Interleukin 1 Receptor Antagonist

A New Member of the Interleukin 1 Family

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Introduction

The precise roles of IL-1α and IL-1β in normal physiology in vivo or in pathophysiology of disease remain unclear. Because of the ubiquitous, and somewhat nonspecific, nature of production and effects of IL-1α and IL-1β in vitro, the importance of these molecules in vivo has been questioned. A new member of the IL-1 family has recently been described. IL-1 receptor antagonist (IL-1ra)[1] is structurally related to IL-1α and IL-1β, but binds to IL-1 receptors on various target cells without inducing any discernible biological responses. IL-1ra represents the first described naturally occurring cytokine or hormone-like molecule that functions as a specific receptor antagonist.

This article will review recent information about IL-1ra, including its discovery and biochemical characterization, production, in vitro properties, and in vivo effects. A further objective is to point out how knowledge about IL-1ra may not only provide answers about the relevance of IL-1α and IL-1β in vivo, but also may introduce new concepts about the function of these other members of the IL-1 family. The reader is referred to three recent more brief reviews on IL-1ra (1–3).

IL-1 inhibitors and discovery of IL-1ra

IL-1 inhibitory bioactivity has been described by many laboratories over the past 10–15 years, both in cultured cell supernatants and in human body fluids (reviewed in 4–6). Some of these "IL-1 inhibitors" may have represented proteins that bound to IL-1 in solution or other molecules that interfered nonspecifically with bioassays for IL-1. However, the nature and mechanisms of action of most of these IL-1 inhibitors remained undetermined.

The molecule now known as IL-1ra was first reported in 1985 as an IL-1 inhibitory bioactivity of 22–25 kD in the supernatants of human monocytes cultured on adherent IgG (7). Dayer and colleagues independently reported studies on an IL-1 inhibitor of similar size in the urine of patients with fever or myelomonocytic leukemia (8). Seckinger et al. (9) established that the 22-kD inhibitor semipurified from urine specifically blocked binding of ^{125}I-IL-1α to IL-1 receptors on the murine thymoma cell line EL4-6.1. Subsequent studies showed that the monocyte-derived molecule also functioned as a specific receptor antagonist of IL-1 (5). Additional effects of IL-1 that are blocked by native IL-1ra are summarized in Table I. In all of these studies, IL-1ra alone failed to exhibit any agonist properties.

Purification, sequencing, cloning, and expression of IL-1ra

Any uncertainties regarding the existence of a specific receptor antagonist of IL-1 were resolved by the purification of this molecule from IgG-induced monocyte supernatants by Hannum et al. (14). Using a combination of anion exchange, gel filtration, and reverse-phase HPLC, three species of native IL-1ra were identified. The monocyte-derived IL-1ra exhibited two species of $\approx$ 22 kD and a smaller species of $\approx$ 18 kD (14). The two larger forms appeared to be glycosylation derivatives of the 18-kD form, as evidenced by reduction in their sizes to 18 kD with *N*-glycanase digestion. All three forms of purified native IL-1ra possessed equivalent IL-1 receptor binding activity per weight, indicating that the N-linked sugar was not essential for this activity (14).

Eisenberg et al. cloned IL-1ra cDNA's from a human monocyte library (15). Characteristics of a 1.8-kb IL-1ra cDNA and of the predicted protein are summarized in Table II. This IL-1ra cDNA was expressed in *Escherichia coli* with production of an 18-kD molecule that blocked IL-1–induced PGE_2 production by fibroblasts (15). The recombinant human IL-1ra inhibited in a dose-responsive fashion ^{125}I-IL-1α binding to EL4-6.1 murine thymoma cells (14). Lastly, the recombinant IL-1ra failed to directly stimulate PGE_2 production by human dermal fibroblasts (14).

An identical IL-1 receptor antagonist protein (termed IRAP) was subsequently purified from the supernatants of the human myelomonocytic cell line U937 after phorbol myristate acetate (PMA) differentiation and stimulation with granulocyte macrophage colony-stimulating factor (GM-CSF). Cloning and expression indicated that recombinant IRAP inhibited IL-1–induced adhesiveness of endothelial cells for neutrophils in vitro, IL-1 augmentation of PHA-induced murine thymocyte proliferation, and IL-1 stimulation of corticosterone production in mice in vivo (18). An identical IL-1 receptor antagonist protein was purified from the supernatants of four other human myelomonocytic cell lines after PHA-differentiation and GM-CSF stimulation: THP-1 (19), H-161 (20), AML-193 (20), and HL-60 (20).

