Biological Properties of Recombinant Human Monocyte-derived Interleukin 1 Receptor Antagonist

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Abstract

Human monocytes cultured on adherent IgG produce a specific IL-1 inhibitor that functions as a receptor antagonist (IL-1ra). This molecule has been purified, sequenced, cloned as a cDNA, and expressed in *Escherichia coli*. Recombinant IL-1ra has 17,000 mol wt and binds to IL-1 receptors on T lymphocytes, synovial cells, and chondrocytes with an affinity nearly equal to that of IL-1. These studies have examined some biological properties of purified recombinant human IL-1ra. This protein exhibits a dose-responsive inhibition of IL-1α and IL-1β augmentation of PHA-induced murine thymocyte proliferation. The recombinant IL-1ra also blocks IL-1α and IL-1β stimulation of PGE_2 production in human synovial cells and rabbit articular chondrocytes, and of collagenase production by the synovial cells. A 50% inhibition of these IL-1-induced biological responses requires amounts of IL-1ra up to 100-fold in excess of the amounts of IL-1α or IL-1β present. IL-1ra may play an important role in normal physiology or in pathophysiological states by functioning as a natural IL-1 receptor antagonist in the cell microenvironment. (*J. Clin. Invest.* 1990. 85:1694–1697.) interleukin 1 • interleukin 1 receptor • monocytes

Introduction

IL-1 has numerous effects on both immune and inflammatory cells, including augmentation of T and B lymphocyte function (1). In addition, IL-1 may be a mediator of tissue destruction in chronic autoimmune or inflammatory diseases. These diseases include juvenile-onset diabetes mellitus, where IL-1 may directly or indirectly induce toxicity to β cells in the islets of Langerhans (2). In rheumatoid arthritis IL-1 may contribute to joint damage through stimulating PGE_2 and collagenase production in synovial fibroblasts and chondrocytes (3).

Because of the pleiotropic effects of IL-1 on many different target cells, and purported roles in normal physiology and in pathophysiology, it has been hypothesized that natural inhibitors of IL-1 might exist (4). IL-1 inhibitory activities have been described in human body fluids and in the supernatants of cultured human or animal cells or cell lines (reviewed in Arend et al. [5]). In most cases, these biological activities have not been further characterized and their mechanisms of action have not been determined. However, IL-1 inhibitors could be acting at multiple levels; these include decreasing synthesis and release of IL-1, adhering to IL-1 in solution, blocking IL-1 receptor binding, or interfering with IL-1–induced signal transduction at a post-receptor level. For example, the ubiquitous urinary protein uromodulin binds to IL-1 in solution (6) and deoxyribonuclease I from human urine interferes with detection of IL-1 in the thymocyte assay, possibly by releasing free thymidine (7).

An IL-1 inhibitory activity has been described in the supernatants of human monocytes cultured on adherent IgG or immune complexes (5, 8) and in the urine of patients with fever or myelomonocytic leukemia (9–11). This specific IL-1 inhibitor of 22,000 mol wt blocks the binding of IL-1α or IL-1β to IL-1 receptors on the murine thymoma cell line EL4-6.1 (5, 11). In recent studies this monocyte-derived molecule has been purified and partially sequenced (12). This native molecule represents a unique new protein that exists in two forms, a 22,000-mol wt glycosylated form with some microheterogeneity, and an nonglycosylated 17,000-mol wt form. A complementary DNA has been cloned and expressed in *Escherichia coli* with production of recombinant 17,000-mol wt molecules (13). Similar to the crude or semipurified IL-1 inhibitor, the recombinant molecule blocks IL-1 binding to the IL-1 receptor on EL4-6.1 cells (5, 11, 12). Furthermore, this molecule has no detectable IL-1 agonist effects on human foreskin fibroblasts and therefore functions as an IL-1 receptor antagonist (IL-1ra)[1] (5, 10, 12). The results of studies on biological properties of purified recombinant 17,000-mol wt IL-1ra are reported in this paper.

