Biologic Basis for Interleukin-1 in Disease

By Charles A. Dinarello

To understand the role of the proinflammatory cytokine interleukin-1 (IL-1) in disease, investigators have studied how production of the different members of the IL-1 family is controlled, the various biologic activities of IL-1, the distinct and various functions of the IL-1 receptor (IL-1R) family, and the complexity of intracellular signaling. Mice deficient in IL-1β, IL-1β converting enzyme, and IL-1R type I have also been studied. Humans have been injected with IL-1 (either IL-1α or IL-1β) for enhancing bone marrow recovery and for cancer treatment. The IL-1-specific receptor antagonist (IL-1Ra) has also been tested in clinical trials. The topics discussed in this review include production and activities of IL-1 and IL-1Ra molecules, the effects of IL-1 on gene expression, functions of cell-bound and soluble IL-1 receptors, the importance in cytokine biology, the intron-exon organization of the three IL-1 genes, the IL-1R accessory protein, IL-1 signal transduction, and IL-1R function as a natural "buffer." It is likely that the heterodimerization of the IL-1R1 and IL-1Ra results in high-affinity binding. It is also possible that the extracellular or "soluble" portions of the IL-1R (IL-1sR) and IL-1R (IL-1sR) circulate in health and disease functioning as natural "buffers." IL-1Ra has been shown to have a negative influence on both the production and activity of IL-1.

As with other cytokines, any importance in health and disease have been shown using gene deletion in mice. Gene expression and synthesis of IL-1α, IL-1β, or IL-1Ra has been shown in various tissues and cells as well as in the dividing embryo. In granulosa cells from in vitro peptide and is readily transported out of the cells and termed secreted IL-1Ra (sIL-1Ra).

There are two IL-1 receptors (IL-1R), the type I receptor (IL-1RI) transduces a signal, whereas the type II receptor (IL-1RII) binds IL-1 but does not transduce a signal. In fact, IL-1RII acts as a sink for IL-1β and has been termed a "decoy" receptor, which is somewhat unique to cytokine biology. When IL-1 binds to IL-1RI, a complex is formed that then binds to the IL-1R accessory protein (IL-1RαCp), resulting in high-affinity binding. It is likely that the heterodimerization of the cytokine domains of IL-1RI and IL-1RαCp triggers IL-1 signal transduction. The extracellular or "soluble" portions of the IL-1R (IL-1sR) and IL-1R (IL-1sR) circulate in health and disease functioning as natural "buffers" binding IL-1α, IL-1β, or IL-1Ra. In addition, several cytokines exert a negative influence on both the production and activity of IL-1.

From the Department of Medicine, Tufts University and New England Medical Center, Boston, MA.

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Address reprint requests to Charles A. Dinarello, MD, Division of Infectious Diseases, B158, University of Colorado Health Science Center, 4200 E Ninth Ave, Denver, CO 80262.

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fertilization, there are 2,000 IL-1 binding sites per cell. Although IL-1 is found in placental trophoblasts and appears to play a role in embryonic development, implantation, and birth, mice deficient in IL-1/β, IL-1/β converting enzyme (ICE), or IL-1R1 suggests that ovulation, fertilization, implantation, and parturition either do not require IL-1 receptor signaling or that compensatory cytokines are used by these mice.

**IL-1α**

**IL-1α promoters.** There are no TATA box motifs in the IL-1α gene promoter region. Inducible gene expression for IL-1α involves both a 4.2-kb upstream region and a proximal promoter region of 200 bp. The upstream sequences are sufficient for induction by phorbol esters and the downstream sequences can be deleted without affecting gene expression. A construct containing sequences 1437 to +19 does not allow for stimulation of specific expression but an additional 731-bp spanning exon I, intron I, and a segment of exon II controls a 20-fold increase in stimulation over background levels in murine macrophagic cells. Interestingly, using the same construct in human leukemic cells, only a twofold increase was observed. These additional 731 bp contain nuclear factor (NF) IL-6 and NFκB within intron I.

**Production of IL-1α.** Nearly all studies on the biologic activities of IL-1α have been performed using the recombinant form of IL-1α that is the mature, 17-kD carboxyl terminal peptide. Even under conditions of cell stimulation, human blood monocytes do not process nor readily secrete IL-1α. As shown in Fig 1, the 31-kD IL-1α precursor (proIL-1α) is synthesized in association with cytoskeletal structures (microtubules), which is unlike most proteins translated in the endoplasmic reticulum. ProIL-1α is fully active as a precursor and remains intracellularly (ProIL-1α) Fig 1). The opposite is the case with the IL-1β precursor (proIL-1β), which is not fully active, and a considerable amount is secreted after cleavage by a specific, intracellular protease (see below). When cells die, proIL-1α is released and can be cleaved by extracellular proteases. ProIL-1α can also be cleaved by activation of the calcium-dependent, membrane-associated cysteine proteases called calpains. In transformed cell lines constitutively synthesizing proIL-1α, the addition of a calcium ionophore stimulates calpain, which cleaves the precursor. Hence, release of the 17-kD IL-1α can take place in the absence of cell death.

Most experimental models of disease use the mouse or rat. Unlike human cells, mouse cells produce and release IL-1α. The relative contribution of IL-1α and IL-1β in these disease models can be resolved by comparing the responses of the IL-1β deficient to the IL-1RI knock-out mouse, assuming differences are due to the role of IL-1α. Specific neutralizing antibodies to either murine IL-1α or murine IL-1β have shown that some disease models are IL-1/β-dependent. IL-1β-deficient mice produce normal amounts of IL-1α, but IL-1β appears to be the critical cytokine for inducing the acute-phase response and fever to a local inflammatory event. IL-1/β knock-out mice do not develop collagen-induced arthritis (M. Tocci, personal communication, September 15, 1995), consistent with the report that anti–IL-1/β but not anti–IL-1/α reduces this disease.

**Intracellular IL-1α.** Because of the lack of a leader peptide, proIL-1α remains in the cytosol soon after translation and there is no appreciable accumulation of IL-1 in any specific organelle. Immunohistochemical studies of IL-1α in endotoxin-stimulated human blood monocytes show a diffuse staining pattern, but, by comparison in the same cell, IL-1Ra is localized to the Golgi. In experimental inflammatory bowel disease, there is a better correlation of disease severity with colonic tissue levels of IL-1α compared with those of IL-1β, presumably due to the cell-associated nature of IL-1α. IL-1α is not commonly found in the circulation or in body fluids except during severe disease, in which case the cytokine may be released from dying cells or by proteolysis after calpain-mediated cleavage.

**ProIL-1α as an autocrine growth factor.** Some investigators have considered that intracellular proIL-1α regulates normal cellular differentiation, particularly in epithelial and ectodermal cells. In the case of keratinocytes, constitutive production of large amounts of proIL-1α is found in healthy human skin. In support of the concept that proIL-1α functions as an intracellular messenger in certain cells, an antisense oligonucleotide to IL-1α reduces senescence in endothelial cells, a prostaglandin-dependent process. In fibroblasts, antisense IL-1α does not have this effect, raising the possibility that an autocrine effect of proIL-1α is cell specific. In the murine Th2 cell line, IL-1α was proposed as an essential autocrine and paracrine growth factor using an antisense IL-1α oligonucleotide and anti–IL-1α antibodies. Thymic epithelium produces IL-1α and a requirement for IL-1α has been shown in the expression of CD25 (IL-2 receptor chain) and maturation of thymocytes.

