Interleukin-6 (IL-6) as an Anti-inflammatory Cytokine: Induction of Circulating IL-1 Receptor Antagonist and Soluble Tumor Necrosis Factor Receptor p55

By Herbert Tilg, Elizabeth Trehu, Michael B. Atkins, Charles A. Dinarello, and James W. Mier

The aim of this study was to investigate whether interleukin (IL)-6 induces the production of IL-1 and tumor necrosis factor (TNF) antagonists. Serial plasma samples were obtained from cancer patients participating in phase I and II trials of recombinant IL-6 administered as a 120-hour continuous intravenous (IV) infusion. Plasma IL-1 receptor antagonist (IL-1Ra) and soluble TNF receptor p55 (TNFsRp55) levels were measured by specific radioimmunoassays (RIAs). IL-1Ra levels increased rapidly, reaching peak values (9.6 ± 1.7 ng/mL) within 2 to 4 hours of beginning treatment. Thereafter, levels promptly declined, reaching near baseline within 24 hours despite continuation of IL-6. TNFsRp55 plasma levels increased within 4 to 8 hours after initiating treatment and increased progressively throughout the duration of therapy. IL-1β and TNF-α plasma levels were below the detection limit in all samples tested. Peripheral blood mononuclear cells (PBMC) exposed to IL-6 produced only small amounts (1.56 ± 0.3 ng/mL) of IL-1Ra, even in the presence of exogenous soluble IL-6 receptor (gp80). TNFsRp55 levels were measured in the supernatants of IL-6–stimulated PBMC were below the detection limit of the assay. Macrophages generated by culturing monocytes in granulocyte-macrophage colony-stimulating factor (GM-CSF) were much more responsive to IL-6 than freshly isolated unfractionated or adherent PBMC and synthesized almost as much IL-1Ra when stimulated with IL-6 as with endotoxin. These results suggest that the antiinflammatory properties of IL-6 may be due, in part, to the induction of IL-1Ra synthesis and the release of soluble TNF receptors. Our findings also suggest that tissue macrophages may be an important source of IL-6–induced IL-1Ra.

© 1994 by The American Society of Hematology.

INTERLEUKIN-6 (IL-6) is a pleiotropic cytokine involved in the regulation of immune responses, the acute-phase reaction, and hematopoiesis.1 This cytokine is produced by a variety of cells after stimulation, such as occurs with infection, trauma, or immunological challenge. IL-6 has a protective role in the lipopolysaccharide (LPS)-galactosamine septic shock model in mice.3 It also suppresses the acute neutrophil exudation caused by an intratracheal instillation of endotoxin in rats.3 Tumor necrosis factor-α (TNF-α) levels measured in the bronchoalveolar fluid of these animals were also significantly reduced. Earlier studies showed that IL-6 inhibits LPS-induced TNF-α and IL-1β production in cultured human monocytes, U937 cells, and in mice in vivo.4,5 Together, these data suggest that IL-6 possesses antiinflammatory properties.

IL-1 receptor antagonist (IL-1Ra) is a member of the IL-1 family that antagonizes the effects of both IL-1α and IL-1β by blocking the binding of IL-1 to cell surface receptors.6,7 IL-1Ra has protective effects against several IL-1–mediated pathological processes, such as septic shock, inflammatory bowel disease, and others.5,7 Several cytokines have been demonstrated to induce IL-1Ra synthesis, including IL-1, IL-2, IL-4, interferon α, interferon γ, granulocyte-macrophage colony stimulating factor (GM-CSF), and transforming growth factor-β (TGF-β).8,13

Naturally occurring TNF antagonists have been identified in the urine of patients with various febrile conditions.11,13 These inhibitors were subsequently shown to represent the extracellular part of the two known TNF receptors (p55 and p75) and have therefore been designated soluble TNF receptors p55 and p75 (TNFsRp55 and TNFsRp75).14-16 Soluble forms of both TNF receptors block LPS-mediated lethality in animal models, particularly when administered as IgG fusion proteins.20,21

The present study was undertaken to determine whether IL-6 immunotherapy in humans is associated with circulating IL-1 and TNF antagonists and to determine the likely cells of origin of these mediators.

