Caspase Inhibitors Attenuate Superantigen-Induced Inflammatory Cytokines, Chemokines, and T-Cell Proliferation

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Received 6 November 2003/Returned for modification 19 December 2003/Accepted 22 January 2004

Proinflammatory cytokines mediate the toxic effect of superantigenic staphylococcal exotoxins (SE). A pan-caspase inhibitor suppressed SE-stimulated T-cell proliferation and the production of cytokines and chemokines by human peripheral blood mononuclear cells. These data suggest that caspase inhibitors may represent a novel therapeutic modality for treating SE-induced toxic shock.

Staphylococcal exotoxins (SE) have been implicated as major virulence factors responsible for toxic shock syndrome (2, 15, 19). This disease is characterized by fever, hypotension, desquamation of skin, and multiple-organ system failure. Staphylococcal toxic shock syndrome toxin 1 (TSST-1) and the structurally related staphylococcal enterotoxin B (SEB) are potent stimulators of the immune system, as they bind directly to the major histocompatibility complex class II molecules on antigen-presenting cells (9). These superantigens polyclonally activate T cells (4), resulting in proliferation and, eventually, apoptosis. Multiple components of the host inflammatory response triggered by SE have been shown to contribute to toxic shock, including the cascade of inflammatory cytokines and chemokines (3, 4, 9, 16, 19). These cytokines include interleukin 1 (IL-1), tumor necrosis factor alpha (TNF-α), and gamma interferon (IFN-γ). These proinflammatory mediators have potent immunoenhancing effects and are known to be pathogenic at high levels (12). The massive production of inflammatory mediators from both T cells and monocytes appears early, within hours after the SE bind to these cells, while T-cell proliferation occurs later.

Recently, caspase inhibitors were used to prevent allergic airway inflammation (7) and to decrease the death rate from acute experimental pancreatitis (18). Caspase inhibitors also blocked murine liver injury (13) and attenuated bleomycin-induced pneumopathy (14). Other studies indicated that caspase activation is required for mitogen- or anti-CD3-stimulated T-cell proliferation (1, 8, 17). Caspases belong to a family of autocatalytic cysteine proteases which regulate many cellular processes, including apoptosis, cell differentiation, and inflammation (5). They share sequence homologies and are grouped into three subfamilies based on their function and substrate specificity (5, 6, 18, 20). Group 1 caspases, which grouped into three subfamilies based on their function and substrate specificity (5, 6, 18, 20). Group 1 caspases, which...
man recombinant cytokines and chemokines (20 to 1,000 pg/ml) were used as calibration standards for each plate. The detection limit of each assay was 20 pg/ml. Cytotoxicity was measured by the release of lactate dehydrogenase (LDH) from the cytosol into the culture supernatant. LDH was measured by using a cytotoxicity kit (Roche) in accordance with the manufacturer’s instructions. T-cell proliferation was assayed with PBMC (10⁵ cells/well), which were plated in triplicate with TSST-1 or SEB (200 ng/ml), with or without caspase inhibitors, 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 (10). Cells were harvested onto glass fiber filters, and the incorporated [³H] thymidine was measured by liquid scintillation. Caspase-1, -3, and -8 activities were determined using, respectively, caspase -1, -3, and -8 assay kits obtained from R&D Systems. Cell lysate was prepared in triplicate from cells stimulated with SE for 48 h, and the specific caspase was measured according to the manufacturer’s instructions. DNA fragmentation was detected by the presence of oligonucleosomes by using a cell death detection ELISA PLUS kit (Roche) according to the manufacturer’s instructions. All data were analyzed for significant difference by Student’s t test with Stata Corp. (College Station, Tex.) software. Differences between treated and untreated control groups were considered significant if P was <0.05.

Caspases have been implicated in the pathogenesis of many inflammatory diseases (5, 13, 14, 21). The stimulatory role of caspases in superantigen-stimulated PBMC was examined by observing the effect of caspase inhibitors on cytokine and chemokine production. Figure 1 shows that the pan-caspase inhibitor Z-D-CH₂-DCB blocked the production of IL-1β, TNF-α, IL-6, and IFN-γ in SEB-stimulated PBMC in a dose-dependent manner. The production of the chemokines MCP-1, MIP-1α, and MIP-1β was also suppressed (Fig. 1C). The inhibitory effect of Z-D-CH₂-DCB on TSST-1-activated PBMC was similar, reducing IL-1β, IL-6, TNF-α, IFN-γ, MCP-1, MIP-1α, and MIP-1β to 10, 36, 25, 10, 11, 25, and 30%, respectively, of levels in untreated cells (Fig. 2A and B). In contrast, another broad-spectrum caspase inhibitor, Z-VAD-fmk, inhibited the SEB- and TSST-induced cytokines and chemokines less effectively (Fig. 2A and B).