There is no dearth of reports that cell lines derived from different cell types spontaneously express IL-1α mRNA (reviewed in Lonnemann et al); however, spontaneous gene expression and synthesis of IL-1α may be due to contamination of tissue culture medium with endotoxins or due to stimulation by serum factors. This latter consideration is important because nearly all cultured cells require either fetal calf serum, defined animal serum substitutes, or human serum—each known to contain platelet-derived and other growth factors. An essential role for IL-1 in the growth of kidney-derived fibroblasts can be shown when the serum concentration is lowered from 5% to 1%; otherwise, cell growth at higher serum concentrations is unaffected by IL-1 receptor blockade. Hence, the concept of proIL-1α as an autocrine growth factor during in vitro culture conditions should be mindful of possible stimulating effects of serum-derived growth factors. Nevertheless, a “downstream” requirement for IL-1 as an essential growth factor in vivo is a therapeutically exploitable area, particularly when demonstrated in cells from diseased tissue.

**Nuclear localization of IL-1α.** The concept that IL-1α can be an autocrine growth factor takes into account three distinct mechanisms: first, that proIL-1α is synthesized and remains inside the cell, where it exerts a direct effect by binding to the nucleus; second, that intracellular proIL-1α complexes to an intracellular pool of IL-1RI before exerting
an effect as a ligand/receptor complex; and third, that either proIL-1α or mature IL-1α bound to surface IL-1RI is internalized with subsequent translocation to the nucleus (similar to steroid receptors). Each mechanism has supporting experimental data.

Initially, Mizel et al reported that radiolabeled 17-kD recombinant IL-1α bound to the cell surface receptors was rapidly internalized and, after 2 to 3 hours, was found to be associated with the nucleus. It was unclear whether the nuclear binding was composed of the IL-1α/IL-1R complex or just the ligand. Curtis et al reported that internalized IL-1α was still bound to its receptor and that internalized IL-1R correlated with increased signal transduction. Using truncated mutants of the cytoplasmic domain of IL-1RI, rapid internalization and nuclear localization of IL-1p was observed with several mutants not capable of transducing a biologic signal. It was later shown that the IL-1α/IL-1R complex but not 17-kD IL-1α bound to immobilized DNA and could be eluted under the same salt conditions as that of the estrogen receptor. The cytoplasmic domain of IL-1RI is highly conserved (see below for discussion of Toll protein) and contains a consensus sequence (residues 517-529) similar to those that transport viral proteins. If proIL-1α plays an essential role in keratinocyte cellular differentiation, it is certainly not in conjunction with the type I IL-1 receptor because mice deficient in this receptor appear to have a normal phenotype, including gross examination of skin and fur (M. Labow, personal communication, June 1995). The response of the IL-1RI-deficient mouse to IL-1 signaling is absent (M. Labow, D. Shuster, K. McIntyre, and R. Chizzonite, unpublished observations, June 1995) and it is anticipated that responses to external challenges will be similarly attenuated as were those in mice treated with neutralizing antibodies to the type I receptor.

Because proIL-1α, whether recombinant or naturally membrane-bound, binds to the extracellular IL-1RI indistinguishably from 17-kD IL-1α, proIL-1α could also be involved in nuclear localization. Using antibodies directed specifically to proIL-1α and transfection with plasmids containing the first 115 amino acids of proIL-1α (also called the IL-1α propiece), it appears that the propiece rather than the carboxyl terminal, mature segment of IL-1α localizes to the nucleus. This concept is supported by the observation that a specific peptide in the propiece of IL-1α binds to DNA. Phosphorylation and myristoylation of the IL-1α propiece may facilitate nuclear localization. Myristoylation takes place on lysine residues 82 and 83 of the IL-1α propiece, which is found in the nuclear localization sequence KVLKKRR. Transfected endothelial cells with a plasmid containing this sequence showed nuclear localization. Transfected cells with the propiece of IL-1α results in slower rate of proliferation, consistent with a role for IL-1α in early endothelial senescence. Transfection of an intracellular IL-1-producing plasmid increases IL-2 production in thymoma cells and this biologic effect is prevented by antisense IL-1, suggesting that IL-1α without its receptor is functional as an intracellular molecule.
**Membrane IL-1α.** ProIL-1α can be found on the surface of several cells, particularly on monocytes and B lymphocytes after stimulation in vitro. Approximately 10% to 15% of the IL-1α is myristoylated, and this form is thought to be transported to the cell surface, where it is called “membrane” IL-1. Myristoylation on specific lysines facilitates passage to the cell membrane. This “membrane” IL-1α is biologically active, its biologic activities are neutralized by anti–IL-1α and not anti–IL-1β antibodies, and it appears to be anchored via a lectin interaction involving mannose residues. Using high concentrations of IL-1Ra to prevent IL-1α binding to the cell surface IL-1R during fixation, the biologic activity of membrane IL-1α was unaffected. In contrast, a mannose-like receptor appears to bind membrane IL-1α. Although IL-1α has glycosylation sites, recombinant forms of mature IL-1α are biologically active when expressed in *Escherichia coli*, which lacks the ability to glycosylate proteins. Because membrane IL-1α is likely a glycosylated or myristoylated form of the cytokine, it accounts for no more than 5% of the total proIL-1α synthesized by the cell. There has been some dispute whether membrane IL-1α represents “leak” of intracellular IL-1, but with prolonged fixation, leakage does not account for the activity of membrane IL-1.

**Commentary on a possible role for intracellular IL-1α.**

Other cytokine growth factors that lack a leader sequence, eg, ciliary neurotropic factor (CNTF) or FGF, stimulate cells via their respective cell-surface receptors. IL-3, on the other hand, can be expressed as a mutant protein lacking the ability to be secreted, in which case intracellular IL-1α functions as an autocrine growth factor. In fact, the large amounts of proIL-1α in normal skin keratinocytes are thought to affect terminal differentiation. If there is a role for intracellular proIL-1α in normal cell function, this should be carefully regulated. The presence of large amounts of an intracellular form of the IL-1Ra (iIL-1Ra) produced in the same cells expressing proIL-1α is thought to compete with the intracellular pool of proIL-1α for nuclear binding sites. At present, there is no IL-1α receptor–deficient mouse to test this concept.

**IL-1β**

**Similarities and differences between IL-1α and IL-1β.** Although several similarities between IL-1α and IL-1β exist, in many respects IL-1β is a different molecule from IL-1α. The mature forms of the two agonists are comprised of similar three-dimensional structures of all β sheets, both molecules are translated in the cytosol associated with cytoskeletal rather than endoplasmic reticulum structures, and both precursors undergo myristoylation on lysine residues in their respective propeptides. However, differences between these two cytokines are remarkable when examining regulation of gene expression, mRNA stability, translation, processing, and secretion. In addition, the affinities of pro and mature IL-1β binding to surface and soluble forms of the receptors are different from those of IL-1α. Once released from cells, mature IL-1β encounters two antagonistic molecules: the soluble form of the type II receptor, which tightly binds IL-1β, and sIL-1Ra, which competes with IL-1β for cell surface receptor occupancy. The effects of these two antagonistic molecules in regulating the activity of IL-1β are discussed below. One is left with the overall impression that IL-1β is a systemic, hormone-like mediator intended to be released from cells, whereas IL-1α is primarily a regulator of intracellular events and mediator of local inflammation.

**Gene expression of IL-1β.** Unlike the promoter of IL-1α, the promoter region for IL-1β contains a clear TATA box, a typical motif of inducible genes. The half-life of IL-1β mRNA depends on the cell type and the conditions of stimulation. The most studied cells are freshly obtained human blood monocytes and macrophage cell lines derived from myelomonocytic leukemias. The initial studies established that endotoxin triggers transient transcription and steady state levels of IL-1β mRNA, which accumulate for 4 hours followed by a rapid decrease due to synthesis of a transcriptional repressor. Using cAMP-inducing agents or IL-1 itself, mRNA levels are sustained for over 24 hours in human blood monocytes. Raising cAMP levels in human monocytes by histamine or prostaglandin (PG) E2 enhances IL-1α–induced IL-1β gene expression and synthesis. In the case of lipopolysaccharide (LPS) stimulation, cAMP levels reduce IL-1β synthesis.

