Divergent Roles of Tumor Necrosis Factor and Platelet-Activating Factor in Endotoxin-Induced Release of Monocyte Chemoattractant Protein 1 and Macrophage Inflammatory Protein 1β in Chimpanzees

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A platelet-activating factor receptor antagonist reduced the release of macrophage inflammatory protein 1β (MIP-1β) during endotoxemia in chimpanzees but did not influence the secretion of monocyte chemoattractant protein 1 (MCP-1). Anti-tumor necrosis factor alpha monoclonal antibody completely prevented MCP-1 release and simultaneously enhanced the secretion of MIP-1β. Levels of MIP-1β and MCP-1 release were differentially regulated during endotoxemia.

Monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein 1β (MIP-1β) are members of the CC chemokine family and exert biological activities on largely similar target cells, including monocytes, T cells, and natural killer cells (7, 10). The serum levels of both chemokines increase after administration of endotoxin to human volunteers (5, 9) and are elevated in the plasma of patients with sepsis (2, 5). Platelet-activating factor (PAF) and tumor necrosis factor alpha (TNF) have been implicated in the activation of inflammatory cascades in the early phase of sepsis. In animals, administration of PAF or TNF results in pathological changes that mimic those found in sepsis, while PAF antagonists or anti-TNF antibodies reduce lethality elicited by systemic administration of endotoxin and/or live bacteria (1, 14). Of interest, PAF and TNF can induce each other’s production and PAF antagonists can reduce the release of endotoxin-induced TNF. Both PAF and TNF can stimulate the production of the CXC chemokine interleukin 8 (IL-8), and inhibition of PAF and neutralization of TNF result in a diminished release of IL-8 in human and nonhuman primate models of low-grade endotoxemia (4, 12, 13, 14).

To determine the role of PAF and TNF in endotoxin-induced release of MCP-1 and MIP-1β, 17 healthy adult chimpanzees, recruited from the primate colony at the Laboratory for Experimental Medicine and Surgery in Primates, New York University School of Medicine, Tuxedo, N.Y., were studied after they were given a bolus intravenous injection of Escherichia coli endotoxin (4 ng/kg of body weight; lot EC-5, kindly provided by D. Hochstein, Bethesda, Md.) (Table 1). The present study was performed simultaneously with studies examining the effects of TCV-309 and anti-TNF monoclonal antibody (MAb) on cytokine release, the results of which have already been published (4, 12, 13). The experimental procedures have been reported in detail previously (4, 12, 13). Six animals received only the bolus injection of purified endotoxin. With four chimpanzees the administration of endotoxin was followed immediately by the administration of the PAF antagonist TCV-309 (provided by Takeda Chemical Industries Ltd., Osaka, Japan), given as a bolus injection of 100 µg/kg and as a continuous infusion of 500 µg/kg/h for 5 h (4). With four other animals a bolus injection of purified endotoxin was immediately followed by the administration of a murine anti-human TNF MAb (provided by Bayer, Wuppertal, Germany), given as a bolus injection of 15 mg/kg (13). With three other animals a bolus injection of purified endotoxin was followed after 30 min by the administration of an anti-TNF F(ab’)2 fragment antibody (MAK 195F; provided by Knoll, Ludwigshafen, Germany), given as a bolus injection of 0.1 mg/kg (12). Venous blood was collected directly before the injection of endotoxin and at 1, 2, 3, 4, and 5 h thereafter. Serum was obtained by centrifugation at 4°C for 20 min at 1,600 × g and stored at −70°C until assayed.

MCP-1 (Pharmingen, San Diego, Calif.) and MIP-1β (R&D Systems, Abingdon, United Kingdom) were measured by enzyme-linked immunosorbent assays according to the instructions of the manufacturer. TNF activity was measured by the WEHI cytotoxicity assay (12, 13). The protocol was approved by the animal health and welfare committee of the Laboratory for Experimental Medicine and Surgery in Primates and was conducted according to the guidelines of the American Physiologic Society. Endotoxin effects were analyzed by one-way analysis of variance. Differences between the results of different treatments were analyzed by repeated-measure analysis of variance (interaction between treatment and time). P values reflect differences between curves. A P < 0.05 was considered significant.

