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Short Communication

Cardiotrophin-1 Inhibits Tumor Necrosis Factor Production in the Heart and Serum of Lipopolysaccharide-Treated Mice and *in Vitro* in Mouse Blood Cells

Fabio Benigni,* Silvano Sacco,*
Diane Pennica,† and Pietro Ghezzi*

From the Laboratory of Neuroimmunology, Mario Negri Institute for Pharmacological Research, Milan, Italy, and the Department of Molecular Biology,† Genentech Inc., South San Francisco, California*

Cardiotrophin-1 (CT-1) is a member of the gp130 family of cytokines that includes interleukin-6, interleukin-11, ciliary neurotrophic factor, leukemia inhibitory factor, and oncostatin M. As interleukin-6, leukemia inhibitory factor, and ciliary neurotrophic factor were previously reported to inhibit the production of tumor necrosis factor (TNF), we studied the effect of CT-1 on serum and heart TNF levels in mice treated with lipopolysaccharide (100 ng/mouse, iv). Co-treatment with CT-1 (5 μg/mouse intravenously) markedly inhibit TNF production both in serum and in the heart. The effect of CT-1 seems to be direct as it also inhibited TNF production when added to whole mouse blood cultured with lipopolysaccharide. Thus, CT-1 might play a protective role in some TNF-mediated diseases. (Am J Pathol 1996, 149:1847–1850)

Cardiotrophin-1 (CT-1) was originally identified as a potent inducer of cardiac myocyte hypertrophy *in vitro*[1] and more recently identified as a promoter of neonatal cardiac myocyte survival.[2] It is structurally related to the family of cytokines including leukemia inhibitory factor, ciliary neurotrophic factor, oncostatin M, interleukin (IL)-6, and IL-11. The receptor for CT-1 shares with all these cytokines the signal transduction protein gp130.[3]

Tumor necrosis factor (TNF) is a proinflammatory cytokine implicated as a pathogenetic mediator in a series of diseases.[4] Elevated TNF levels have been detected in patients with severe acute myocardial infarction or chronic heart failure and have been shown to correlate with the severity of the disease.[5] IL-6 is also elevated in patients with acute myocardial infarction and was suggested to be an important endogenous mediator of the induction of the acute-phase response observed in these patients.[6] In fact, IL-6 is a well known inducer of acute-phase protein synthesis, an activity that is also shared by the other gp130 cytokines, including CT-1, which induces acute-phase proteins both *in vitro* and *in vivo*.[7–9] We and others reported that IL-6, leukemia inhibitory factor, and ciliary neurotrophic factor inhibit the production of TNF induced *in vivo* by injection of lipopolysaccharide (LPS).[10–14] IL-6 has also been reported to inhibit TNF production *in vitro*.[10]

In this paper we have investigated whether recombinant mouse CT-1 inhibits the production of TNF *in vitro* and/or *in vivo*. For this purpose, mice were injected with CT-1 along with a classical inducer of TNF production, LPS, and TNF levels were measured in the serum and in the heart. We also studied the effect of CT-1 on LPS-induced TNF production in mouse blood cultures *in vitro*. The results show that CT-1 is an inhibitor of TNF production.

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Address reprint requests to Dr. Pietro Ghezzi, Mario Negri Institute, via Eritrea 62, 20157 Milan, Italy.

Materials and Methods

Animals and Treatments

Male CD-1 mice (25 g body weight; Charles River, Calco, Como, Italy) were used. Recombinant mouse CT-1 (Genentech, South San Francisco, CA), prepared as described,[1] was administered as a single dose of 5 μg/mouse intravenously[7] and LPS (from *Escherichia coli* O55:B5, Sigma Chemical Co., St Louis, MO) at 80 μg/kg intravenously. Blood was taken from the retro-orbital plexus at various times under ether anesthesia, and serum was prepared. TNF was measured by cytotoxicity on L929 cells as previously described,[15] using mouse recombinant TNF-α as a standard (Genzyme, Cambridge, MA). When indicated, the hearts were removed, rinsed extensively in ice-cold saline, and homogenized with an Ultra Turrax in 4 vol (w/v) of ice-cold saline. The homogenate was then centrifuged 10 minutes at 13,000 rpm in a microfuge, and the supernatant was used for TNF assay.

