Interferon-α Induces Circulating Tumor Necrosis Factor Receptor p55 in Humans

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In the present studies we investigated the effect of interferon-α (IFNα) on the release of the soluble (extracellular) form of the tumor necrosis factor p55 receptor (TNFSRp55), because TNFSRp55 is a natural antagonist of tumor necrosis factor (TNF)-induced inflammation and also might be part of the antiinflammatory properties of IFNα. Plasma levels of TNFSRp55 were measured by a specific radioimmunoassay in five healthy volunteers and in five patients with chronic hepatitis C treated with IFNα. Levels showed a significant increase after a single injection of 5.0 million U IFNα in both healthy and hepatitis patient groups. Peak values (3.5 to 4.5 ng/mL) were observed within 12 hours of beginning treatment. Thereafter, levels promptly declined, reaching baseline values within 24 hours. TNFα and C-reactive protein (CRP) levels were below the detection limit in the same plasma samples. In addition, IFNα suppressed significantly interleukin (IL)-1α-induced TNFα protein synthesis by human peripheral blood mononuclear cells. These results suggest that the antiinflammatory properties of IFNα may be, in part, also due to the induction and/or release of TNF soluble receptors and the suppression of TNFα synthesis.

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TUMOR NECROSIS FACTOR (TNF) is a pleiotropic cytokine, which possesses a wide range of biologic properties. TNF plays a major role in the pathogenesis of sepsis, the progression of acquired immune deficiency syndrome (AIDS), as well as in a number of inflammatory disorders. Naturally occurring TNF inhibitors have been identified by their ability to bind to TNF and to neutralize its biologic activity. These inhibitors were subsequently purified and sequenced, which revealed that the proteins represented the extracellular portions of the two membrane-associated TNF receptors p55 and p75. Both TNF-binding proteins are presumably derived from their respective membrane receptors by proteolytic cleavage and have, therefore, been termed soluble TNF receptors (TNFSRp55 and TNFSRp75). Soluble forms of both TNF receptors block TNF-mediated effects such as lethality in septic shock, replication of human immunodeficiency virus in vitro, and others. The present study aimed to investigate whether interferon-α (IFNα) therapy is associated with the induction of circulating TNFSRp55 and whether IFNα modulates TNFα protein synthesis in vitro.

MATERIALS AND METHODS

Five healthy male volunteers and five patients with histologically and serologically confirmed chronic hepatitis C, but without evidence of cirrhosis, were included in an outpatient study with IFNα-2b (Aesca-Scherering-Plough Corp, Vienna, Austria). Liver histology showed chronic persistent hepatitis in four and chronic active hepatitis in one of the patients. The protocol was approved by the Ethics Committee of the Faculty of Medicine of the University of Innsbruck and informed consent was obtained from each participant. All participants received increasing doses of IFNα subcutaneously. IFNα was injected as a single dose of 1 × 10⁸, 3 × 10⁸, or 5 × 10⁸ at weekly intervals. Venous blood was drawn from each participant immediately before, and 2, 12, 48, and 72 hours after the injection. Blood was collected in EDTA-containing venojet tubes placed on ice, and centrifuged within 20 minutes of venipuncture at 2,000g for 10 minutes. Plasma samples were stored at −70°C until assayed. Plasma samples obtained after injection of 1 × 10⁸ and 5 × 10⁸ U IFNα were assayed for TNFSRp55, TNFα and C-reactive protein (CRP).

Peripheral blood mononuclear cells (PBMC) for in vitro studies were isolated from the heparinized blood of six healthy volunteers and then 1 mL incubated in 5 mL polypropylene tubes at a density of 2.5 × 10⁶ cells/mL for 24 hours in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10 mmol/L HEPES (Sigma), 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Irvine Scientific, Santa Ana, CA). PBMC were incubated for 24 hours at 37°C with the same preparation of IFNα used in the patients. PBMC cultures contained 5 μg/mL polymyxin B (Pfizer Inc, New York, NY). Human recombinant interleukin (IL)-1α was kindly provided by Dr P. Lomedico (Hoffmann-LaRoche Inc, Nutley, NJ). Lipopolysaccharide (LPS) (from Escherichia coli 055:B5) and phorbol myristate acetate (PMA) were purchased from Sigma.

The radioimmunoassay (RIA) for the TNFSRp55 has been described. This RIA is highly specific and is not affected by TNFα. The lower limit of detection is 80 to 160 pg/mL. A glycosylated form of TNFSRp55 expressed in Chinese hamster ovary cells was used to immunize New Zealand white rabbits for the generation of the anti-TNFα antibodies used in the RIA. TNFα was measured by a previously described RIA. The lower limit of detection of this assay is 80 pg/mL. This TNFα RIA is unaffected by the presence of TNFSRp55 at concentrations up to 10 μg/mL. CRP was measured with a nephelometric method (QM 300, Kallestad, Austin, TX). The lower detection limit for CRP using this assay is 6 μg/mL. Data are presented as mean ± SE. Two-tailed paired t-tests were used. P values less than .05 were considered significant.