Unlike most cytokine promoters, IL-1β regulatory regions can be found distributed over several thousand basepairs upstream and a few basepairs downstream from the transcriptional start site. The topic of IL-1β gene regulation has recently been reviewed in detail, concluding that IL-1β gene expression is regulated at different levels. Studies have shown sequences in the IL-1β promoter required for transcription using a reporter gene transfected into human and mouse macrophage cell lines. There are two independent enhancer regions, −2782 to −2729 and −2896 to −2846, that appear to act cooperatively. The latter contains a cAMP response element, whereas the former is a composite cAMP response element–NFκB–6 that is responsive to LPS. The 80-bp fragment (−2782 to −2729) is required for transcription and contains, in addition to a cAMP response element, an NFκB-like site. Activating protein-1 (AP-1) sites also participate in endotoxin-induced IL-1β gene expression.

Proximal promoter elements between −131 and +14 have also been identified. Sequences in this region contain the binding sites for the novel nuclear factor NFβA, which appears to be similar to nuclear factors termed NFβ1 and NFβ2. This proximal promoter is required for maximal IL-1β gene expression. Recently, the nucleotide binding sequences of NFβA were found to be identical to those of the transcription factor Spi-1/PU.1, a well-established NFκB in cells of myeloid and monocyte lineage. The requirement for Spi-1/PU.1 for IL-1β gene expression imparts tissue specificity because not all cells constitutively express this NF. Human blood monocytes, which constitutively express Spi-1/PU.1, are exquisitely sensitive to gene expression of IL-1β by 1-10 pg/mL of LPS. Interestingly, the IL-1Ra promoter contains the proximal Spi-1/PU.1 site, which is also highly sensitive to LPS. Although disease-derived fibroblasts express IL-1β, these cells do not constitutively express Spi-1/PU.1 and are highly resistant to stimulation by LPS.

**Nonmicrobial inducers of IL-1.** Nearly all microbes and
BIOLOGIC BASIS FOR IL-1 IN DISEASE

Table 1. Non-Microbial Inducers of IL-1

<table>
<thead>
<tr>
<th>Stress factors</th>
<th>Hyperosmolarity</th>
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<tbody>
<tr>
<td>Hypoxia/hyperoxia*; ischemia-reperfusion</td>
<td></td>
</tr>
<tr>
<td>Ultraviolet B; laser*; gamma radiation*</td>
<td></td>
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<tr>
<td>Thermal injury</td>
<td></td>
</tr>
<tr>
<td>Neuroactive substances</td>
<td>Substance P; isoprotorernal; methamphetamine</td>
</tr>
<tr>
<td>Kainic acid (convulsant)<em>; phenytoin</em>; melatonin</td>
<td></td>
</tr>
<tr>
<td>Inflammatory substances</td>
<td>C5a*; C5b-9*; factor H*; retinoic acid*</td>
</tr>
<tr>
<td>Urate crystals; Ca pyrophosphate crystals</td>
<td></td>
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<tr>
<td>Advanced glycosylated end-products</td>
<td></td>
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<tr>
<td>Phthalate; dioxin; silica/ asbestos</td>
<td></td>
</tr>
<tr>
<td>Polynucleosides</td>
<td>C-reactive protein; α-1-antitrypsin</td>
</tr>
<tr>
<td>Tobacco antigen</td>
<td>Amphotericin-B</td>
</tr>
<tr>
<td>Cell matrix</td>
<td>Fibronectin; collagen</td>
</tr>
<tr>
<td>Clotting factors</td>
<td>Fibrin degradation products; plasmin; thrombin</td>
</tr>
<tr>
<td>Lipids</td>
<td>9-hydroxyoctadecadienoic acid; oxidized low density lipoprotein</td>
</tr>
<tr>
<td></td>
<td>Platelet activating factor</td>
</tr>
<tr>
<td>Cytokines</td>
<td>IL-1; TNF; IL-2; IL-3; IL-12, GM-CSF; M-CSF; stem cell factor; PDGF</td>
</tr>
<tr>
<td>Others</td>
<td>Phorbol esters</td>
</tr>
<tr>
<td>Bleomycin; ricin; taxol/colchicine</td>
<td></td>
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<tr>
<td>β-1 integrins; LFA-3; anti-HLA-DR</td>
<td></td>
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<tr>
<td>Antibiotics (arbekacin; ciprofloxacin*)</td>
<td></td>
</tr>
<tr>
<td>Phytohemagglutinin (CD2); concanavalin A</td>
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<tr>
<td>Anti-IgM (B cells); anti-CD3 (T cells); anti-thymocyte globulin</td>
<td></td>
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<tr>
<td>Sensitized red blood cells; sCD23</td>
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</table>

Data are derived from human and animal cells; some inducers are species specific.
* Gene expression but not IL-1 protein.

Table 1 lists several nonmicrobial inducers of both IL-1α and IL-1β. Depending on the stimulant, IL-1β mRNA levels increase rapidly within 15 minutes, but start to decrease after 4 hours. This decrease is thought to be due to the synthesis of a transcriptional repressor and/or a decrease in mRNA half-life. Using IL-1 as a stimulant of its own expression, IL-1β mRNA levels were sustained for over 24 hours. Raising cAMP levels in these same cells with histamine increases IL-1α–induced IL-1β gene expression and protein synthesis. In human peripheral blood mononuclear cells (PBMC), retinoic acid induces IL-1β gene expression, but the primary precursor transcripts fail to yield mature mRNA. Inhibition of translation by cycloheximide results in enhanced splicing of exons, excision of introns, and increased levels of mature mRNA (superinduction) by two orders of magnitude. Thus, synthesis of mature IL-1β mRNA requires an activation step to overcome an apparently intrinsic inhibition to process precursor mRNA.

Dissociation of transcription from translation. Stimulants such as the complement component C5a, hypoxia, adherence to surfaces or cloting of blood induce the synthesis of large amounts of IL-1β mRNA in monocytes without significant translation into the IL-1β protein. This dissociation between transcription and translation is characteristic of IL-1β but also of tumor necrosis factor α (TNFα). It appears that the above stimuli are not sufficient to provide a signal for translation despite a vigorous signal for transcription. Without translation, most of the IL-1β mRNA is degraded and this has been observed in humans undergoing hemodialysis with complement-activating membranes. Although the IL-1β mRNA assembles into large polyribosomes, there is little significant elongation of the peptide. However, adding bacterial endotoxin or IL-1 itself to cells with high levels of steady state IL-1β mRNA results in augmented translation in a similar manner to the removal of cycloheximide after superinduction. One explanation is that stabilization of the AU-rich 3' untranslated region takes place in cells stimulated with LPS. These AU-rich sequences are known to suppress normal hemoglobin synthesis. The stabilization of mRNA by microbial products may explain why low concentrations of LPS or a few bacteria or Borrelia organisms per cell induce the translation of large amounts of IL-1β.

Another explanation is that IL-1 stabilizes its own mRNA by preventing deadenylation as it does for the chemokine gro-α. Removal of IL-1 from cells after 2 hours increases the shortening of poly (A) and IL-1 apparently is an important regulator of gro synthesis because it prevents deadenylation. In fact, of the several cytokines induced by IL-1, large amounts of the chemokine family are produced in response to low concentrations of IL-1. For example, 1 pmol/L of IL-1 stimulates fibroblasts to synthesize 10 nmol/L of IL-8.