Experiments were performed with five mice per group and repeated three times. One representative experiment is shown in each figure.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, December 12, 1987; Italian Legislative Decree 116/92, Gazzetta Ufficiale della Repubblica Italiana 40, February 18, 1992; National Institutes of Health Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 85–23, 1985).

Whole Blood

Heparinized (Liquemin, Roche, Milano, Italy, 14 U/ml) whole blood obtained from CD-1 mice was diluted 1:4 (v/v) in RPMI 1640 medium (without bovine serum), plated in 96-well tissue culture plates (100 μl/well), and incubated for 4 hours at 37°C/5% CO_2 in the presence of 1 μg/ml LPS with and without CT-1 at the concentrations indicated. After the incubation, plates were centrifuged and the supernatant collected for TNF determination.

Results

We first investigated the possible inhibitory effect of CT-1 on TNF production, based on previous results obtained with leukemia inhibitory factor, ciliary neurotrophic factor, and IL-6.

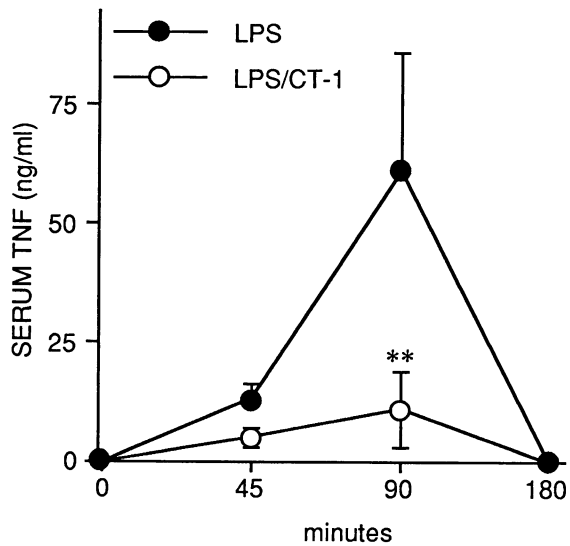

Figure 1. *Effect of CT-1 on serum TNF levels in LPS-treated mice. Mice were treated with CT-1 (5 μg/mouse, iv) or saline alone (controls). LPS was given iv at the same time at the dose of 100 ng/mouse. Serum TNF was measured at the indicated times. Data are the mean ± SD from five mice per group.* **P < *0.01* versus *LPS alone by Student's* t-*test.*

Figure 1 shows the effect of CT-1 pretreatment on the time course of serum TNF levels induced by LPS. LPS-treated mice induced high serum TNF levels with a peak at 90 minutes. Pretreatment with CT-1 significantly inhibited serum TNF without changing the kinetics of its induction.

The effect of different doses of CT-1 (0.1, 1, or 5 μg per mouse) on LPS-induced TNF production was then determined. In these experiments, TNF was measured at the peak time of 90 minutes, in the serum as well as in heart homogenates. No TNF was detected either in sera or heart homogenates in the absence of LPS (data not shown). CT-1 inhibited serum TNF production (by approximately 60%) only at the dose of 5 μg, although there was a trend toward decreased TNF levels with the dose of 1 μg (Figure 2A). Injection of LPS induced high levels of TNF in the heart, which was also inhibited by 5 μg of CT-1 (Figure 2B). Maximal inhibition obtained at this dose was 77%. As IL-6 was reported to directly inhibit TNF production *in vitro*, the ability of CT-1 to inhibit TNF production also *in vitro* in mouse blood was studied, and the results are shown in Figure 3. CT-1 at 10 μg/ml markedly inhibited (by 80%) TNF production.

Discussion

The present study shows that CT-1 is an inhibitor of TNF production *in vivo*. Inhibition of TNF production was also observed *in vitro*, indicating that this effect