MATERIALS AND METHODS

Clinical materials. Patients who participated in this study were enrolled in phase I and II trials performed by the Cytokine Working Group. These clinical trials were approved by the Human Investigation Review Committee at the New England Medical Center, and all study participants gave written, informed consent. Patients received 30 µg/kg/24 hours of IL-6 (Sandoz, East Hanover, NJ) intravenously (IV) for 5 consecutive days as a 120-hour continuous infusion. Blood samples for cytokine assays were collected in EDTA-containing tubes immediately before and 1, 2, 4, 8, 24, 48, 72, 96, and 120 hours after therapy start. Samples were centrifuged within 20 minutes of venipuncture at 2,000g for 10 minutes and the plasma stored at –70°C.

Reagents. The IL-6 used in both in vivo and in vitro studies was recombinant, derived from Escherichia coli, and provided by the Cytokine Development Unit, Sandoz Inc. Soluble IL-6 receptor (sIL-6R) was obtained from R & D Systems (Minneapolis, MN) and as a gift from D. Novick, Weizmann Institute of Science, Rehovot.

From the Divisions of Hematology-Oncology, and Geographic Medicine and Infectious Disease, Department of Medicine, New England Medical Center and Tufts University School of Medicine, Boston, MA.

Submitted June 15, 1993; accepted September 1, 1993.

Supported by National Institutes of Health Grants No. CA 43950, UO1 CA 07190, AI 15614, and C5140-CA 07190-28, and by American Cancer Society Grant No. IM 643. H.T. is a recipient of a fellowship from the Max Kade Foundation. J.W.M. is a Burroughs Wellcome Scholar in Experimental Therapeutics. The Tufts/New England Medical Center Clinical Study Unit is supported by General Clinical Research Center Grant No. RR-00088.

Address reprint requests to James W. Mier, MD, Division of Hematology-Oncology, Department of Medicine, New England Medical Center, 750 Washington St, Box 245, Boston, MA 02111.

The publication costs of this article were defrayed in part by charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology.

0006-4971/94/8301-0027$3.00/0
were quantitated with specific radioimmunoassays (RIAs) as previously described. The RIA for IL-1Ra is unaffected by the presence of IL-1β and the IL-1β assay is not influenced by IL-1Ra. Like-
wise, the RIA for TNF-α is not affected by TNFsRp55 and the RIA for TNFsRp55 is unaffected by TNF-α. A glycosylated form of TNFsRp55 expressed in Chinese hamster ovary cells was used to
immunize New Zealand white rabbits in the generation of the anti-
TNFsRp55 antibody used in the RIA. This glycosylated form of
TNFsRp55 was also used to generate the standard curve for the
RIA. The limit of detection was 80 to 160 pg/mL for all RIAs used.

**Statistics.** All results are shown as the mean ± SEM. Student’s
i-test was used to determine statistical significance.

**RESULTS**

**Plasma IL-1Ra and TNFsRp55 levels during IL-6 immunotherapy.** Six patients with advanced malignancy were
studied. Before therapy, plasma IL-1Ra levels in the patients
were slightly, but not significantly, elevated compared with
those of healthy controls (3.2 ± 0.07 v 0.29 ± 0.08 ng/mL,
n = 10). Levels increased rapidly, reaching a peak of 9.6 ± 1.7 ng/mL within 2 to 4 hours of initiating the IL-6 infusion
(Fig 1). Thereafter, levels gradually declined to nearly baseline
values within 24 hours (0.67 ± 0.14 ng/mL). Levels remained
slightly elevated compared with pretreatment val-
ues until the end of therapy.

Pretreatment TNFsRp55 levels were significantly higher
in patients than in healthy controls (3.2 ± 0.4 v 1.4 ± 0.2 ng/
/mL, n = 10; P < .05). Levels increased within 4 hours of
beginning treatment and thereafter increased continuously
until the end of the 120-hour infusion. Peak levels (12.2 ± 1.5 ng/mL) were observed on day 5 (Fig 2). IL-1β and TNF-
α levels were assayed at the same time points as the IL-1Ra
and TNFsRp55 measurements and were consistently below
the detection limit of the assays (data not shown).