Translation requires an additional signal. Studies have taken advantage of pyridinyl-imidazol compounds that block the synthesis of IL-1β and TNFα without affecting transcription or steady state levels of mRNA. mRNA levels for either IL-1 or TNFα in PBMC stimulated with LPS in the presence of these compounds are indistinguishable from those in PBMC stimulated with C5a, hypoxia, or adherene. In other words, there is ample cytokine mRNA but no cytokine protein. These are interesting compounds that have entered clinical medicine. Some are cyclooxygenase/lipoxygenase inhibitors because, by classification, they block these enzymes. Hence, they are often called "dual inhibitors." However, their mechanism of action in suppressing IL-1 and TNFα has never been linked to their ability to suppress either cyclooxygenase or lipoxygenase. These and other imidazol-like drugs have recently been called "cytokine suppressing anti-inflammatory drugs" or CSAIDs.

Two agents have received careful study for their ability to inhibit cytokine translation. These CSAID compounds include:...
reduce IL-1 and TNF translation because they bind and inactivate a mitogen activating protein (MAP) kinase.66 Like most MAP kinases, phosphorylation of serine-threonine residues on various proteins is observed; however, these CSAID-associated MAP kinases apparently phosphorylate proteins that are required for translation of cytokine mRNAs into their respective proteins.68 They share homology with that of the yeast hyperosmolarity glycerol-1 gene (HOG-1) and in the human are identical to the 38-kD MAP kinase that is phosphorylated in cells stimulated with LPS or hyperosmolar concentrations of NaCl.77 79 These HOG-1-related MAP kinases are also the same as those that are phosphorylated during IL-1 signal transduction.80,81 Thus, these observations are consistent with the ability of IL-1 or LPS to augment the translation of cytokine mRNA.82 Of relevance is that initiation factor eIF-4E requires a MAP-like phosphorylation step to dissociate from a translational regulatory molecule.83

Processing and secretion. After synthesis, proIL-1β remains primarily cytosolic until it is cleaved and transported out of the cell (Fig 2). The IL-1β propiece (amino acids 1-116) is also myristoylated on lysine residues,99 but, unlike IL-1α, proIL-1β has no membrane form and proIL-1β is only marginally active.99 Some IL-1β is found in lysosomes34 or associated with microtubules98 and either localization may play a role in the secretion of IL-1β. In mononuclear phagocytes, a small amount of proIL-1β is secreted from intact cells,89,90 but the pathway for this secretion remains unknown. On the other hand, release of mature IL-1β appears to be linked to processing at the aspartic acid-alanine (116-117) peptide cleavage by the ICE96 (see below).

Although well-controlled in the setting of laboratory cell culture, death and rupture of inflammatory cells is not an unusual occurrence in vivo. There are several sites in the N-terminal 16-kD part of proIL-1β that are vulnerable to cleavage by enzymes in the vicinity of alanine 117. These are trypsin,11 elastase,86 chymotrypsin,99 a mast cell chymase,100 and a variety of proteases101,102 that are commonly found in inflammatory fluids. The extent of the role that these proteases play in the in vivo conversion of proIL-1β to mature forms is uncertain, but in each case, a biologically active IL-1β species is produced. In the discussion on the soluble IL-1 receptor type II (see below), the affinity of proIL-1β for this constitutively produced soluble receptor is high and may prevent haphazard cleavage of the precursor by these enzymes in inflammatory fluids.

Commentary on regulating IL-1β production. Although ICE is constitutively expressed in most cells, not all cells process proIL-1β and secrete mature IL-1β. However, in cells of monocytic lineage, proIL-1β cleavage takes place and mature IL-1β is secreted from stimulated cells. This is in sharp contrast to the same cell synthesizing proIL-1α, which is generally not secreted or found in body fluids during pathologic events. In some levels for regulating IL-1β synthesis, including the large and complex nature of the IL-1β promoter, regulation of mRNA splicing processes, phosphorylations of proteins required for translation, and the stabilization of the 3′ untranslated region. Once synthesized, proIL-1β requires the activated form of ICE to be processed and secreted. With microbial agents as stimulators, each of these events take place in rapid succession and, once released...
from cells, IL-1β can trigger IL-1RI on cells both near and distant from the site of its synthesis. This implicates IL-1β in systemic as well as local disease. Naturally occurring inhibitors of ICE likely exist in mammalian cells, similar to those of some viral gene products. One can speculate that tight regulation of IL-10 production evolved to reduce the interaction of IL-1β with IL-1RI.

**ICE**

As shown in Fig 2, proIL-1β requires cleavage before the mature form is secreted. The percentage of mature IL-1β secreted into the supernatants during the 24 hours from LPS-stimulated human PBMC is variable (30% to 70%), suggesting that cleavage and secretion are differentially regulated in these cells. Secretion is affected by the presence of cyclooxygenase inhibitors or interferon γ (IFNγ). In addition, there is a polymorphism of a Taq I site in the IL-1β gene that is located in the coding sequence of proIL-1β. The cleavage of proIL-1β at the aspartic acid–alanine positions (amino acids 116-117) is a property of a specific intracellular cysteine protease known as the ICE. This enzyme generates the N-terminal IL-1β molecule commonly found in supernatants from human PBMCs and inflammatory fluids. There is another aspartic acid site at position 27 in proIL-1β that likely accounts for a 22-kD form observed in monocyte cultures. The cDNA encoding ICE has been reported. The 45-kD precursor of ICE requires two internal cleavages before becoming the enzymatically active heterodimer composed of a 10- and 20-kD chain. The active site cysteine is located on the 20-kD chain. ICE itself contributes to autoprocessing of the ICE precursor by undergoing oligomerization with itself or homologs of ICE (Fig 2).

The tertiary structure of the active site has been reported. Two molecules of the ICE heterodimer form a tetramer with two molecules of proIL-1β for cleavage. The aspartic acid at position 116 of the proIL-1β is the recognition amino acid for ICE cleavage. ICE does not cleave the IL-1α precursor. Enzymes such as elastase and granzyme A cleave proIL-1β at amino acid 112 and 120, respectively, yielding biologically active IL-1β. The pro-piece of IL-1β can be found both inside and outside the cell. In addition, the pro-piece exhibits biologic activity as a chemoattractant for fibroblasts via an IL-1R-mediated event.

In the presence of a tetrapeptide competitive substrate inhibitor of ICE, the generation and secretion of mature IL-1β is reduced and proIL-1β accumulates mostly inside but also outside the cell. This latter finding supports the concept that proIL-1β can be released from a cell independent of processing by ICE. Similar to that of thioredoxin and basic FGF, exocytosis has been proposed as a possible mechanism of proIL-1β release. However, as shown in Fig 2, a putative membrane "channel" in which active ICE is localized has also been proposed. In this model, mature IL-1β is released through this channel. When ICE activity is blocked by a reversible competitive substrate inhibitor, greater amounts of proIL-1β are found in the supernatants; thus, the putative channel may provide a passive secretory pathway for both proIL-1β and mature IL-1β. Macrophages from ICE-deficient mice do not release mature IL-1β on stimulation in vitro. Although neutrophil enzymes such as elastase and granzyme A cleave proIL-1β at sites close to alanine 117, proIL-1β accumulates in cells from ICE-deficient mice. Interestingly, IL-1α production in macrophages from ICE-deficient mice is reduced, the latter finding being consistent with self-induction of IL-1 gene expression and synthesis. ICE-deficient mice can be either resistant or susceptible to lethal endotoxemia. Recent studies suggest that mice deficient in ICE fail to develop collagen-induced arthritis (M. Tocci, personal communication, September 15, 1995).

