Effect of Quinupristin/Dalfopristin on Production of Cytokines by Human Monocytes

Anis A. Khan,1,2 Teri R. Slifer,1 Fausto G. Araujo,1 and Jack S. Remington1,2

The effect of the novel streptogramin antibiotic quinupristin/dalfopristin (synercid) on cytokine production in vitro was examined in monocytes obtained from healthy human volunteers and stimulated with either lipopolysaccharide or heat-killed Staphylococcus aureus (Pansorbin). Synercid at concentrations that are achievable in humans (1, 5, and 10 μg/mL) significantly suppressed production of interleukin (IL)-1α, IL-1β, IL-6, IL-10, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor-α in a concentration-dependent manner. Thus, synercid possesses significant immunomodulatory activity, in addition to its antimicrobial activity.

Materials and Methods

Reagents. Synercid (lot C98-26) was obtained from Rhône-Poulenc Rorer (Collegeville, PA). The freeze-dried powder was dissolved in deionized distilled water and further diluted in RPMI 1640 tissue culture medium. Lipopolysaccharide (LPS; Escherichia coli O26:B6) was purchased from Difco Laboratories (Detroit). Pansorbin, heat-killed S. aureus Cowan strain I was obtained from Calbiochem-Behring (La Jolla, CA).

Isolation of monocyte-enriched peripheral blood mononuclear cells (PBMC). Monocytes were isolated as described elsewhere [7]. In brief, blood was obtained by venipuncture from healthy volunteers (4 men, 25–38 years old). PBMC were separated on Ficoll-Paque density gradients (Pharmacia Biotech, Uppsala, Sweden), washed twice with calcium and magnesium-free PBS (Mediatech, Herndon, VA), and fractionated by centrifugation over discontinuous Percoll gradients (Pharmacia Biotech). The monocyte-enriched cell fraction was collected, washed 3 times with calcium- and magnesium-free PBS, and resuspended in RPMI 1640 (with 25 mM HEPES, 1-glutamine; Mediatech) containing 10% fetal bovine serum (Gibco BRL Products, Grand Island, NY) at a density of 10^6 cells/mL. At least 90% of the cells thus obtained were monocytes, as determined by esterase staining.

Cytokine assays. Cells were seeded into 24-well plates (Costar, Cambridge, MA) at a density of 10^6 cells/mL (1 mL/well) and incubated in the presence of LPS (100 ng/mL) or Pansorbin (0.0075%, wt/vol) with or without various concentrations of synercid for 24 h at 37°C in a 5% CO2 incubator. Cell-free supernatants were recovered by centrifugation and stored at −20°C until assayed. The concentrations of each cytokine (interleukin [IL]-1α, IL-6, IL-10, granulocyte-macrophage colony-stimulating factor [GM-CSF], and tumor necrosis factor [TNF]-α) were determined by ELISA with commercial reagents (PharMingen, San Diego). The concentration of IL-1β was determined by ELISA with matched antibody pairs and supporting reagents from Endogen (Woburn, MA). Quantification was performed on the basis of a standard curve derived by linear dilution of the cytokine standards included in the respective kits. The detection limits were 8 pg/mL for IL-1α, IL-10, and GM-CSF; 20 pg/mL for IL-6 and TNF-α; and 3.9 pg/mL for IL-1β. Cytokine assays were performed in quadruplicate by using the supernatant samples or appropriate dilutions of the supernatants, as determined in preliminary studies.
Table 1. Decrease in cytokine production after synercid exposure (10 μg/ml) of monocytes stimulated with LPS or heat-killed Staphylococcus aureus Cowan strain 1 (Pansorbin) in vitro.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>No. of persons evaluated</th>
<th>Stimulus</th>
<th>% Decreasea (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>5</td>
<td>LPS</td>
<td>87.6 ± 92.3</td>
</tr>
<tr>
<td>IL-1β</td>
<td>3</td>
<td>LPS</td>
<td>93.7 ± 93.5</td>
</tr>
<tr>
<td>IL-6</td>
<td>5</td>
<td>LPS</td>
<td>74.9 ± 99.9</td>
</tr>
<tr>
<td>IL-10</td>
<td>5</td>
<td>LPS</td>
<td>99.3 ± 95.7</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>5</td>
<td>LPS</td>
<td>90.9 ± 97.0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5</td>
<td>LPS</td>
<td>91.1 ± 99.5</td>
</tr>
</tbody>
</table>

NOTE: LPS, lipopolysaccharide; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor.

a P values, calculated by using Welch’s test, were <.0001–.0054.

Cellular toxicity assay. The toxicity of the antibiotic for the purified monocytes was determined by the MTT cytotoxicity assay (Cell Titer 96 Kit; Promega, Madison, WI) as described elsewhere [7].