Injection of endotoxin induced transient increases in the concentrations of MCP-1 and MIP-1β (Fig. 1 and 2) in sera. Peak MCP-1 levels were reached after 3 h (mean ± standard error [SE], 15.74 ± 4.07 ng/ml; P < 0.05), while peak MIP-1β levels were reached after 2 h (9.68 ± 1.72 ng/ml; P < 0.05). PAF concentrations were not measured in this study, since PAF is difficult to measure in biological fluids and PAF detected in the circulation may not have great biological significance, since most PAF that is produced by a cell is never excreted by that cell (1). TCV-309 modestly inhibited endotoxin-induced release of MIP-1β (peak levels, 5.00 ± 0.69 ng/ml; P < 0.05 versus levels produced with endotoxin only), without influencing endotoxin-induced release of MCP-1.
(peak levels, 10.93 ± 3.69 ng/ml, reflecting a nonsignificant difference from levels produced with endotoxin only). Injection of endotoxin only resulted in a transient increase in TNF bioactivity, which peaked after 1.5 h (156 ± 53 pg/ml, \( P < 0.05 \)) (data derived from references 12 and 13). Infusion of anti-TNF MAb, given either directly after or 30 min after endotoxin injection, resulted in a complete neutralization of endotoxin-induced TNF activity (12, 13). Both anti-TNF MAb regimens completely prevented the release of MCP-1 during endotoxemia (Fig. 2) (with results of both regimens reflecting a \( P \) of <0.05 versus results with endotoxin only). Remarkably, anti-TNF MAb given directly after endotoxin significantly enhanced MIP-1\(\beta\) release (peak levels, 25.29 ± 7.41 ng/ml; \( P < 0.05 \) versus levels produced with endotoxin only) while postponed treatment with anti-TNF MAb tended only to increase MIP-1\(\beta\) concentrations (peak levels, 13.38 ± 6.38 ng/ml, reflecting a nonsignificant difference from levels produced with endotoxin only).

MCP-1 can be produced in response to various stimuli, including TNF and endotoxin (7, 10). PAF can stimulate MCP-1 production in glomeruli in isolated perfused rat kidneys (3) and can increase MCP-1 secretion by monocytes that have adhered to endothelial cells by an interaction with P-selection (15). MCP-1 has an antiinflammatory role during endotoxemia (16). Indeed, passive immunization of mice with anti-MCP-1 antiserum enhanced endotoxin-induced mortality, while treatment with recombinant MCP-1 protected mice from lethality. Moreover, anti-MCP-1 antiserum increased endotoxin-induced TNF release when compared with the TNF release in animals treated with endotoxin and control antiserum (16). We now report that neutralization of endogenous TNF is associated with a complete prevention of MCP-1 release during endotoxemia. These data extend earlier findings from baboons intravenously infused with a lethal dose of \textit{E. coli}, in which anti-TNF MAb treatment significantly attenuated MCP-1 release (6). Together, these data suggest that TNF produced early after administration of endotoxin induces the production of a mediator (i.e., MCP-1) that exerts a negative-feedback effect on ongoing TNF production. We previously reported a similar negative-feedback loop involving IL-10, an antiinflammatory cytokine of which the production is also in part regulated by endogenous TNF during endotoxemia (11).

In a previous study of human volunteers, infusion of a recombinant TNF receptor fusion protein did not influence endotoxin-induced release of MIP-1\(\alpha\) or MIP-1\(\beta\) (5, 8). However, although the TNF receptor fusion protein completely prevented TNF activity, the infusion was associated with a number of paradoxical unexplained proinflammatory effects, hampering a straightforward interpretation of the results (8). In light of the fact that TNF can induce MIP-1\(\beta\) production by various

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**TABLE 1. Treatment groups**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Timing (h) relative to that with endotoxin alone</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>TCV-309 (PAF antagonist)</td>
<td>0–5</td>
<td>4</td>
</tr>
<tr>
<td>Anti-TNF MAb</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Anti-TNF MAb</td>
<td>0.5</td>
<td>3</td>
</tr>
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\(a\) All animals received a bolus intravenous injection of endotoxin (4 ng/kg) at time zero.
cell types in vitro (10), our finding that anti-TNF MAb enhanced (when it was given directly after endotoxin) or did not influence (postponed treatment) MIP-1β release was unexpected. It is remarkable that delayed treatment with anti-TNF MAb exerted an effect on MIP-1β release slightly different from the effect of anti-TNF MAb given directly after endotoxin, especially in consideration of the fact that both regimens were associated with a complete neutralization of TNF activity. In an earlier study we also found differential effects of early and postponed anti-TNF MAb treatment on endotoxin-induced IL-10 release (11). Together, these data suggest that within 30 min after injection of endotoxin, some TNF is induced at the tissue level and/or in a cell-associated form and can influence MIP-1β and IL-10 release. Nonetheless, it seems clear that TCV-309 reduced MIP-1β release independently from its effect on TNF.

REFERENCES
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