**IL-6 induction of IL-1Ra synthesis and TNFsRp55 release by PBMC.** PBMC were incubated for 24 hours with increasing concentrations of IL-6. TNFsRp55 levels measured

---

**Graphs:**

**Fig 1.** In vivo production of IL-1Ra in patients treated with a
120-hour continuous infusion of IL-6. Data represent the mean ±
SEM from six patients. EDTA-containing specimens were obtained
before, and 1, 2, 4, 8, 24, 48, 72, 96, and 120 hours after therapy
start. *P < .05 compared with time = 0; **P < .005 compared
with time = 0.***

**Fig 2.** Circulating TNFsRp55 levels in patients treated with a
continuous IL-6 infusion. Plasma samples used in these
measurements were identical to those in Fig 1. *P < .05 compared
with time = 0; **P < .005 compared with time = 0.
with low LPS concentrations was not statistically significant. On the other hand, neither did IL-6 suppress LPS-induced IL-1Ra production as previously demonstrated for LPS-induced IL-1 and TNF synthesis.42

The weak response of PBMC to IL-6 could have been due to a lack of IL-6 receptors (gp80). In circumstances in which a low level of gp80 expression limits IL-6 signaling, exogenous sIL-6R has been shown to enhance the response to this cytokine. For example, sIL-6R augments IL-6-induced acute-phase protein synthesis by hepatocytes.27 However, this truncated receptor had no effect on IL-6–induced IL-1Ra synthesis by PBMC at sIL-6R concentrations of up to 500 ng/mL (data not shown), suggesting that gp80 expression was not a limiting factor in IL-6–induced IL-1Ra synthesis. The poor response to IL-6 was also not due to a dependency on glucocorticoids. Whereas IL-6–induced acute-phase protein synthesis by hepatocytes is steroid-dependent,28 glucocorticoids (10−7 mol/L dexamethasone) actually suppress IL-6–induced IL-1Ra production by PBMC (data not shown).

PBMC from four of six patients treated with a 5-day continuous IL-6 infusion were isolated before therapy and on day 3. Unstimulated PBMC obtained before treatment from these patients produced significantly more IL-1Ra (2.95 ± 0.97 ng/mL) than did PBMC from healthy controls (0.5 ± 0.1 ng/mL, n = 8; P < .01). Despite increased constitutive production, PBMC from the cancer patients were refractory to stimulation with IL-6 alone or in combination with sIL-6R (Fig 4). The response to LPS was comparable to that of PBMC from healthy donors. Constitutive production of IL-1Ra in vitro by PBMC obtained 48 hours into the treatment

in the supernatants were below the detection limit of the assay (data not shown). IL-6 was a weak inducer of IL-1Ra synthesis, yielding levels rarely in excess of 1.5 ng/mL, even with high IL-6 concentrations (100 ng/mL) (Fig 3A). In the same experiments, LPS (10 ng/mL) induced 8.3 ± 1.3 ng/mL of IL-1Ra. IL-6 (10 ng/mL) did not enhance the response to LPS at LPS concentrations of 0.1 ng/mL or greater (Fig 3B). Even the modest enhancement apparent
course was even greater than that of pretreatment PBMC. As with the pretreatment cells, those obtained on day 3 were unresponsive to stimulation with IL-6 alone or in conjunction with sIL-6R (Fig 4).

**IL-6-induced IL-1Ra synthesis by GM-CSF–primed macrophages.** We studied the possibility that macrophages, rather than monocytes, could synthesize IL-1Ra in response to IL-6. Macrophages (5 x 10⁶ cells) were obtained by culturing monocytes from healthy donors in GM-CSF–containing medium for 6 days. After three washes, cells were incubated for 24 hours in medium alone or medium containing IL-6, sIL-6R, IL-6 plus sIL-6R, or LPS. As shown in Fig 5, macrophages synthesized more IL-1Ra than did equal numbers of freshly isolated adherent PBMC from the same donors (P < .001). Furthermore, these cells were highly responsive to IL-6, generating as much IL-1Ra in response to this cytokine (46.2 ± 4.3 ng/mL) as they did to LPS (50.3 ± 3.8 ng/mL). As with PBMC, the addition of sIL-6R to the cultures did not further enhance IL-6–induced IL-1Ra production.

**DISCUSSION**

Cytokines such as IL-4, IL-10, and tumor growth factor-$\beta$ (TGF-$\beta$) exert their antiinflammatory effects, in part, by suppressing IL-1 and TNF synthesis. IL-4 and TGF-$\beta$ are also potent inducers of IL-1Ra, which may contribute to their antiinflammatory actions by neutralizing the biological effects of IL-1. Our results indicate that IL-6 belongs to this category of antiinflammatory cytokines, which both suppress IL-1 and TNF synthesis and induce the production or release of IL-1 and TNF antagonists.