Thus, IL-1ra is produced by IgG-stimulated human monocytes and by a variety of human myelomonocytic cell lines

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1. *Abbreviations used in this paper:* AM, alveolar macrophages; GM-CSF, granulocyte macrophage colony-stimulating factor; IL-1ra, interleukin 1 receptor antagonist.

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Table I. Effects of IL-1 Which Are Blocked by Native IL-1 Receptor Antagonist

Binding of ^{125}I-IL-1α to IL-1 receptors on murine thymoma cells EL4-6.1 (5, 9)
Augmentation of PHA-induced murine thymocyte proliferation (5, 10)
PGE_2 production by human synovial cells and fibroblasts (5, 10)
Collagenase production from rabbit articular chondrocytes (5)
Stimulation of hyaluronic acid synthesis by human synovial cells (11)
Inhibition of glycosaminoglycan synthesis in human articular cartilage cultures (11)
Resorption of and PGE_2 production in rat or mouse bones in vitro (12)
Lowering of insulin content in and decrease of insulin release from cultured rat pancreatic islet cells (13)

under the influence of PMA and GM-CSF. What is the evidence that the IL-1 inhibitory activity in human urine represents the same protein? This molecule was purified from the urine of febrile patients, although no amino acid sequence analysis was reported (21). The urine-derived molecule demonstrated similar biological properties of an IL-1 receptor antagonist as did the native or recombinant monocyte molecule. Furthermore, the urine molecule appeared to exist in a larger glycosylated form (21). Lastly, antibodies specific for monocyte IL-1ra recognized the urine IL-1 inhibitor (12). Thus, although not proven definitively, the monocyte and urinary IL-1 inhibitors most likely represent the same protein. The evidence indicating that IL-1ra is a member of the IL-1 family is summarized in Table II.

IL-1ra structural variants

Recent studies using Western blot analysis of monocyte and macrophage lysates and supernatants have clarified some of the structural aspects of IL-1ra. All of the structural variants of extracellular monocyte-derived IL-1ra appear to represent varying degrees of glycosylation of a single peptide of 17,115 predicted mol wt. However, this molecule migrates anomalously by PAGE with apparent sizes for nonglycosylated IL-1ra of 20–22 kD. In addition, monocyte lysates also exhibited a major smaller band of 15–16 kD, representing either a synthetic form or a product of proteolysis (22). The heterogeneous 22–25 kD glycosylated forms predominated in monocyte supernatants over a small amount of nonglycosylated IL-1ra (22). Human macrophages exhibited the same size distributions of IL-1ra in supernatants and lysates as described for monocytes (23). IL-1ra is significantly conserved between species as the mouse and rat proteins are 77% and 75% identical, respectively, to the human protein (16).

Table II. Structural Characteristics of Human Monocyte IL-1 Receptor Antagonist

1.8-kb cDNA has a single open reading frame coding for 177 amino acids with a short 3′-untranslated region and a long 5′-untranslated region (15)
Mature protein is a single nonglycosylated peptide of 152 amino acids (mol wt 17,115) with a 25-amino acid leader sequence (15)
Amino acid sequence homology is 26–30% to IL-1β and 19% to IL-1α (15)
Similar hydrophilicity plot to IL-1β, indicating possible similarities in tertiary structure (15)
Gene structure similar to that of IL-1α and IL-1β (16)
Gene located on chromosome 2 in the mouse, same as IL-1α and IL-1β (17)
Produced by monocytes and macrophages with heterogeneous 22–25-kD glycosylated derivatives representing a major extracellular form (14)

A structural variant of monocyte IL-1ra is present in keratinocytes and other epithelial cells (22). This form of IL-1ra possessed the same 152-amino acid structure as mature monocyte IL-1ra but contained an additional seven residues at the NH_2 terminus. The first four amino acids in this extension of keratinocyte IL-1ra were identical to the first four residues in the leader sequence of the synthesized monocyte form; however, the remaining three amino acids were different. The keratinocyte IL-1ra is probably encoded by the same gene as monocyte IL-1ra, with an alternative first exon spliced into an internal acceptor site between bp 87 and 88 of the monocyte cDNA (22). Lacking a leader peptide, 18-kD keratinocyte IL-1ra remains almost completely intracellular and is not glycosylated (22, 24). A smaller structural variant of the keratinocyte IL-1ra also may exist (22, 24).