Statistics. All values are expressed as mean ± SD. Welch’s test was utilized to determine statistical differences. A 2-tailed P value <.05 was considered statistically significant.

Results

LPS-stimulated monocytes induced production of each of the cytokines examined—IL-1α, IL-1β, IL-6, IL-10, GM-CSF, and TNF-α. The 24-h accumulation, in nanograms per milliliter, in supernatants of LPS-stimulated monocyte cultures was 0.157 ± 0.133 (mean ± SD) for IL-1α, 1.372 ± 0.987 for IL-1β, 28.702 ± 24.336 for IL-6, 1.265 ± 0.662 for IL-10, 0.096 ± 0.098 for GM-CSF, and 1.340 ± 0.449 for TNF-α (figure 1A). Cytokine levels in control (sham-stimulated) cells were undetectable or insignificant when compared with the effect of LPS stimulation. Exposure of the LPS-stimulated monocytes to 1, 5, or 10 μg/mL synercid for 24 h resulted in a concentration-dependent suppression of production of each cytokine (figure 1A). The inhibition of each cytokine after exposure to 10 μg/mL synercid was as follows: 87.6%–99.7% for IL-1α, 97.4%–99.7% for IL-1β, 74.9%–99.9% for IL-6, 95.7%–99.5% for IL-10, 27.3%–96.6% for GM-CSF, and 91.1%–99.6% for TNF-α and was statistically significant for each cytokine (P values of <.0001–.0054; table 1). Although in some persons production of IL-1α, IL-10, and GM-CSF was increased after exposure to 1 μg/mL synercid, a significant decrease was observed at higher concentrations of the antibiotic (figure 1A and table 1).

Pansorbin-stimulated monocytes produced amounts of IL-1α, IL-1β, IL-10, GM-CSF, and TNF-α that were, on average, 3.0-, 1.9-, 2.6-, 2.0-, and 1.7-fold higher, respectively, than those produced by LPS-stimulated monocytes (figure 1B). In contrast, the amounts of IL-6 produced by Pansorbin-stimulated cells were 2.5-fold lower than those produced by LPS-stimulated monocytes (figure 1B). Similar to the observations with LPS-stimulated monocytes, synthesis and accumulation of each of the cytokines by Pansorbin-stimulated monocytes also was suppressed in a concentration-dependent manner after a 24-h exposure to 1, 5, or 10 μg/mL synercid (figure 1B and table 1).

To determine whether this concentration-dependent suppression of cytokine production by synercid was due to toxicity of the cells, monocytes cultured in the absence of LPS or Pansorbin were exposed to the same concentrations of synercid and were examined for cytotoxicity by the MTT assay. The results revealed that the viability of monocytes was not affected significantly at any of the concentrations used (data not shown).

Discussion

These results reveal that synercid significantly modulates the in vitro production of IL-1α, IL-1β, IL-6, IL-10, GM-CSF, and TNF-α by human monocytes. These effects were observed at synercid concentrations that are achievable in human serum with conventional dosing [8] and are not the result of a direct cytotoxic effect on monocyte function. This suggests that, in addition to its antimicrobial effects, synercid may have a significant regulatory effect on the immune response in vivo. Moreover, the suppression of cytokine production by synercid is markedly different from that observed with azithromycin or clarithromycin [6]. Exposure to either of these latter antibiotics resulted in an increase or decrease in production of different cytokines by stimulated monocytes, and there was a wide variation in individual responses.

Since each of the cytokines examined is not similarly regulated and the suppressive effect of synercid was noted after 2 different stimuli, it is likely that synercid exerts its nonspecific suppressive effect through multiple mechanisms. The downregulatory effects that we observed are unlikely to be due to IL-10, because synercid down-regulated production of IL-10 in our studies. Of interest, we found a similar remarkable nonspecific suppression of cytokine production by monocytes treated with trovafloxacin, a fluoroquinolone antibiotic [7]. Other antibiotics we studied did not result in such a profound effect on cytokine production by human monocytes [6].

Our results suggest that, in addition to its antimicrobial action, synercid may have an immunomodulatory effect on host response to infection. Whether this will be helpful in the treatment of infected patients can be answered only through appropriate studies.

References

2. Caron F, Gold HS, Wennersten CB, Farris MG, Moellering RC Jr, Eliopoulos...
Figure 1. Effect of synercid on cytokine production by lipopolysaccharide-stimulated human monocytes in vitro (A) and Pansorbin (heat-killed *Staphylococcus aureus* Cowan strain I)-stimulated human monocytes in vitro (B). Symbols represent different healthy human volunteers. IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor.