Methods

Recombinant IL-1ra. Expression of IL-1ra in *Escherichia coli* was carried out as recently described (13). The recombinant IL-1ra of 17,000 mol wt was purified from the bacterial lysates by successive cation and anion-exchange column chromatography. The IL-1ra was > 95% pure as determined by silver staining after SDS-PAGE. The IL-1ra protein concentration was determined by OD_{280} using a calculated extinction coefficient of 0.776 $(mg/ml)^{-1}$. The recombinant IL-1ra was stored at −70°C in small aliquots in PBS with 10% glycerol.

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1. *Abbreviation used in this paper:* IL-1ra, interleukin 1 receptor antagonist.

A fresh aliquot was thawed for each experiment. Stored in this fashion for up to 6 mo, the recombinant IL-1ra demonstrated a full maintenance of biological activities.

Effects of IL-1ra on thymocyte proliferation. The recombinant IL-1ra was assayed against IL-1α or -β augmentation of PHA-induced proliferation of thymocytes from C3H/HeJ mice, as recently described (5). Recombinant human IL-1α (compliments of Dr. Peter T. Lomedico, Hoffmann-La Roche, Inc., Nutley, NJ) and recombinant human IL-1β (compliments of Dr. John Childs, Synergen, Inc., Boulder, CO) were used at baseline concentrations of 56 pg/ml IL-1α and 207 pg/ml IL-1β, respectively. These concentrations were functionally equivalent to 3 U/ml of both types of IL-1, reflecting the fact that the recombinant IL-1α possessed a higher specific activity than did the IL-1β. A unit of IL-1 activity was defined as that amount giving 50% maximal augmentation in the murine thymocyte assay. A concentration of 3 U/ml of IL-1α or IL-1β yielded $\approx$ 80% of maximal stimulation of thymocytes. Serial dilutions of the recombinant IL-1ra were mixed with the single amount of IL-1α or IL-1β before adding to the thymocytes in 96-well plates. The cells were cultured in RPMI-1640 medium (Gibco Laboratories, Grand Island, NY) with 10% low-endotoxin FCS (HyClone Laboratories, Logan, UT) for 4 d at 37°C and 5% CO_2. Thymocyte proliferation was measured by the colorimetric method with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), as described (5, 14). Recombinant IL-1ra also was tested against IL-2 (Amgen Biologicals, Thousand Oaks, CA) effects in the murine thymocyte assay.

Effects of IL-1ra on IL-1 stimulation of synovial cells and chondrocytes. The recombinant IL-1ra also was assayed for inhibitory activity against IL-1 induction of biological responses in synovial cells and chondrocytes. Human synovial cells (15) and rabbit articular chondrocytes (16) were obtained and cultured adherent to plastic as recently described (5). Chondrocytes were used either in the original culture or first passage to avoid problems with dedifferentiation. Rheumatoid synovial cells were used at confluency in the first through third passage. Again, 3 U/ml of human IL-1α or IL-1β were mixed with increasing amounts of IL-1ra and added to the cells in DME with 5% NuSerum (Collaborative Research, Inc., Waltham, MA). These amounts of IL-1α and IL-1β yielded submaximal stimulation of the synovial cells and chondrocytes. After 16 h of culture at 37°C and 5% CO_2 supernatants were harvested for determination of PGE_2 concentration by a specific ELISA (17). Initial experiments performed in the absence of any added serum indicated that PGE_2 levels in cell supernatants were low and highly variable. ELISAs also were used to measure protein concentrations of latent and active collagenase (18) and tissue inhibitor of metalloproteinases (19) in supernatants of cultured rheumatoid synovial cells.

Results

Inhibition of IL-1α and IL-1β augmentation of murine thymocyte proliferation. The recombinant IL-1ra first was assayed against 3 U/ml IL-1α or IL-1β in the murine thymocyte assay. Parallel dose-responsive inhibition curves were obtained with IL-1ra and either form of IL-1 (Fig. 1). 50% inhibition of IL-1α and IL-1β effects was observed with $\approx$ 2 and 1 ng/ml IL-1ra, respectively. These amounts of IL-1ra are 40 times the concentration of IL-1α and 5 times the concentration of IL-1β used to stimulate the thymocytes. The effects of a submaximal amount of IL-2 (10 U/ml) in the thymocyte assay were not inhibited by IL-1ra in concentrations up to 100 ng/ml (data not shown). In addition, these amounts of IL-1ra alone exhibited no stimulatory or agonist effects on the murine thymocytes.

