toxin 1 (TSST-1) and the distantly related staphylococcal enterotoxins A and B (SEA and SEB, respectively) are superantigens that potently stimulate T-cell proliferation and cytokine production (5). These toxins bind with high affinity to the major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APC) and subsequently interact with specific Vβ regions of the T-cell antigen receptors (3, 5, 13, 14). In vitro and in vivo studies show that these superantigens induce high levels of a variety of proinflammatory mediators, including tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), IL-6, and gamma interferon (IFN-γ) (5, 6, 11, 12, 15). Both TNF-α and IL-1 have potent immunostimulating activities (8). They promote leukocyte cellular interactions by increasing the expression of MHC class II as well as adhesion molecules. In addition, TNF-α and IL-1 also activate endothelial cells, increase procoagulant activities, and promote tissue injury. These cytokines act synergistically to enhance immune reactions. Consequently, these cytokines are pathogenic when present at high concentrations in vivo and are responsible for fever and toxic shock induced by SE.

Previous studies identified the involvement of other cell surface molecules on both T cells and APC in the interaction with superantigens (4, 7, 9). It is not known to what extent the interaction of MHC class II and CD4 contributes to the production of these cytokines. Recently, a computational analysis

FIG. 1. Dose response inhibition of TNF-α (A) and IFN-γ (B) production by PBMC stimulated with SEB in the presence of various concentrations of TJU103. Values represent the means ± SD of duplicate samples from two experiments. Inhibition at all concentrations of TJU103, with the exception of 0.05 mM, was statistically significant by comparisons with control SEB-stimulated cells (P < 0.01).

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was used to deduce the interactions between MHC class II on APC and CD4 on T lymphocytes (10). Based on the contact residues, small nonpeptidic molecules were then synthesized to block the interactions of these cells. One such compound, TJU103, was immunosuppressive and was useful for inhibiting graft rejection (10). This study was undertaken to determine the effect of TJU103 on staphylococcal superantigen-induced T-cell activation and cytokine production from human peripheral blood mononuclear cells (PBMC).

Purified SEA, 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 (rTNF-α), peroxidase-conjugated anti-rabbit immunoglobulin G (IgG), and peroxidase-conjugated anti-goat IgG were obtained from Boehringer Mannheim (Indianapolis, Ind.). Antibodies against human TNF-α (hTNF-α) and hIL-6 were purchased from R&D Systems (Minneapolis, Minn.). Human rIL-1β was kindly provided by J. Oppenheim (National Cancer Institute, Frederick, Md.). Human rIFN-γ, rIL-6, and antibodies against hIL-1β were obtained from Collaborative Research (Boston, Mass.). Anti-hIFN-γ and IgG, with and without biotin, were obtained from Pharmingen (San Diego, Calif.). TJU103 was obtained from Calbiochem (San Diego, Calif.) and was dissolved in dimethyl sulfoxide (DMSO). All other reagents were from Sigma (St. Louis, Mo.).

Human PBMC were isolated by Ficoll-Hypaque density gradient centrifugation of heparinized blood from healthy human donors. Among the 12 donors tested, the magnitude of inhibition of the SE-induced cytokine production or T-cell proliferation in response to TJU103 was very consistent in all. PBMC (10⁶ cells/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 SEA, SEB, or TSST-1 (100 ng/ml) for 16 h, and the supernatants were harvested and analyzed for TNF-α, IL-1β, IL-6, and IFN-γ. Cytokines were measured by enzyme-linked immunosorbent assay with cytokine-specific antibodies according to the manufacturer’s instructions (6, 7). Recombinant cytokines (20 to 1,000 pg/ml) represented the standards for calibration, and the detection limit of all assays was 20 pg/ml. The cytokine data are expressed as the mean readings ± standard deviations (SD). TJU103, when present, was added simultaneously with the exotoxins. For T-cell proliferation assays, PBMC (10⁵ cells/well) were plated in triplicate with SEA, SEB, or TSST-1 (100 ng/ml), with or without TJU103, 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 previously described (6, 7).
superantigen-mediated production of TNF-α, IL-1β, IL-6, and IFN-γ by human PBMC in vitro. Downregulation of proinflammatory cytokines by TJU103 in SEA-, SEB-, and TSST-1-stimulated PBMC suggested that blocking the interaction of MHC class II and CD4 can drastically decrease proinflammatory cytokine induction and T-cell proliferation by staphylococcal superantigens. Preliminary experiments indicated that TJU103 does not inhibit proinflammatory cytokine production by endotoxin-activated monocytes. Thus, TJU103 has anti-inflammatory effects and interferes with the activation of both T cells and APC, as monocyte-derived cytokines (IL-1β, TNF-α, and IL-6) as well as T-cell cytokines (IFN-γ, TNF-α, and IL-6) were inhibited.

In conclusion, the promising findings of this study suggest that TJU103 is a potential therapeutic agent to mitigate SEA-, SEB-, and TSST-1-mediated shock in humans.

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