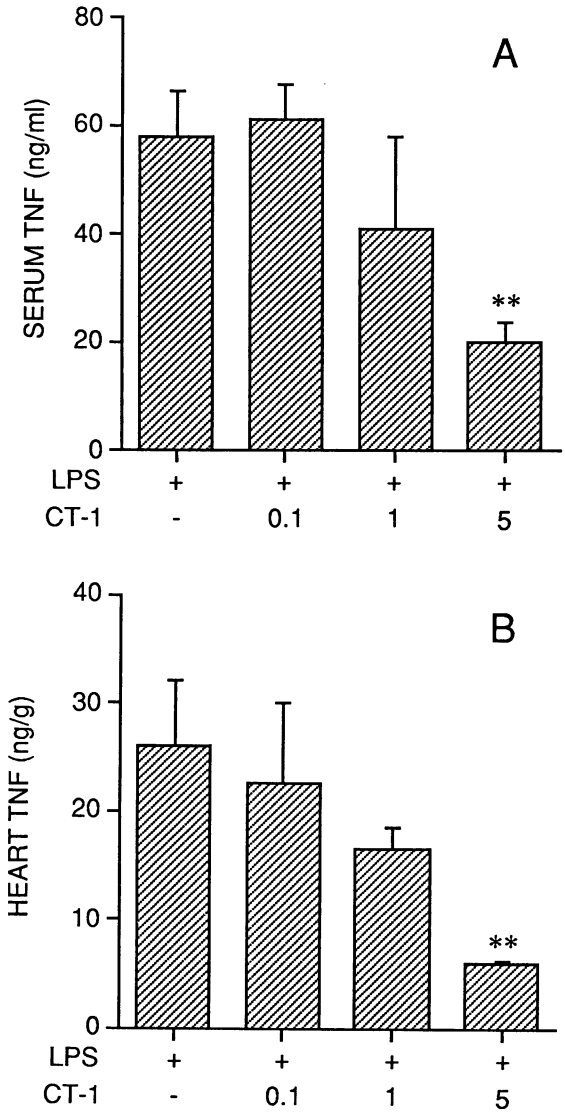

Figure 2. *Effect of various doses of CT-1 on serum and heart TNF production. Mice were treated with CT-1 at the indicated dose. LPS was given iv at the same time at the dose of 100 ng/mouse. TNF was measured 90 minutes later in serum or in heart homogenate. Data are the mean ± SD from five mice per group.* **P < *0.01* versus *LPS alone by Student's* t-*test.*

is not mediated by an increase in serum corticosteroids due to activation of the hypothalamus-pituitary-adrenal axis, a response that is potentiated by CT-1 as well as other gp130 cytokines[7]

Although this finding adds to the list of biological effects of CT-1, the biological significance of it is far from being established. In particular, it is not known whether CT-1 is increased, in the circulation or in specific tissues, after LPS treatment. Interestingly, it has been suggested that CT-1 is induced in cardiac hypertrophy associated with genetic hypertension in rats.[16] It should be noted, however, that we could not show any preferential susceptibility of TNF production in the heart (in terms of minimal effective dose of CT-1) compared with circulating TNF, in terms of inhibition by CT-1.

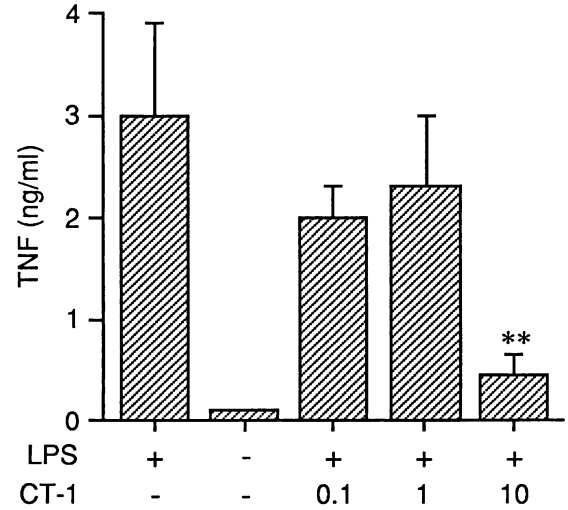

Figure 3. *Effect of CT-1 on TNF production in mouse blood* in vitro. *Whole blood from control mice was stimulated for 4 hours with LPS (1 μg/ml) in the presence or absence of CT-1. TNF was measured in the supernatant. TNF production of unstimulated samples (without LPS) was <4 pg/ml. Data are mean ± SD (n = 6).* **P < *0.01* versus *LPS alone by Duncan test.*

Thus, CT-1 shares with ciliary neurotrophic factor, IL-6, and leukemia inhibitory factor the ability to inhibit TNF production. It remains to be established whether CT-1 could be an endogenous inhibitor of TNF production. This was the case with IL-6 as we reported that IL-6-deficient mice induce higher TNF levels than normal mice upon injection with LPS.[17] Additional studies will be required to investigate whether there are pathological conditions in which CT-1 and TNF might be co-induced.

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