**Regulation of ICE and IL-1β secretion.** Methods for measuring IL-1β detect primarily the mature form of IL-1β. Hence, agents and conditions reported to reduce the synthesis of IL-1β may, in fact, only inhibit secretion of mature IL-1β. Furthermore, prevention of the cleavage of the ICE precursor may also account for an apparent inhibition of IL-1β synthesis. Due to alternate RNA splicing, there are five isoforms of human ICE (ICEα, β, γ, δ, and ε). ICEα cleaves the ICE precursor and proIL-1β. It is presumed that ICEβ and γ also process precursor ICE. ICEε is a truncated form of ICE that may inhibit ICE activity by binding to the p20 chain of ICE to form an inactive ICE complex. Although there are no data concerning the effect of overexpressing ICEε on the cleavage and secretion of IL-1β, cells overexpressing ICEε are resistant to apoptosis (see below). Enhancement of processing and secretion can also be regulated; adding ATP to LPS-stimulated cells increases IL-1β secretion, whereas blocking anion transport reduces the secretion of mature IL-1β, an effect that may be due to inactivation of ICE. A reduction in cellular potassium is associated with increased processing of proIL-1β. Incubation of LPS-stimulated murine macrophages with the potassium ionophore nigericin activates the conversion of proIL-1β to the mature form. Blocking PGE2 synthesis in vitro with IFNγ or cyclooxygenase inhibitors enhances secretion of IL-1β. In PBMC from patients undergoing hemodialysis with complement activating membranes, increased endogenous PGE2 synthesis correlated with decreased secretion but not total synthesis of IL-1β. In general, as blood monocytes are cultured and take on a macrophage phenotype, there is a loss in the ability to secrete IL-1β, whereas intracellular levels remain unchanged. A similar observation has been made for human peritoneal macrophages. Each of these studies suggest that ICE activity and/or IL-1β secretion are regulated by events associated with inflammation.

**ICE, ICE homologs, and apoptosis.** The gene ced-3 in the nematode, Caenorhabditis elegans, codes for a protein homologous to human ICE. During embryonic development of the worm, this gene is expressed in specialized cells and is thought to be responsible for programmed cell death (apoptosis). In the worm, ced-9 protects against apoptosis; in the human, the homologous death protecting gene is bcl-2. There is a remarkably conserved homology of the five amino acids required for ced-3 and human ICE activity. Other homologs of ICE have been discovered and each has a similar aspartic acid substrate specificity. Nedd2 is a mouse
gene also expressed in cells undergoing apoptosis during development and was recently shown to be homologous to ced-3 and ICE. In human T-lymphocytes, a 32-kD cysteine protease protein (CPP32) with significant homology to ICE, ced-3, and Nedd2 has been reported. Transcription X is a novel human protease with 30% homology to ICE but does not cleave proIL-1β. There are at least seven ICE homologs described.

Overexpression of ICE or any of its homologs in transiently transfected cells is associated with increased apoptosis. However, intracellular overexpression of most proteases induces apoptosis. Nevertheless, cotransfection with the proto-oncogene bcl-2 suppresses cell death due to overexpression of ICE or its homologs. Cell death induced by ICE or its homologs can also be reduced by cotransfection with crmA, a cow pox viral gene coding for an inhibitor of proteases including ICE. For example, transfection with crmA in neurons prevents programmed cell death due to removal of nerve growth factor. However, CrmA is not specific for inhibiting ICE.

Although one substrate for ICE is known (proIL-1β), the substrate(s) for the enzymes encoded by ced-3, Tx, Nedd2, and other ICE homologs is unknown. A putative, non-proIL-1β substrate candidate for ICE and ICE homologs is poly-ADP-ribose polymerase (PARP), which is important for maintaining the integrity and repair of DNA. An intracellular protease that cleaves PARP resembles ICE. The active form of this protease cleaves a tetrapeptide sequence in its substrate identical to the aspartic acid cleavage site in human protease that cleaves PARP resembles ICE. A cow pox viral gene coding for an inhibitor of proteases including ICE. For example, transfection with crmA in neurons prevents programmed cell death due to removal of nerve growth factor. However, CrmA is not specific for inhibiting ICE.

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ICE and FAS-mediated cell death. Recent studies in ICE-deficient mice have shed light on the relationship of ICE to programmed cell death. In these mice, the thymus develops normally; furthermore, stressed-induced apoptosis (cortisosteroids or radiation) of thymocytes and macrophages in vitro is also normal in these mice. However, apoptosis in thymocytes by an activating antibody to FAS, a TNF-related receptor, is diminished in ICE-deficient mice. Blocking ICE activity with a specific substrate inhibitor also reduces FAS-induced as well as TNF-induced apoptosis. Apoptosis triggered by activating FAS or TNF receptors is associated with a "death domain" on the cytoplasmic segment of these receptors. During this time, a novel intracellular protein called MORT 1 associates with the death domains. The MORT 1 precursor contains an ICE-like aspartic acid cleavage site and may be a substrate for ICE or its homologs. The overall findings suggest that, in the absence of ICE, cell death can take place when triggered by cellular stress pathways. However, FAS/TNF receptor-induced apoptosis apparently requires an ICE-sensitive pathway. Because ICE-deficient mice do not spontaneously develop autoimmune diseases, the role of ICE in immune-mediated cell death remains uncertain.

IL-1 and apoptosis. Is the ICE apoptosis-promoting activity and the ICE-homologs a coincidental finding or could IL-1β itself play a role in apoptosis? To resolve this question, it is important to note that exogenous IL-1 induces programmed cell death in the insulin-producing β cell of the pancreatic islet and in other cells (reviewed in Mandrup-Poulsen et al). This is a specific event requiring a metabolically active cell. The glucagon-producing α cells adjacent to the β cells are unaffected by IL-1. IL-1-induced death in the β cell is mediated by nitric oxide (NO) and the ability of IL-1 to increase inducible nitric oxide synthase (iNOS) is a well-established property of the cytokine. As discussed below, the cytokidal effect of IL-1 on neoplastic or normal cells may also be due to IL-1 induction of NO.

Contrary to studies on IL-1-induced cell death, IL-1 can be a growth factor for a variety of cells, particularly when IL-1-induced PGE2 synthesis is inhibited. With the notable exception of an autocrine effect of intracellular IL-1α in endothelial cells, IL-1-induced cell growth are IL-1RI mediated. In some cells, IL-1 protects against TNF-mediated cell death by arresting cell cycling. At the present time, although receptor-mediated cell death can be a property of mature IL-1β, proIL-1β is probably not the substrate for ICE or its homologs responsible for apoptosis. Because thy-

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<th>Table 2. Nitric Oxide-Dependent Effects of IL-1</th>
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<td>Effects of IL-1 reduced by inhibition of NO generation</td>
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<tr>
<td>Fever in rats</td>
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<td>Increased slow wave sleep in rabbits*</td>
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<td>Hyperglycemia in rats</td>
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<td>Hyperglucagonemia in rats</td>
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<td>Neutrophilia in rats (†)</td>
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<td>Increased COX-2 expression in fibroblasts in vitro</td>
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<td>Inhibition of PAF release from rat peritoneal mast cells</td>
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<td>Programmed cell death in β-islet cells in vitro</td>
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<td>Inhibition of insulin release from islet cultures</td>
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<td>Increased release of ACTH, vasopressin and oxytocin in rats†</td>
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<td>Inhibition of cartilage proteoglycan synthesis</td>
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<td>Rat hypothalamic release of CRH and ACTH in vitro</td>
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<td>Suppression of melanoma cell line A375 growth in vitro</td>
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<td>Cytotoxicity in the rat ovary</td>
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<td>Inhibition of chondrocyte proliferation</td>
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<td>Enhancement of N-methylaspartate neurotoxicity</td>
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<td>Reduction in electric contraction of myocardial muscle</td>
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<td>Effects of IL-1 enhanced by inhibition of NO generation</td>
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<td>ACTH release</td>
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* IL-1-induced fever in rabbits is NO-independent. † Inhibition of iNOS augments the response to IL-1.
mocyte apoptosis is normal in ICE-deficient mice,\(^{108,109}\) this observation further supports the concept that apoptosis in cells overexpressing ICE is independent of the cleavage of proIL-1\(\beta\).