Measurements of IL-1Ra levels during an IL-6 infusion showed an unexpected pattern. Levels promptly increased and then rapidly declined to nearly baseline levels, despite continuing the IL-6 infusion. In contrast, plasma IL-1Ra levels in patients receiving repeated bolus injections of IL-2 remain persistently elevated throughout the treatment course. The reason for the rapid decline in IL-1Ra levels despite continuous IL-6 administration is not clear. The administration of IL-6 results in the release of glucocorticoids. The release of endogenous hydrocortisone has been shown to limit the levels of circulating TNF and IL-6 resulting from an injection of LPS, and it is likely that a similar process is responsible for the kinetics of IL-1Ra release induced by IL-6. The contention that endogenous steroids may be involved in this short-lived increase in IL-1Ra levels is further supported by our finding that IL-6–induced IL-1Ra synthesis in PBMC is completely abolished by dexamethasone. An alternative explanation for the rapid decline in IL-1Ra levels could be the downregulation of the IL-6–binding subunit (gp80) of IL-6R, which has been demonstrated to occur in hepatoma cells in response to IL-6. However, low levels of membrane gp80 may not appreciably compromise IL-6 responses in vivo, as sIL-6R present in plasma may be able to substitute for the membrane-bound receptor in cells in which gp80 expression is limited. This is in fact the case with hepatocytes in which exogenous sIL-6R has been shown to augment IL-6–induced acute-phase protein synthesis.

The constitutive production of IL-1Ra by PBMC isolated on day 3 of treatment was much greater than that of pretreatment PBMC. We have recently shown that acute-phase proteins such as C-reactive protein are potent inducers of IL-1Ra synthesis in vitro. These are present in high concentrations in the plasma during IL-6 treatment and could be responsible for the enhanced constitutive production of IL-1Ra by PBMC isolated during treatment. The lack of correlation between the increased constitutive production in vitro of IL-1Ra and the low plasma levels is most likely due to high plasma hydrocortisone levels.

Although transient, the peak plasma levels of IL-1Ra achieved with an IL-6 infusion are similar to those observed after IL-2 or LPS administration. However, IL-2 or LPS induces five times more IL-1Ra than does IL-6 in vitro. The basis for this weak in vitro response to IL-6 is unknown. Human monocytes express high numbers of IL-6R (gp80). Furthermore, IL-6 suppresses LPS-induced IL-1 and TNF synthesis in human PBMC. Together, these data suggest that the weak induction of IL-1Ra by PBMC is not likely due to a defect in IL-6 signaling.

Our data suggest that PBMC are most likely not the primary source of the circulating IL-1Ra in patients undergoing IL-6 treatment. Macrophages produce large amounts of IL-1Ra, especially when they are GM-CSF–primed. High levels of constitutive IL-1Ra production have also been demonstrated in alveolar macrophages, and gut macrophages from healthy people. Our data showing that macrophages generated from GM-CSF–primed monocytes synthesize large amounts of IL-1Ra after stimulation with IL-6 or LPS further support the view that tissue macrophages are...
an important source of this cytokine antagonist. In our studies, macrophages produced five to 10 times more IL-1Ra than did fresh monocytes, and were highly sensitive to IL-6, indicating that IL-1Ra production changes during macrophage differentiation both with respect to the quantities synthesized and to the range of inducing agents to which the cells are responsive.

IL-6 is known to enhance the expression of TNF receptors on hepatoma cells and hepatocytes. We now show that IL-6 immunotherapy is associated with the shedding of TNFsR55 into the circulation. However, IL-6 failed to induce the release of TNFsR55 from PBMC in vitro. This lack of in vitro activity has been observed with both PBMC and neutrophils exposed to LPS, IL-1, TNF, and other cytokines that induce high levels of TNFsR55 when administered in vivo. The basis for this discrepancy is so far unexplained.

IL-6 has been shown to suppress inflammation in several animal models. These effects have been attributed to the inhibition of IL-1 and TNF production. Our results suggest that the induction of IL-1Ra synthesis by IL-6, as well as the release of soluble TNF receptors into the circulation, may be equally important factors in the modulation of inflammatory responses.

ACKNOWLEDGMENT

We thank the Clinical Study Unit nurses, Hematology-Oncology fellows, and Department of Medicine housestaff for the excellent care provided to the patients participating in this study. We gratefully acknowledge D. Novick from the Weizmann Institute of Science, Rehovot, Israel for providing sIL-6R.

REFERENCES

26. Granowitz EV, Wang MZ, Dinarello CA, Skolnik PR: Solu-
tumor necrosis factor receptor p55 inhibits constitutive and phorbol myristate acetate-induced human immunodeficiency virus type I expression in U1 cells. (submitted)
Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55

H Tilg, E Trehu, MB Atkins, CA Dinarello and JW Mier