Tumor necrosis factor α (TNF-α) (also called cachectin) exhibits a wide range of biological activities (1). When injected into animals, TNF-α may induce circulatory failure and death, and the lethality induced by LPS or Gram-negative bacteria may be inhibited by antibodies to TNF-α (2-4). The protein thus appears to play a role in the pathogenesis of septic shock (5). The multiple biological activities of TNF-α are overlapping with those of IL-1 (6). Both cytokines may increase the adherence of neutrophils, induce fever in the rabbit, and induce a procoagulant activity in endothelial cells (7-9). However, the role of IL-1 in septic shock remains to be clarified.

We have previously reported that the presence of TNF-α in serum is associated with fatal outcome in patients with meningococcal disease (10). Recently, we have detected IL-1 activity in serum from three patients with septic shock (Waage, A., P. Brandtzaeg, A. Halstensen, and T. Espevik, manuscript in preparation). These patients had high serum levels of TNF-α and rapid fatal outcomes. This observation led us to the hypothesis that TNF-α and IL-1 may have synergistic lethal effect in septic shock. The present study gives support to this hypothesis by demonstrating that recombinant IL-1α (rIL-1α) and IL-1β (rIL-1β) markedly potentiate the lethal effect of recombinant TNF-α (rTNF-α) in mice.

Materials and Methods

Reagents. Mouse rTNF-α (Dr. G. R. Adolf, Boehringer Ingelheim, Vienna, Austria) had a specific activity of $7 \times 10^7$ U/mg as determined by a cytotoxicity assay using L-M cells, and contained <0.025 ng of endotoxin/mg of recombinant protein by the limulus amoebocyte lysate test (11, 12). Human rIL-1α (Glaxo Institute for Molecular Biology, Geneva, Switzerland) had a specific activity of $2.5 \times 10^7$ U/mg as determined in the thymocyte proliferation assay (13), and human rIL-1β (Glaxo) had a specific activity of $5 \times 10^5$ U/mg (14). The contents of endotoxin were specified to be 0.4–0.7 ng/mg of rIL-1α and 0.007–0.014 ng/mg of rIL-1β (limulus amoebocyte lysate test) (13, 14). The cytokines were diluted in endotoxin-free 0.9% saline to appropriate concentrations, and injected in a volume of 0.1 ml into each mouse via the tail vein. An antiserum against rIL-1α has been raised in sheep, and neutralized at least 20 μg rIL-1α/ml antiserum (a gift from Dr. A. Shaw, Glaxo). Control serum was taken from a normal sheep. LPS from Escherichia coli (026:B6) was purchased from Sigma Chemical Co., St. Louis, MO.

Animals. Male NMRI mice (Bomholt gård, Ry, Denmark) weighing 22–24 g were caged in groups at room temperature 22°C, and were allowed to eat and drink ad libitum.
Results

Mice receiving doses of rIL-1α or rIL-1β from 0.15 to 1.8 μg/mouse were active and appeared to be healthy during the observation period. No deaths were recorded in these groups of animals (Fig. 1).

A dose-response curve for rTNF-α with respect to lethality was determined (Fig. 1). The lowest dose of rTNF-α causing death was 0.5 μg/mouse, and the dose of rTNF-α causing 50% death (LD₅₀) was 1.5 μg/mouse, as estimated by the regression line on the curve in Fig. 1. 98% of all the deaths occurred within 12 h of injection.

Concomitant with the administration of rTNF-α to one group of mice, animals in a parallel group received the same dose of rTNF-α combined with 0.5 μg rIL-1α. The combination of the two cytokines was far more toxic than any of them given alone, and the LD₅₀ of rTNF-α in this combination was calculated to be 0.4 μg/mouse (Fig. 1). A similar effect of rIL-1β (0.5 μg/mouse) and rTNF-α was observed, as the LD₅₀ of rTNF-α administered in this combination was calculated to be 0.5 μg/mouse. The analysis of these results showed that the effects of the cytokines were not merely additive, but that rIL-1α and rIL-1β potentiated the lethal effect of TNF-α (15).
TABLE 1

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Number of dead mice</th>
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<tbody>
<tr>
<td>rTNF-a/rIL-1α and rIL-1α antiserum</td>
<td>1/5</td>
</tr>
<tr>
<td>rTNF-a/rIL-1α and control serum</td>
<td>4/5</td>
</tr>
<tr>
<td>rTNF-a and control serum</td>
<td>1/5</td>
</tr>
</tbody>
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* 0.6 μg of rTNF-a, 0.4 μg of rIL-1α and 90 μl rIL-1α antiserum or control serum was injected in a volume of 100 μl into each animal. The mice were observed for 24 h.

Fig. 2 shows survival curves that clearly demonstrate the potentiating effect of rIL-1α. The combination of a near nonlethal dose of rTNF-a and a nonlethal dose of rIL-1α induced 100% lethality within 10 h of injection of the cytokines. In comparison, 1 μg of rTNF-a induced 30% lethality.