Commentary on ICE, IL-1\(\beta\), and programmed cell death. Several reports show that overexpressing ICE leads to programmed cell death. If there is a link between ICE, IL-1\(\beta\), and cell death, it is most likely due to an IL-1R-mediated event rather than to an intracellular mechanism. Although ICE-induced intracellular generation of the IL-1\(\beta\) "pro-piece" may contribute to cell death, at the present time there is no evidence that the IL-1\(\beta\) "pro-piece" is active in initiating apoptosis. On the other hand, blocking the cleavage of proIL-1\(\beta\) in some cells, eg, the islets of Langerhans in the pancreas, would result in reduced secretion of active IL-1\(\beta\) and less NO production. Therefore, in any cell or tissue in which IL-1\(\beta\) is produced under conditions of disease, agents inhibiting ICE will probably reduce IL-1\(\beta\)-mediated NO synthesis and any NO-mediated cell death. Under those conditions, inhibiting ICE should reduce cell death to the same extent as a neutralizing antibody to IL-1\(\beta\) or receptor blockade. Overall, the data support the concept that there are other substrates for ICE and its homologs and that overexpressing the ICE family of proteases cleaves non-IL-1\(\beta\) intracellular proteins that trigger cell death. Because ICE-deficient mice have normal neuronal and immune cell development, this enzyme does not participate in cell death required for embryonic development.

There is concern that ICE inhibitors that reduce inflammation by inhibiting processing and secretion of IL-1\(\beta\) may inadvertently prolong the life of malignant cells. Is there a risk of aggravating autoimmune or malignant disease using ICE inhibitors? Data support the opposite view; ICE inhibition reduces inflammation without a change in cell death. One explanation may be that cleavage of molecules such as PARP may require 50- to 100-fold more ICE compared with cleavage of proIL-1\(\beta\).\(^{127}\) Incubation of leukemic blasts from patients with acute myeloblastic leukemia (AML) in the presence of ICE inhibitors reduced IL-1\(\beta\) secretion\(^{146,151}\) and reduced spontaneous proliferation,\(^{156}\) but did not increase cell survival. Antisense ICE also reduced spontaneous proliferation of AML cells without increasing survival.\(^{158}\) These were anticipated results because IL-1\(\beta\) is a growth factor for AML cells.\(^{159}\) In another experiment, pretreatment of murine melanoma cells with ICE inhibitors before intrasplenic injection reduced the number of hepatic metastases and increased survival of mice (Vidal-Vanaclocha and Dinarello, unpublished observations). Hence, inhibition of ICE in these models does not worsen but rather improves disease outcome. Nevertheless, the clinical benefit of reducing the release of mature IL-1\(\beta\) will require an inhibitor with a high degree of specificity for the cleavage sites of proIL-1\(\beta\) without affecting the cleavage of substrates of other members of the ICE family.

**IL-1Ra**

**Production of IL-1Ra.** Although sIL-1Ra is an inducible gene in most cells, icIL-1Ra is expressed constitutively in keratinocytes and intestinal epithelial cells. Two forms of the icIL-1Ra exist (icIL-1Raa and icIL-1RaII) and are inducible in various cell types.\(^{146,147}\) Like sIL-1Ra, icIL-1Ra blocks IL-1Ra and does not trigger a biologic response. Natural sIL-1Ra is a 22-kD glycosylated protein,\(^{142,144}\) but the recombinant (non-glycosylated) 17-kD form of IL-1Ra retains a comparable ability to inhibit IL-1 binding in vitro.\(^{143,144}\) As shown in Fig 3, pro(s)IL-1Ra, which possesses a leader sequence, is synthesized, processed, and secreted from the cell. Upon stimulation with LPS, human blood monocytes initially express the gene for sIL-1Ra.\(^{149}\) During the first 4 to 6 hours, sIL-1Ra protein can be visualized in the Golgi.\(^{150}\) After 24 hours, the primary transcript in these cells is icIL-1Ra, which, lacking a leader peptide, stains diffusely in the cytosol and remains intracellular.\(^{151}\) It has been proposed that icIL-1Ra constitutively produced in keratinocytes and epithelial cells may block the binding of IL-1\(\alpha\) to nuclear DNA.\(^{46,140}\)

**IL-1Ra does not possess agonist activities.** The primary amino acid homology of mature human IL-1\(\beta\) to IL-1Ra is 26%, which is greater than that between IL-1\(\alpha\) and IL-1\(\beta\). Each member of the human IL-1 family is composed of an all \(\beta\) strand molecule that forms an open barrel-like structure closely related to structure of FGF.\(^{147-149}\) Because each member of the IL-1 family binds to the IL-1RI, it is not surprising that IL-1\(\alpha\), IL-1\(\beta\), and IL-1Ra share structural similarities. How does IL-1Ra bind to IL-1RI with nearly the same affinity as IL-1\(\alpha\) or IL-1\(\beta\) and yet not trigger a response? Crystal structural analysis of the IL-1RI-IL-1Ra complex shows that IL-1Ra contacts all three domains of the IL-1RI.\(^{150}\)

IL-1\(\beta\) has two sites of binding to IL-1RI. There is a primary binding site located at the open top of its barrel shape that is similar but not identical to that of IL-1\(\alpha\).\(^{47}\) There is a second site on the back side of the IL-1\(\beta\) molecule.\(^{48}\) IL-1Ra also has two binding sites similar to those of IL-1\(\beta\).\(^{149,152}\) However, the back side site in IL-1Ra is more homologous to that of IL-1\(\beta\) than the primary binding site.\(^{153}\) Thus, the present interpretation is that the back side site of IL-1Ra binds to IL-1RI and occupies the receptor. Lacking the second binding site, IL-1Ra does not trigger a signal (see below for discussion of IL-1 receptor dimerization). After IL-1Ra binds to IL-1RI-bearing cells, there is no phosphorylation of the epithelial growth factor receptor,\(^{153}\) a well-established and sensitive assessment of IL-1 signal transduction.\(^{154}\) Moreover, when injected intravenously into humans at doses 1,000,000-fold greater than that of IL-1\(\alpha\) or IL-1\(\beta\),\(^{155,156}\) IL-1Ra has no agonist activity.\(^{157}\)

The formation of the heterodimer consisting of the IL-1RI and IL-1R accessory protein (IL-1R-Acp)\(^{2}\) likely explains the failure of IL-1Ra to trigger a signal. From the structural differences described above between IL-1\(\beta\) and IL-1Ra, one can propose that the second binding site missing from the IL-1Ra is, in fact, the site that binds the accessory protein. The cross-linked complex of radiolabeled IL-1Ra to the type I receptor was not precipitated by a specific antibody to the accessory protein.\(^{2}\) As shown in Fig 4, IL-1Ra binds to the type I receptor with the same affinity as that of IL-1, but, lacking the second binding site, the IL-1R-Acp does not dock to the IL-1Ra and the heterodimer is not formed. The
binding of IL-1Ra to the type I receptor likely prevents or disrupts the complex between IL-1 and the type I receptor. This model implies that signal transduction takes place only when the heterodimer is formed. A triple mutation in IL-1Ra may have partially reconstituted the second binding domain so that a degree of dimerization takes place between the cytosolic domains of IL-1RI and IL-1R-AcP, resulting in increased agonist mRNA levels but steady state mRNA levels remain elevated for several hours. After 48 hours, this cell takes on a more macrophagic phenotype that can be facilitated by GM-CSF. The macrophagic cell uses the icIL-1Ra promoter and transcribes icIL-1Ra, which lacks the leader sequence. icIL-1Ra is translated and remains intracellularly, where it is thought to inhibit intracellular binding of IL-1α to nuclear receptors.