Inhibition of IL-1 stimulation of synovial cells and fibroblasts. The recombinant IL-1ra also was evaluated for effects on IL-1 stimulation of adherent human synovial cells and rabbit articular chondrocytes. These cells were stimulated with 3 U/ml of IL-1α or IL-1β. Examination of PGE_2 production revealed similar dose-responsive inhibition curves with serial concentrations of IL-1ra cultured with IL-1 and either synovial cells (Fig. 2) or chondrocytes (Fig. 3). In addition, the IL-1ra inhibited IL-1 stimulation of collagenase production by the human synovial cells in a similar fashion (Fig. 4). IL-1ra alone in concentrations up to 100 ng/ml exhibited no agonist effects on either synovial cells or chondrocytes. Unstimulated synovial cells produced large amounts of tissue inhibitor of metalloproteinases with no alterations observed with IL-1α or IL-1β or with IL-1ra (data not shown).

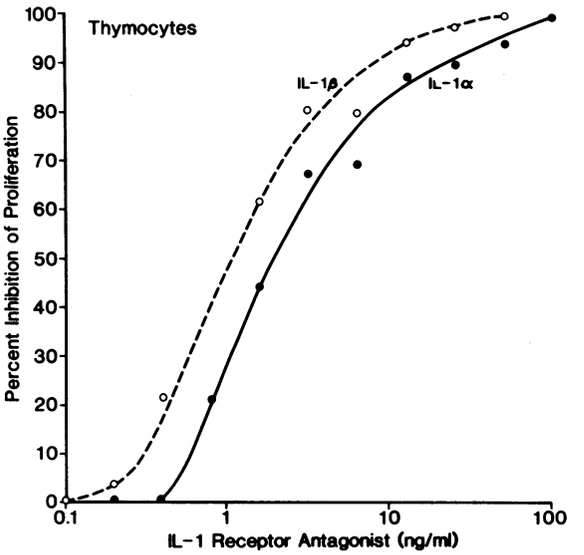

Figure 1. The effects of recombinant IL-1ra on IL-1α or IL-1β augmentation of PHA-induced murine thymocyte proliferation. 3 U/ml of IL-1α (●, 56 pg/ml) and of IL-1β (○, 207 pg/ml) were combined with serially increasing concentrations of IL-1ra, then cultured with thymocytes for 4 d. Thymocyte proliferation was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method. The data are expressed as percent inhibition of thymocyte proliferation, on a linear scale, vs. nanograms per milliliter IL-1 receptor antagonist on a log scale. The baseline OD (570 nm) for IL-1α and IL-1β stimulation were 0.538 and 0.562, respectively. Representative results are presented from one of five experiments.

Comparison of the relative potencies of IL-1ra against IL-1α or IL-1β stimulation of the two cell types revealed some interesting differences. 50% inhibition of IL-1α or IL-1β induction of PGE_2 production by the synovial cells was observed with $\approx$ 1.6 and 2.8 ng/ml IL-1ra, respectively (Table 1). These protein concentrations were $\approx$ 30- and 10-fold greater than the concentrations of IL-1α or IL-1β used to stimulate the cells. The rabbit chondrocytes required higher amounts of IL-1ra, 5.7 and 15.5 ng/ml, respectively, to yield 50% inhibition of IL-1α and IL-1β stimulation (Table I). These concentrations are up to 100-fold higher than the stimulating levels of IL-1.