RESULTS

Pretreatment levels of TNFSRp55 in the five hepatitis C patients (1.38 ± 0.23 ng/mL) were not significantly higher from those measured in five healthy volunteers (1.12 ± 0.19 ng/mL). IFNα at a concentration of 1.0 million U did not result in an increase of circulating TNFSRp55 levels (data not shown). A higher dose (5 × 10⁸ U), however, resulted
in a significant induction of circulating TNFsRp55 within 12 hours in both the hepatitis C patients (3.84 ± 0.36 ng/mL, P < .001) and in healthy volunteers (3.76 ± 0.37 ng/mL, P < .001) (Fig 1). Thereafter, levels declined, reaching pretreatment values within 24 hours. TNFα and CRP levels were measured at the same time intervals as those of TNFsRp55, but were below the detection limit in each plasma sample (data not shown).

PBMC from six normal donors incubated with IFNα in concentrations of up to 1,000 U/mL or with endotoxin (100 ng/mL) did not release detectable TNFsRp55 into the supernatants after a 3-hour incubation period (data not shown). PBMC released TNFsRp55 into the supernatants after incubation with 100 ng/mL PMA for 10 minutes (0.31 ± 0.05 ng/mL, P < .05). PBMC incubated with increasing concentrations of IFNα (up to 1,000 U/mL) failed also to induce the synthesis of TNFα (data not shown). In contrast, IFNα suppressed IL-1-induced TNFα synthesis by 56% at a concentration of 100 U/mL IFNα (P < .01) and by 53% at a concentration of 1,000 U/mL (P < .01) (Table 1).

DISCUSSION

IFNα is clinically useful in the treatment of diseases of diverse pathogenesis and manifestations. There is increasing evidence that IFNα interferes with the synthesis of various cytokines and in some cases acts as an antiinflammatory agent. IFNα also induces interleukin-1 receptor antagonist (IL-1Ra) in vitro and in vivo and suppresses IL-1-induced IL-1 synthesis by PBMC. The gene expression of another proinflammatory cytokine, namely IL-8, is suppressed in human fibroblasts by IFNα. Constitutive IL-8 mRNA expression in PBMC from patients with chronic myelogenous leukemia is also downregulated during IFNα therapy. Several reports show that IFNα may suppress TNFα gene expression and protein synthesis. Expression of the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) is mainly regulated by IL-1 and TNF. IFNα treatment of patients with chronic viral hepatitis leads to downregulation of ICAM-1 on hepatocytes. In addition, IFNα has been shown to prevent endotoxin-induced mortality in mice. We now show that two additional mechanisms might contribute to its anti-inflammatory properties: (1) induction of circulating TNFsRp55 during IFNα therapy and (2) suppression of IL-1α-induced TNFα synthesis in vitro by PBMC.

The induction of circulating TNFsRp55 during IFNα therapy may provide a regulatory mechanism for the modulation of endogenous circulating TNF activity. Van Zee et al recently showed that TNFsRp55 concentrations of 5 ng/mL are required to neutralize the cytotoxicity of endogenously produced TNF in the plasma of normal donors after endotoxin challenge. It is difficult to establish from the published literature whether the concentrations of TNFsRp55 released into the plasma are sufficient to alter the biologic activity of the TNF circulating in various inflammatory diseases. Given the fact that the TNFsRp55 levels measured in patients receiving IFNα were approximately twofold below the peak levels achieved in endotoxin-treated normal volunteers, one assumes that the TNFsRp55 circulating in IFNα-treated patients might be biologically significant. As IFNα induces both circulating IL-1 and TNF antagonists and the proinflammatory cytokines IL-1 and TNF may act as growth factors for malignant cells and several viruses, part of the clinical success of IFNα may not be only its antiinflammatory effects but also its interference with growth-promoting cytokines.

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| Table 1. Suppression of IL-1α-induced TNFα Synthesis by IFNα |
|-----------------|-----------------|-----------------|
| IL-1α (100 ng/mL) | IFNα (U/mL) | TNFα (ng/mL) |
| +                | -              | 3.2 ± 0.3      |
| +                | 1              | 3.3 ± 0.4      |
| +                | 10             | 2.5 ± 0.2      |
| +                | 100            | 1.4 ± 0.2*     |
| +                | 1000           | 1.5 ± 0.3*     |

PBMC were incubated for 24 hours and data show mean ± SE of PBMC from six donors. The total amount of TNFα was measured after PBMC cultures were frozen and thawed three times.

* P < .01.


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