More-specific caspase inhibitors were used to further determine which enzymes were involved in SE-stimulated PBMC. The caspase 1 specific inhibitor, Ac-YVAD-cmk, attenuated IL-1β and monocyte chemotactic protein production in PBMC stimulated by SEB and TSST-1 to 95 and 18%, respectively, but had minimal effects on the other cytokines (Fig. 2A and B). The caspase 3-specific inhibitor, Z-DEVD-fmk, and the caspase 8 inhibitor, Z-IETD-fmk, did not significantly affect the levels of cytokines or chemokines from SE-stimulated cells (data not shown). Neither the caspase inhibitors nor dimethyl sulfoxide (0.26%), the solvent used in preparations of these inhibitors, affected the viability of the cells at the concentrations used, as confirmed by a trypan blue dye exclusion test and the release of cytosol LDH. The two pan-caspase inhibitors did not inhibit [³⁵S]Met incorporation into proteins in PBMC cultures (data not shown) and therefore are not general inhibitors of protein synthesis.

Because superantigens also cause T-cell proliferation, the effect of these caspase inhibitors on SE-induced T-cell proliferation was investigated next. Figure 3 shows that Z-D-CH₂-DCB was also the most potent inhibitor and reduced SEB-stimulated T-cell proliferation in a dose-dependent manner, achieving 98% inhibition at 100 µM. The pan-caspase inhibitor

![Graph](image1.png)

**FIG. 1.** Dose-response inhibition of IL-1β, IL-6, and TNF-α (A); IFN-γ (B); and MCP-1, MIP-1α, and MIP-1β (C) production by PBMC stimulated with 200 ng of SEB/ml in the presence of various concentrations of Z-D-CH2-DCB. Values are the means ± standard deviations (SD) of values for duplicate samples from three experiments.
Z-VAD-fmk blocked SEB-activated cells by 32%, whereas inhibitors specific for caspase-1, -3, and -8 failed to have an effect on SEB-induced T-cell proliferation.

The presence of three different caspases, representing the three major groups of caspases, was examined next. Caspase-1 was found in SEB- and TSST-1-stimulated cell lysate (80 ± 6 and 68 ± 6 pg/ml, respectively, versus 14 ± 4 pg/ml in the unstimulated control). Insignificant amounts of caspase-8 were present in the SEB- and the TSST-1-treated cells (23 ± 7 and 28 ± 6 pg/ml versus 12 ± 5 pg/ml in the control lysate), whereas caspase-3 was completely absent in these cells. Thus, in agreement with previous reports, caspase-1 was required for
the proteolytic processing of the cytokine IL-1β, as caspase-1 inhibitor suppressed its release (5, 6, 21). The presence of oligonucleosomes in cell lysate was also examined, because some caspasas promote apoptosis. SEB- and TSST-1-stimulated cells were not apoptotic, as oligonucleosomes were absent in these cells (data not shown).

The novel observations presented here indicate that the broad-spectrum caspase inhibitor Z-D-CH₂-DCB suppressed multiple proinflammatory cytokines and chemokines in SEB- or TSST-1-stimulated human mononuclear cells. T-cell proliferation was also blocked by this pan-caspase inhibitor but not by specific inhibitors of caspase-1, -3, or -8. Moderate caspase-1 activity was found in SE-stimulated PBMC, and a specific caspase-1 inhibitor blocked SE-induced IL-1β but not other cytokines or chemokines. While the inhibition mechanism has not been identified, it is clear that multiple caspasas aside from caspase 1, perhaps unidentified, are required in the activation of T cells and the production of these inflammatory cytokines and chemokines. These results suggest that the inactivation of caspasas represents a previously unrecognized modality for blocking superantigen activation of cellular responses and treatment of toxic shock.

I thank Marilyn Buckley for excellent technical assistance.

REFERENCES