Discussion

The experiments reported herein examine the biological activities of purified recombinant monocyte-derived IL-1ra, a receptor antagonist of IL-1. The results indicate that the purified recombinant IL-1ra possesses an identical spectrum of biological activities as does the unpurified IL-1ra in IgG-induced

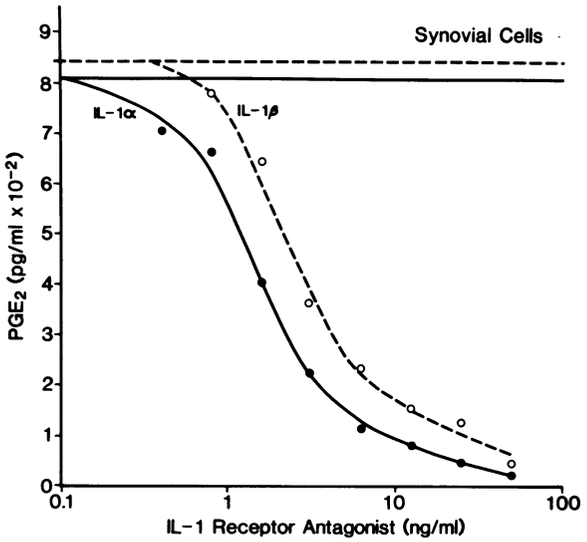

Figure 2. Inhibition of IL-1–induced PGE_2 production in cultured adherent human synovial cells by recombinant IL-1ra. Serially increasing amounts of recombinant IL-1ra and 3 U/ml of IL-1α (●), or β (○), were incubated with the cells for 16 h. PGE_2 concentrations in cell supernatants were determined by an ELISA. The data are expressed as picograms per milliliter PGE_2 produced vs. nanograms per milliliter IL-1 receptor antagonist present during culture. The horizontal lines represent the baseline levels of IL-1–induced PGE_2 production. Representative results are shown from one of three experiments.

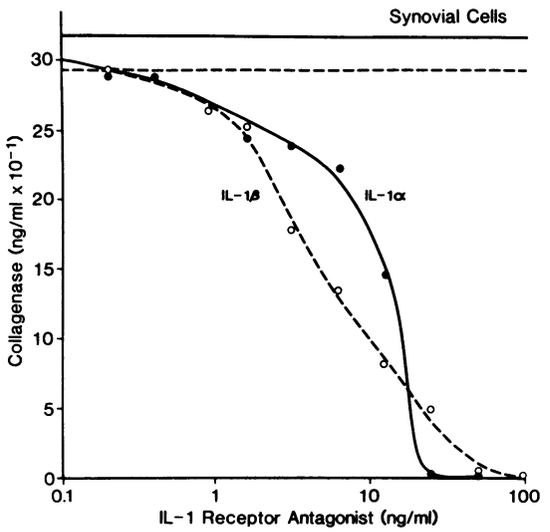

Figure 4. Inhibition of IL-1–induced collagenase production in cultured adherent human synovial cells by recombinant IL-1ra. Collagenase concentrations in cell supernatants were determined by an ELISA. The data are expressed as nanograms per milliliter collagenase produced vs. nanograms per milliliter IL-1 receptor antagonist present during culture.

monocyte supernatants (5). The recombinant IL-1ra exhibited a dose-responsive inhibition of IL-1 effects on murine thymocytes, human synovial cells, and rabbit articular chondrocytes. The inhibition of IL-2 augmentation of murine thymocyte proliferation observed in the previous studies probably was due to another protein than IL-1ra in the crude monocyte supernatant. This conclusion is supported by the observation that highly purified native IL-1ra, like the recombinant molecule, does not inhibit IL-2 effects on thymocytes (Arend, W. P., and C. H. Hannum, unpublished observations).

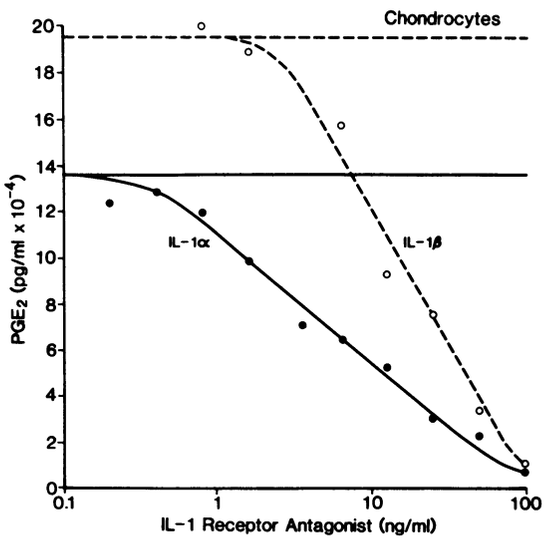

Figure 3. Inhibition of IL-1–stimulated PGE_2 production in cultured adherent rabbit articular chondrocytes by recombinant IL-1ra. The experiment was performed and the data are expressed as described in the legend for Fig. 2. Representative results are depicted from one of four experiments.