To evaluate the specificity of the potentiating effect of rIL-1α, mixtures of rTNF-a, rIL-1α, and antiserum to rIL-1α or control serum were incubated at 37°C for 1 h before injection. In the group of mice receiving a mixture containing antiserum, 1 of 5 mice died, whereas 4 of 5 mice died in the group receiving control serum (Table I). Also, LPS has been reported to potentiate the lethal effect of TNF-a in mice (16), and control experiments were conducted to assess the possible influence of LPS contamination. Solutions containing 0.5 μg of rTNF-a and various amounts of LPS were injected into mice, and enhanced lethality was observed when the LPS doses were >1 μg/mouse (data not shown). Injections of LPS alone, or LPS together with 0.5 μg of rIL-1α, did not cause death for LPS doses up to 200 μg/mouse. Altogether, these results indicate that the rTNF-a potentiating effect of the rIL-1 preparations was caused by the IL-1 molecule itself and not by the LPS content in the preparations of cytokines.

In mice, a dual effect of rTNF-a on the temperature has been reported, as low doses may induce fever and high doses produce a marked hypothermia (17). In all our experiments, rTNF-a had a hypothermic effect, and results from one experiment are shown in Fig. 3. The lowest mean temperature recorded after injection of 0.375 μg of rTNF-a was 33.4°C. When rTNF-a was combined with 0.5 μg/mouse

![Figure 3. Temperatures in mice receiving various combinations of cytokines. The doses given were 0.375 μg/mouse of rTNF-a and 0.5 μg/mouse of rIL-1. Results are mean ± SEM.](image-url)
of rIL-1α or rIL-1β, the lowest mean temperatures detected were 28.4°C and 31.6°C, respectively. A similar pattern in the temperature responses was observed in all experiments.

**Discussion**

The results demonstrate that the LD$_{50}$ of rTNF-α was reduced by $\sim$70% when rTNF-α was administered together with a nonlethal dose of rIL-1α or rIL-1β. A similar effect was observed on the temperature response, and we conclude that the rIL-1 markedly potentiate the lethal and hypothermic effects of rTNF-α. By comparing rIL-1α and rIL-1β on a weight basis, rIL-1α seemed to have a slightly stronger potentiating effect than rIL-1β.

It has been reported that also LPS may potentiate the lethal effect of TNF-α in mice (16), and it appears to be important to evaluate the possibility of LPS contamination in our experiments. We found that it was required to inject $>1$ μg/mouse of LPS in order to potentiate the lethal effect of rTNF-α. Rothstein and Schreiber have reported a similar potentiating effect with LPS doses at 24 ng/mouse, or higher (16). In comparison, the maximum dose of LPS injected in a mixture of 0.5 μg of rTNF-α and 0.5 μg of rIL-1α was 0.4 pg. This dose is $\sim 10^6$ times lower than the critical LPS dose determined in our control experiments, and could hardly be the cause of the potentiating effect of the rIL-1 preparations.

Furthermore, we demonstrated that neutralization of rIL-1α resulted in a protection from the lethal effect of the combination of rTNF-α and rIL-1α. Altogether, the control experiments strongly indicate that the potentiating effect observed in the present study can be assigned to IL-1.

Ruggiero and Baglioni (18) have previously reported a synergistic antiproliferative effect of TNF-α and IL-1 on a human melanoma cell line. In contrast, additive effects of TNF-α and IL-1 have been observed with respect to the suppression of a lipoprotein lipase in 3T3-L1 cells, and the induction of procoagulant activity in endothelial cells (9, 19). At the cellular level, it thus appears that the effects of IL-1 and TNF-α may be either additive or synergistic. Despite many similarities in the biological activities, TNF-α and IL-1 appears to bind to different receptors (19, 20). What happens beyond the receptor level after binding of their respective cytokines, and the basis of the observed synergistic effects, is incompletely understood. The in vivo responses observed in the present study are probably mediated via complex biochemical and cellular reactions, and even more difficult to explain.

During the initial phase of septic shock, a number of cytokines are probably released into the circulation. The identification of these cytokines and their actions and interactions in septic shock appears to be important in order to direct new strategies for the treatment of these patients. We have observed that patients with high levels of TNF-α and detectable amount of IL-1 in the circulation rapidly died from septic shock. The results in the present study support our suggestion that IL-1 and TNF-α may have synergistic lethal effect in human septic shock.

**Summary**

Human rIL-1α and human rIL-1β were examined for their ability to potentiate the lethal and hypothermic effects of mouse rTNF-α in mice. The LD$_{50}$ of rTNF-α was 1.5 μg/mouse, whereas the LD$_{50}$ of rTNF-α was reduced to 0.4 μg/mouse and
0.5 μg/mouse when rTNF-α was administered in combination with a nonlethal dose of rIL-1α or rIL-1β, respectively. A similar rTNF-α enhancing effect of the rIL-1 was observed on the temperature response. The results show that the rIL-1 markedly potentiate the effects of rTNF-α on lethality and temperature in mice, and support our suggestion that TNF-α and IL-1 may have synergistic lethal effect in human septic shock.

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References