Fig 3. A human blood monocyte stimulated with LPS producing IL-1Ra. During the first 24 hours, this cell uses the sIL-1Ra promoter. The primary transcript is pro(s)IL-1Ra, which is translated in the endoplasmic reticulum and transported to the Golgi. After cleavage of the leader sequence, sIL-1Ra is readily secreted from the cell. Compared with IL-1β, the gene for sIL-1Ra is expressed 1 to 2 hours later, but steady state mRNA levels remain elevated for several hours. After 48 hours, this cell takes on a more macrophagic phenotype that can be facilitated by GM-CSF. The macrophagic cell uses the icIL-1Ra promoter and transcribes icIL-1Ra, which lacks the leader sequence. icIL-1Ra is translated and remains intracellularly, where it is thought to inhibit intracellular binding of IL-1α to nuclear receptors.

Blocking IL-1 receptors with IL-1Ra. In equilibrium binding assays performed at 4°C, human natural and nonglycosylated recombinant IL-1Ra has nearly the same affinity (~200 pmol/L) for the murine IL-1RI as that of human IL-1α and IL-1β. Although the type II IL-1R is not a signaling receptor, IL-1Ra binds to this receptor on neutrophils, monocytes, and B-cell lines with an affinity that is approximately 100- to 500-fold less than that for IL-1RI. The rate of association of IL-1Ra with IL-1RI is slower than that of either form of IL-1, and, as discussed by Arend, when IL-1Ra dissociates from its cell receptor, IL-1 rather than IL-1Ra will occupy the empty receptor. Despite the near equal affinities of IL-1 and IL-1Ra for IL-1RI, a 10- to 100-fold molar excess of IL-1Ra is often required to inhibit IL-1 activity. In animals, a preinjection of 100- to 1,000-fold molar excess of IL-1Ra over that of IL-1 is needed to block systemic responses to IL-1.

Other receptor antagonists. Single point mutations or deletions in IL-1β have resulted in molecules with greater than a 100-fold loss in biologic activity but only a small decrease in IL-1RI binding. A single point mutation in the carboxyl end of the IL-1β molecule (lysine to aspartic acid) converts IL-1Ra from an antagonist to a partial agonist, but changes in this and other mutants may be due to misfolding in IL-1β. In general, no single amino acid substitution completely accounts for the differences between the binding and signal transduction of IL-1 compared with IL-1Ra. Subpeptides of either IL-1α or IL-1β have been synthesized and shown to possess biologic activities in various assays. A nonpeptide derived from the mature IL-1β sequence but absent from IL-1Ra possesses immunostimulatory but no inflammatory properties. Although this peptide (also known as the β-bulge) does not displace the binding of IL-1, when inserted into IL-1Ra, it helps convert IL-1Ra to a partial agonist.

Commentary on IL-1Ra. Healthy humans are the most sensitive indicators of IL-1 agonist activity; 1 ng/kg of intravenous IL-1β produces symptoms. In contrast, the intravenous infusion of 10 mg/kg of IL-1Ra in healthy humans, a 10 million-fold molar excess, is without effects. What are the structural requirements of the respective molecules that account for this dramatic difference? The ability of IL-1β to optimally trigger cell signaling requires stability of the overall tertiary structure of the cytokine so that mutations in

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one amino acid may unfold the molecule resulting in a several hundred-fold loss in activity but without a loss in receptor binding. This suggests that biologic activity requires binding of IL-1β to a relatively broad area on the receptor. The tertiary structure of IL-1Ra, which is closely related to that of IL-1β, allows for tight binding to the IL-1RI but IL-1Ra clearly lacks the second binding site that allows docking of the IL-1R-AcP to form the heterodimer. Without dimerization, no signal is transduced, but occupancy of the IL-1RI by IL-1Ra results in a very effective prevention of IL-1 signal transduction. Small molecules may mimic the near perfect receptor antagonism of IL-1Ra, but, to date, none has been reported.

**IL-1 RECEPTORS**

**IL-1 receptors and receptor accessory proteins.** At present, two primary IL-1 binding proteins (receptors) and one receptor accessory protein (IL-1R-AcP) have been identified. The extracellular domains of the two receptors and the IL-1R-AcP are members of the Ig superfamily, they are each composed of three IgG-like domains, and they share a significant homology to each other. Although cross-linking studies have shown the existence of large molecular weight complexes with IL-1 suggesting the presence of other binding molecules, the IL-1R-AcP exhibits functionality. The two IL-1 receptors are distinct gene products. In the human, the genes for IL-1RI and IL-1RII are located on the long arm of chromosome 2.
Another member of the IL-1 receptor family\(^{184}\) has various homologs in different species. In the rat, it is called Fit-1, an estrogen-inducible, c-fos–dependent transmembrane protein that shares 26% to 29% amino acid homology to the mouse IL-1RI and II, respectively. In the mouse, the Fit-1 protein is called ST2 and in the human it is called T1. The organization of the two IL-1 receptors and the Fit-1/ST2/T1 genes indicate they are derived from a common ancestor.\(^{183}\) Fit-1 exists in two forms: a membrane form (Fit-1M) with a cytosolic domain similar to that of the IL-1RI and Fit-1S, which is secreted and composed of the extracellular domain of Fit-1M. In many ways, these two forms of the Fit-1 protein are similar to those of the membrane-bound and soluble IL-1RI. It has been shown that the IL-1sRI is derived from proteolytic cleavage of the cell-bound form.\(^{183}\) On the other hand, the Fit-1 gene is under the control of two promoters, which results in two isoforms coding for either the membrane or soluble form of the receptor. Two RNA transcripts result from alternative RNA splicing of the 3’ end of the gene. Although IL-1\(\beta\) binds weakly to Fit-1 and does not transduce a signal,\(^{185}\) a chimeric receptor consisting of the extracellular murine IL-1RI fused to the cytosolic Fit-1 transduces an IL-1 signal.\(^{185}\) It is also unclear whether the Fit-1/ST2/T1 forms a complex with IL-1/IL-1RI/IL-1R-Acp. The cytosolic portion of Fit-1 align with GTPase-like sequences of IL-1RI\(^{186}\) (see below).

**IL-1 receptor type I.** IL-1RI is an 80-kD glycoprotein found prominently on endothelial cells, smooth muscle cells, epithelial cells, hepatocytes, fibroblasts, keratinocytes, epidermal dendritic cells, and T lymphocytes. IL-1RI is heavily glycosylated and blocking the glycosylation sites reduces the binding of IL-1.\(^{187}\) Surface expression of this receptor is likely on most IL-1–responsive cells because biologic activity of IL-1 is a better assessment of receptor expression than ligand binding to cell surfaces.\(^{188}\) Failure to show specific and saturable IL-1 binding is often due to the low numbers of surface IL-1RI on primary cells. In cell lines, the number of IL-1RI can reach 5,000 per cell but primary cells usually express less that 200 receptors per cell. In some primary cells there are less than 50 per cell\(^{189}\) and IL-1 signal transduction has been observed in cells expressing less than 10 type I receptors per cell.\(^{190}\) The low number of IL-1RI on cells and the discrepancy between binding affinities and biologic activity can be explained by the increased binding affinity of IL-1 in the complex with the IL-1R-Acp.\(^{2}\)

Using in situ hybridization, IL-1RI gene expression can be detected in the endocrine pancreas, cardiac endothelium, epidermis, hair follicles, uterine serosa, developing oocytes, and granulosa cells from ruptured ovarian follicles of healthy mice.\(^{191}\) In contrast, the spleen and thymus are largely negative. IL-1RI has also been localized to specific hippocampal areas of the normal mouse brain, adrenal glands, and testis.\(^{192}\) There is an uneven distribution with localization in the hippocampus, the anterior lobe of the pituitary gland, and the dentate gyrus.\(^{193}\) The importance of constitutive gene expression for IL-1RI in ontogeny and homeostasis is unclear because mice deficient in this gene exhibit a grossly normal phenotype (M. Labow, personal communication, June 1995).