The monocyte IL-1ra has been termed sIL-1ra (secretory) and the keratinocyte variant icIL-1ra (intracellular) (22). However, the monocyte molecule, although possessing a leader peptide, is not completely secreted. Up to 50% of IL-1ra produced by monocytes remains cell-associated at any time during synthesis, while up to 80% of IL-1ra is found in the lysates of in vitro-derived macrophages (23) and 50% in alveolar macrophages (Janson, R. W., and W. P. Arend, unpublished observations). Whether the cell-associated IL-1ra in keratinocytes, monocytes, and macrophages is free in the cytoplasm or is membrane bound, in part, has not been determined.

Binding of IL-1ra to IL-1 receptors

IL-1ra appears to be a pure receptor antagonist, binding to IL-1 receptors but not activating target cells. Many laboratories have examined the biological properties of IL-1ra over the past year and no unequivocal agonist effects have yet been reported. How IL-1ra functions as a receptor antagonist is not known as it remains unclear how any of the three members of the IL-1 family bind to IL-1 receptors. Human IL-1ra binds to the type I IL-1 receptor on murine EL4-6.1 cells with an affinity of $\approx$ 150 pM, equal to the binding of human IL-1α and IL-1β (25). In addition, human IL-1ra binds to human synovial cells with a K_d of $\approx$ 200 pM, again identical to IL-1α (26). However, some evidence suggests that IL-1ra may not bind identically to type I IL-1 receptors in comparison to binding of IL-1α or IL-1β (26). Furthermore, IL-1ra does not induce receptor interiorization after binding to EL4-6.1 cells and fails to activate the protein kinase responsible for down-modulation of the epidermal growth factor receptor (25).

Studies on structural mutants of IL-1α, IL-1β, and IL-1ra indicate the relative nature of agonist or receptor antagonist properties. A conversion of Arg to Gly at residue 127 in the mature IL-1β molecule reduced binding affinity by only 25% while IL-1 bioactivity decreased 100-fold; thus, a relative IL-1 receptor antagonist was generated by this mutation (27). In a similar fashion, conversion of Asp to Tyr at residue 151 in human IL-1α led to no change in binding affinity but produced

a variable decrease in bioactivity, depending on the assay (28). Conversely, a point mutation of Lys to Asp at residue 145 in human IL-1ra produced a partial agonist for both T cells and fibroblasts (29). However, this mutant IL-1ra molecule was still 100- to 1,000-fold less active as an agonist in comparison to IL-1α or IL-1β. Thus, it is likely that multiple residues are involved in receptor binding of these three forms of IL-1, or in induction of biological responses by IL-1α or IL-1β. A single point mutation may influence these properties by removing one contact point or by changing the tertiary structures.

Two unrelated IL-1 receptors exist on human cells: type I, an 80-kD single chain protein on T cells, fibroblasts, endothelial cells, and other cells; and type II, a 60-kD single chain protein present on B cells, neutrophils, and macrophages. Human type I IL-1 receptors bind human IL-1α, IL-1β, and IL-1ra with equal affinity, while type II IL-1 receptors bind IL-1β with greater avidity than IL-1α or IL-1ra (S. K. Dower, personal communication). Human IL-1ra has been shown to bind to type II IL-1 receptors on human PMN and Raji human B lymphoma cells, although much less avidly than IL-1α or IL-1β (30). There are species differences, however, as human IL-1ra binds to murine type II IL-1 receptors very weakly, if at all (14, 31). These and other properties of recombinant IL-1ra are summarized in Table III.

IL-1ra production

The observation that the same population of human monocytes produces both the agonists IL-1α and IL-1β as well as IL-1ra raises the question of whether production of these apparently opposing molecules is differentially regulated. That a single monocyte can synthesize both IL-1 and IL-1ra simultaneously has not been proven, but would appear to be likely since myelomonocytic cell lines exhibit this capacity. During maturation of monocytes into macrophages IL-1β production is downregulated while IL-1ra production is greatly enhanced. Recent studies have clarified the mechanisms of regulation of production of IL-1ra by human monocytes. These studies have been aided by the development of a specific ELISA for IL-1ra, permitting quantification of this protein in fluids also containing IL-1α or IL-1β (32).