The majority of the native monocyte-derived IL-1ra is a 22,000-mol wt form that is glycosylated (12). Small amounts of the nonglycosylated form of the same unique molecule (17,000 mol wt) also are present in IgG-induced monocyte supernatants (12, 13). The nonglycosylated recombinant 17,000-mol wt IL-1ra possesses an identical specific activity as the 22,000-mol wt form of the purified native molecule in the murine thymocyte assay (Arend, W. P., and C. H. Hannum, unpublished observations). Therefore, the presence of carbohydrate is not necessary for maintenance of full biological activity.

An important observation is that 5- to 100-fold greater amounts of IL-1ra are necessary to observe 50% inhibition of IL-1α and IL-1β stimulation of murine thymocytes, human synovial cells, or rabbit articular chondrocytes. This result is even more striking when considering that the IL-1ra binds to IL-1 receptors on all three cell types with nearly the same

*Table I. Inhibition of IL-1-induced PGE_2 Production by IL-1ra**

Cell	Inducing protein	50% inhibition
		ng/ml IL-1ra‡
Human synovial cells	IL-1α	1.6
	IL-1β	2.8
Rabbit chondrocytes	IL-1α	5.7
	IL-1β	15.5

* Cultured human synovial cells or rabbit articular chondrocytes were incubated for 16 h with 3 U/ml IL-1 (56 pg/ml IL-1α and 207 pg/ml IL-1β) and serially increasing concentrations of recombinant IL-1ra. PGE_2 concentrations in cell supernatants were measured using an ELISA.

‡ The concentrations of IL-1ra giving 50% inhibition of IL-1-induced PGE_2 production were extrapolated from Figs. 2 and 3.

affinity as IL-1α or IL-1β (12).[2] An explanation for the apparent differences in IL-1ra potency between direct binding to the IL-1 receptor and in biological activities probably lies in the fact that all these cell types are exquisitely responsive to tiny amounts of IL-1. Maximal biological responses can be observed when < 5% of available receptors are occupied by IL-1. Thus, although IL-1ra and IL-1 bind to receptors in an equimolar fashion, much higher concentrations of IL-1ra are necessary to block IL-1 occupancy of only a few receptors.

IL-1ra alone exhibited no agonist effects on murine thymocytes, human synovial cells and rabbit articular chondrocytes. Furthermore, IL-1ra blocked IL-1–induced PGE_2 production by human foreskin fibroblasts (13) and adhesion molecule mRNA increases in human umbilical vein endothelial cells (20) without exhibiting any agonist effects on these cells. Although this molecule has been characterized as an IL-1 receptor antagonist, further studies are necessary to investigate whether IL-1ra has agonist effects on any target cells responsive to IL-1.

The biological relevance of IL-1ra to normal physiology of IL-1, or to the possible role of IL-1 in pathophysiology, remains to be established. IL-1ra binds to IL-1 receptors on T lymphocytes (5, 11, 12), synovial cells and chondrocytes,[2] but not to the second class of IL-1 receptors that is present on a murine pre-B lymphocyte cell line (12, 21, 22). As monocytes mature into macrophages, the cells produce less IL-1 and more IL-1ra (23, 24). IL-1ra production by human macrophages is further stimulated by culture in granulocyte/macrophage colony-stimulating factor (GM-CSF) (23, 24). IL-1ra may represent a natural receptor antagonist that limits the effects of IL-1 primarily in the microenvironment of tissue macrophages. IL-1ra may be of therapeutic value in human diseases possibly mediated by pathophysiological effects of IL-1.

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