Stimulation of monocytes with LPS or culture on adherent IgG led to different patterns of IL-1β or IL-1ra production (33). LPS-stimulated monocytes produced near equal amounts of IL-1β and IL-1ra protein over 24 h in culture with parallel steady-state mRNA levels. In addition, transcriptional rates and mRNA stability were similar for both IL-1β and IL-1ra in LPS-induced monocytes. However, monocytes cultured on adherent IgG exhibited low levels of IL-1β transcription but failed to synthesize any detectable protein. In contrast, IgG-stimulated monocytes exhibited a high and prolonged rate of IL-1ra protein production due both to enhanced transcription and prolonged mRNA stability (33). These effects of IgG appeared to be Fc receptor-specific (34), although which Fc receptor is involved has not been determined. The results of additional studies further emphasize the different regulatory patterns of IL-1β and IL-1ra production by monocytes (35). The presence of 1% AB serum, soluble IgG, or GM-CSF all enhanced IL-1ra production but not that of IL-1β. In addition, nonadherent monocytes produced less IL-1ra but the same amounts of IL-1β. The mechanisms responsible for this different, and possibly differential, regulation of IL-1β and IL-1ra production by monocytes remain to be more completely determined.

The mechanisms of transcriptional regulation of IL-1ra production by monocytes are being approached in ongoing studies (36). 1,680 bp of 5′-flanking DNA for the IL-1ra gene have been isolated, mapped, and sequenced. The results of initial studies indicate that DNA elements responsible for both baseline and LPS-induced expression of IL-1ra are located in the most proximal 294 bp of the IL-1ra promoter (36). Additional studies are necessary to delineate the specific DNA sequences that both bind regulatory proteins and exhibit function. A comparison will be made with similar studies being performed on regulation of IL-1β transcription to explore whether a differential mechanism may exist.

What other stimuli induce IL-1ra production in monocytes and what other cells synthesize this protein has not yet been completely determined. As was observed with myelomonocytic cell lines, differentiation of human monocytes by PMA and/or GM-CSF enhanced IL-1ra production (37, 38). Adherent monocytes differentiated in vitro into macrophages produced large amounts of IL-1ra without any further stimulus (23). Furthermore, these in vitro-derived macrophages were no longer responsive to LPS or to adherent IgG (23). The results of recent studies indicate that IL-3 (39) and transforming growth factor β (TGFβ) (40) also induce IL-1ra production in freshly isolated human monocytes. However, the presence of TGFβ during monocyte differentiation into macrophages in vitro appears to downregulate IL-1ra production (Lotz, M., and W. P. Arend, unpublished observations). The effects of other cytokines on monocyte production of IL-1ra remain to be systematically examined.

In addition to in vitro-derived macrophages, alveolar macrophages (AM) (41) and synovial tissue macrophages (42) also produced IL-1ra. IL-1ra production by AM was modestly enhanced by GM-CSF but, unlike peripheral monocytes, these cells failed to respond to LPS or adherent IgG (Janson, R. W., and W. P. Arend, unpublished observations). Thus, during differentiation from monocytes into macrophages in vitro or in vivo, the ability of these cells to enhance IL-1ra production after stimulation with LPS or adherent IgG has been lost. The mechanisms responsible for this alteration in cell function have not been established. AM obtained from patients with interstitial lung disease produced more IL-1ra than AM from nonsmoking or smoking normal donors, suggesting that these cells may have been activated in vivo. Thus, IL-1ra is a major product of tissue macrophages, particularly in disease states. Lastly,

Table III. Some Properties of Recombinant IL-1 Receptor Antagonist

Binds to human types I and II IL-1 receptors (more avidly to type I) (30)
Binding affinity to type I IL-1 receptors equal to that of IL-1α and IL-1β (25, 26)
Fails to trigger receptor interiorization or to induce detectable biological responses in target cells (25)
50% inhibition of IL-1 biological effects in vitro requires 10- to 500-fold excess amounts of IL-1ra (43)
Does not alter in vitro T cell responses to mitogens, antigens, or allogeneic determinants (45)
Ameliorates various animal models of arthritis (49, 50), septic shock (55–57), and inflammatory bowel disease (59)

cultured human peripheral neutrophils also contained both mRNA and protein for IL-1ra at ≈ 5% of the amount per cell observed in monocytes (Malyak, M., and W. P. Arend, unpublished observations).

The production of IL-1α and the variant IL-1ra by keratinocytes also appear to be regulated differently (24). Keratinocytes produced ≈ 390 ng IL-1ra/mg total protein, nearly equivalent to IL-1ra production by monocytes (≈ 300 ng IL-1ra/mg total protein). During differentiation of keratinocytes by culture in increasing concentrations of Ca^{++}, IL-1ra production increased more than twofold while IL-1α and IL-1β production remained unchanged. During differentiation the ratio of IL-1ra to IL-1α in keratinocytes increased from 12 to over 25 (24). Lastly, IL-1ra extracted from keratinocytes exhibited identical biological activity per weight in comparison to recombinant monocyte IL-1ra.

In vitro properties of IL-1ra

The effects of unpurified or purified preparations of native IL-1ra on blocking of various IL-1–induced cell responses have been summarized in Table I. Additional information about IL-1ra functions in vitro have resulted from studies using the recombinant IL-1ra molecule. Most of the inhibitory effects observed with native IL-1ra have been confirmed with the recombinant molecule, indicating that possible contaminants in the native IL-1ra preparations were not responsible for these observations.

One universal theme that has emerged from recent studies is that excess amounts of IL-1ra over IL-1 must be present in various in vitro systems in order to yield 50% inhibition of IL-1–induced responses (43) (Table III). This observation probably reflects the fact that occupancy of a small number of IL-1 receptors on a target cell is sufficient to induce a complete biological response. Because of this exquisite sensitivity to tiny amounts of IL-1, excess amounts of the receptor antagonist must be present in order to flood the system. The ratio of IL-1ra to IL-1 necessary to block 50% of IL-1 responses in vitro varies between ≈ 10 in rheumatoid synovial cells to over 500 for IL-1–dependent cell lines such as D10.G4.1 and LBRM.33. This variation probably reflects both the relative affinities of these molecules for IL-1 receptors on each target cell as well as the relative abilities of the cells to mount a biological response.

Another important observation is that human IL-1ra appears not to affect human T cell responses in vitro. In contrast to the results of studies with murine Th2 clones (44), IL-1ra did not inhibit the proliferation of human peripheral blood T cells induced by mitogen, soluble antigens, or allogeneic determinants (45). This result suggests that IL-1 may not be a necessary accessory molecule for proliferation of human T cells in vitro. These in vitro experiments need to be confirmed by similar in vivo studies. If IL-1ra exhibits an absence of effects on human T and B cell responses in vivo, its usefulness as a therapeutic agent in human diseases may be enhanced.

In vivo effects of IL-1ra

As stated earlier in this review, one of the important unanswered questions about IL-1 relates to its role in normal physiology. Although IL-1 is produced by many cells in vitro, its true role in functioning of the intact organism remains unclear. A second unanswered question concerns the relevance of IL-1 to pathophysiology of various diseases. IL-1 is one of many cytokines possibly produced in excess in infectious, inflammatory, or autoimmune human diseases. Although IL-1 certainly has the potential to mediate events of tissue destruction, its primary role in this regard has not been established. Because of the redundant and overlapping nature of the cytokine network, inhibition of IL-1 effects in vivo may not be sufficient to alter disease processes. The availability of recombinant IL-1ra allows these important questions to be examined, first in animal models of disease and ultimately in human diseases.

The possible role of various cytokines and their inhibitors or antagonists in rheumatoid arthritis has recently been reviewed (46, 47). In recent in vitro studies, IL-1ra blocked IL-1–induced degradation of proteoglycans and inhibition of glycosaminoglycan synthesis in bovine nasal cartilage explants (48). In addition, rabbits given an intraarticular injection of IL-1β and then an intravenous injection of IL-1ra exhibited decreased leukocyte migration into the inflamed joint and reduced proteoglycan release from the articular cartilage (49). Lastly, IL-1ra blocked type II collagen-induced arthritis in mice but not antigen-induced arthritis (50). These results indicate that IL-1ra may modify some animal models of acute arthritis but not others, suggesting some variation in a possible primary role for IL-1 in these disease models.

Unfortunately, no animal model of arthritis exactly resembles RA, primarily in the waxing and waning nature and chronicity of the human disease. Therefore, there is no substitute for direct observations on the human disease. IL-1ra was found in the urine of children with juvenile RA, particularly during periods of fever (51). One-half or more of patients with active RA exhibited high synovial fluid levels of IL-1ra in the absence of detectable IL-1β (52). In addition, IL-1ra mRNA was present in RA tissues, both in synovial lining cells and in perivascular lymphoid aggregates, and IL-1ra protein was produced by cultured synovial tissue cells (42). The relevance of these observations to the pathophysiology of RA, or to the possible therapeutic application of IL-1ra in this disease, remains unclear. IL-1ra in rheumatoid synovial fluid may not influence events in the tissue at the pannus–cartilage interface.

Both tumor necrosis factor α (TNFα) and IL-1 have been incriminated as mediators of inflammation in septic shock. The results of recent studies on the effects of IL-1ra in animal models of this disease suggest that IL-1 may play a primary role. IL-1ra coinjected with LPS or IL-1 into the trachea of rats reduced the acute inflammatory response; moreover, LPS injected alone led to endogenous IL-1ra mRNA production by the lung (53). IL-1ra also blocked LPS-induced colony-stimulating factor production and early endotoxin tolerance in mice (54). Three recent studies showed ameliorative effects of IL-1ra in septic shock in rabbits (55, 56) or mice (57). Fig. 1 reproduces data from reference 55 indicating the marked improvement in mortality from endotoxin-induced shock in rabbits after administration of IL-1ra. What was not established by any of these studies was how long after induction of septic shock IL-1ra could be administered and beneficial effects still be seen. Endotoxin injection of normal volunteers led to the appearance of circulating IL-1 inhibitor bioactivity (58); so IL-1ra may normally be synthesized in response to infections.

IL-1ra may also have relevance to two other human diseases, inflammatory bowel disease and chronic myelogenous leukemia. IL-1ra reduces inflammation and tissue destruction in a rabbit model of colitis induced by the instillation of formalin and immune complexes into the colon (59). Whether administration of IL-1ra will alter the course of this chronic hu-

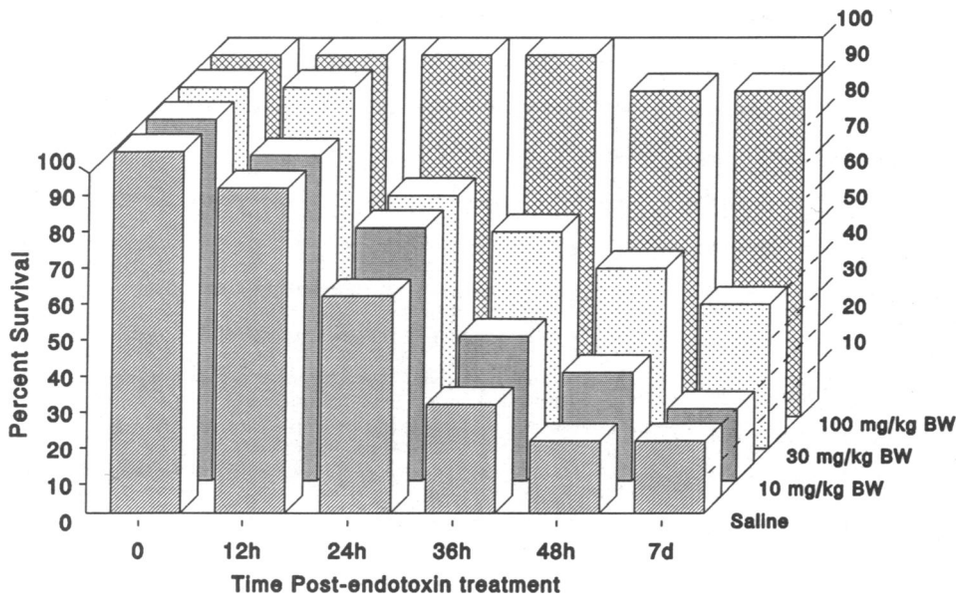

Figure 1. The effect of IL-1ra on the survival rate in endotoxin-induced shock in rabbits. IL-1ra was injected in equal doses just before the injection of endotoxin (*E. coli* 026,B26; Sigma Chemical Co., St. Louis, MO) and every 2 h thereafter for 24 h. The rabbits were not anesthetized but were under constant observation for 48 h and were then observed during the daytime for up to 7 d. The animals had free access to water and food. Injections and blood sampling were made through the ear veins. *BW,* body wt; *d,* days. (Reprinted by permission. *Nature* [*Lond.*]. 348:550–552. Copyright [C] 1991 Macmillan Magazines, Ltd.)

man inflammatory disease remains to be determined. Lastly, the growth of acute myelogenous leukemia cells in vitro may be enhanced by the autocrine and paracrine production of IL-1 and GM-CSF. In one preliminary study, IL-1ra inhibited by 30–85% both the spontaneous and IL-1–induced proliferation of leukemic blast cells from 12 patients (60). The effect of IL-1ra on growth of chronic myelogenous leukemia cells in vitro is currently being studied.

The in vivo studies summarized above all concern the possible therapeutic usefulness of IL-1ra in human diseases. A separate issue is what can IL-1ra clarify about the possible role of IL-1 in normal physiology? In addition, what is the role of IL-1ra in normal physiology? One example may come from a study of brain function. IL-1 is produced by normal brain cells in vivo and may regulate non–rapid-eye-movement sleep (NREMS) as well as body temperature. Administration of IL-1ra into the lateral ventricle of rabbit brains reduced spontaneous NREMS sleep activity as well as blocked IL-1-induced NREMS and fever (61). These fascinating observations suggest that the balance between IL-1 and IL-1ra may influence central nervous system events in vivo.

The skin represents another organ where some information exists on the presence of IL-1 and IL-1ra, although functional relevance remains unclear. Cultured keratinocytes synthesized large amounts of precursor 31-kD IL-1α and 18-kD IL-1ra, both of which remained intracellular (24). In recent studies IL-1ra was found in large amounts in extracts of normal skin, in $\approx$ 100-fold higher concentrations than IL-1α (62). The IL-1ra was concentrated in the stratum granulosum of normal skin, consistent with the presence of more differentiated cells. Interestingly, IL-1ra levels were slightly lower in skin lesions of psoriasis patients but IL-1α levels decreased more dramatically so that the ratio of IL-1ra to IL-1α in psoriatic skin exceeded 1,000 (62). This alteration in cytokine production in psoriatic skin may play a primary role in the abnormal differentiation of epithelial cells in this disease.

Summary

This review has summarized recent information derived from many laboratories on the discovery, characteristics, and properties of a new member of the IL-1 family, IL-1 receptor antagonist. In addition to information, an emphasis has been placed on unanswered questions and new concepts. The existence of this first-described naturally occurring specific cytokine receptor antagonist may lead to a different perspective on the cytokine network.

A major unanswered question emphasized throughout this review, that now can be addressed more directly, concerns what are the physiological roles of members of the IL-1 family. Although IL-1β is presumed to function primarily as an extracellular cytokine, this molecule lacks a leader peptide, is synthesized and handled by the cells in a manner suggestive of a cytoplasmic (not secretory) protein (63), and may only be released after cellular injury (64). Furthermore, although IL-1ra possesses a leader sequence, 50% or more of this protein remains cell associated. Do these observations suggest that members of the IL-1 family possess important intracellular functions, as yet undetermined?

IL-1α may play an intracellular role in regulating senescence; an IL-1α antisense oligodeoxynucleotide was shown to prolong the life span of cultured human endothelial cells (65). Whether intracellular IL-1ra plays a role in influencing life span has not been determined. The discovery of IL-1ra has led to a first level of assumptions that this molecule may be functioning in vivo to regulate the pleiotropic extracellular effects of IL-1 in physiological or pathophysiological processes. Although enticing, these assumptions have not yet been proven to be true. Perhaps we need to look beyond, or within, and consider that IL-1ra and other members of the IL-1 family may have additional roles in normal or abnormal cell growth and development.

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