As shown in Fig 4, IL-1RI has a single transmembrane segment and a cytoplasmic domain. Using specific neutralizing antibodies, IL-1RI but not IL-1RII is the primary signal transducing receptor.\(^{190,193,195}\) Antisense oligonucleotides directed against IL-1RI block IL-1 activities in vitro and in vivo.\(^{196}\) The cytoplasmic domain of IL-1RI has no apparent intrinsic tyrosine kinase activity, but, when IL-1 binds to only a few receptors, the remaining unoccupied receptors appear to undergo phosphorylation,\(^{197}\) probably by a member of the MAP kinase family. Interestingly, the cytosolic domain of IL-1RI has a 45% amino acid homology with the cytosolic domain of the Drosophila Toll gene.\(^{198}\) Toll is a transmembrane protein acting like a receptor, although the ligand for the Toll protein is unknown. Gene organization and amino acid homology suggests that the IL-1RI and the cytosolic Toll are derived from a common ancestor and trigger similar signals.\(^{199,200}\)

**IL-1 receptor accessory protein.** Both the extracellular domain of the IL-1R-Acp and its cytoplasmic segment share homology with the IL-1RI.\(^{2}\) There is a perfectly conserved protein kinase C (PKC) acceptor site in both cytoplasmic domains, although agents activating PKC do not mimic IL-1 signal transduction. Limited sequence homology of the gp130 cytoplasmic domain with those of IL-1RI\(^{201}\) and IL-1R-Acp suggest that complex formation of the IL-1R/IL-1/IL-1R-Acp transduces a signal similar to that observed with ligands that cause the dimerization of gp130. In fact, deletion of the gp130 shared sequences from the IL-1RI cytoplasmic domain results in a reduced response to IL-1.\(^{202}\) IL-1 shares some prominent biologic properties with gp130 ligands; eg, fever, hematopoietic stem cell activation, and the stimulation of the hypothalamic-pituitary-adrenal axis are common to IL-1 and IL-6. Other biologic activities of IL-1 and IL-6 are distinctly antagonistic.

High levels of IL-1R-Acp are expressed in mouse and human brain tissue. The discovery and function of the IL-1R-Acp has placed IL-1 receptor biology and signaling mechanisms into the same arena as other cytokines and growth factors. The IL-1R-Acp also explains previous studies describing low and high binding affinities of IL-1 to various cells. As shown in Fig 4, like other models of two-chain receptors, IL-1 binds first to the IL-1RI with a low affinity. Although there is no direct evidence, a structural change may take place in IL-1 allowing for docking of IL-1R-Acp to the IL-1RI/IL-1 complex. Once IL-1RI/IL-1 binds to IL-1R-Acp, a high-affinity binding is observed. Antibodies to the type I receptor and to the IL-1R-Acp block IL-1 binding and activity.\(^{2}\) Therefore, IL-1 may bind to the type I receptor with a low affinity causing a structural change in the ligand followed by recognition by the IL-1R-Acp. Alternatively, cells express IL-1RI/IL-1R-Acp already complexed and the high-affinity binding takes place on the preformed complexes. However, this is unlikely (S. Greenfeder, personal communication, August 1995).

Similar to IL-1RI and IL-1RII, a soluble form of the IL-1R-Acp exists but this form appears to result from an RNA splice donor/acceptor site resulting in a truncated protein ending before the transmembrane region. Unlike the soluble
forms of the IL-1RI and IL-1RII, the soluble IL-1R-AcP is not formed by proteolytic cleavage of the full-length accessory protein. It is unclear how soluble IL-1R-AcP mRNA is expressed compared with the cell-bound protein. Furthermore, because the IL-1R-AcP does not bind IL-1 itself; the effect of the soluble IL-1R-AcP on the binding of IL-1 remains unclear. As discussed above and shown in Fig 4, IL-1Ra does not form a complex with the IL-1RI/IL-1R-AcP and this likely explains how the IL-1Ra can bind so tightly to the IL-1RI and yet not exhibit any agonist activity. One thus concludes that the IL-1RI/IL-1RII/IL-1R-AcP complex triggers the cell and that, without the IL-1R-AcP participation, the IL-1 signal via the IL-1RI is weak or nonexistent. It is unlikely that the complex IL-1RII/IL-1RI/IL-1R-AcP exists.

Gene and surface regulation of IL-1RI. The genomic organization of the human type I receptor shows three distinct transcription initiation sites contained in three separate segments of the first exon that is distributed over 29 kb of the gene. Each part of this first exon is thought to possess a separate promoter which functions independently in different cells. Despite evidence that type I receptor gene expression can be upregulated in vitro, the most proximal (5') promoter region lacks a TATA or CAAT box. In fact, this promoter region for the human IL-1RI shares striking similarity to those of house-keeping genes rather than highly regulated genes. The transcriptional initiation start site contains nearly the same motif as that for the TdT gene. There is a guaninose-cytosine rich segment (75%) following the transcriptional initiation site of exon I that accounts for considerable secondary RNA structure. Low numbers of surface IL-1RI may, in fact, be due to multiple secondary RNA structures that reduce optimal translation of the mRNA.

Surface expression of IL-1RI clearly impacts on the biologic response to IL-1. Similar to IL-1β, cells can express high steady state levels of mRNA for IL-1RI but low levels of the protein. This may be due to the amount of secondary structure in each of the polyadenylated RNA species. Studies on IL-1RI surface expression have mostly used binding of labeled ligands rather than assessment of surface receptor density using specific antibodies. Nevertheless, phorbol esters, PGE2, dexamethasone, epidermal growth factor, IL-2, and IL-4 increase surface expression of IL-1RI. In cells that synthesize PGE2, IL-1 upregulates its own receptor via PGE2; however, when PGE2 synthesis is inhibited, IL-1 downregulates IL-1RI in the same cells. In the case of Th2 lymphocytes, IL-1 downregulates IL-1RII surface expression; this downregulation is associated with a decrease in mRNA half-life. Therefore, despite the housekeeping nature of its promoters, IL-1RI is regulated in the context of inflammation and immune responses.

IL-1 receptor type II. The cell-bound IL-1RII does not appear to form a complex with the type I receptor and also does not transduce a signal. In the human and mouse, IL-1RII has a short cytosolic domain consisting of 29 amino acids; in the rat, there are an additional 6 charged amino acids. In general, antibodies to IL-1RII block IL-1-mediated activities in vitro and in vivo, whereas antibodies specific for the IL-1RII have no effect. An antibody (ALVA 42) that recognizes α and β subunits of HLA-DR also binds to cells expressing type II receptors. The ability of this antibody to inhibit IL-1-mediated effects in vivo may be due to inhibition of IL-1-induced production of IL-1. For example, anti-HLA-DR monoclonal antibodies stimulate the production of IL-1β by macrophages and enhance (or suppress) IL-1β induced by either superantigens or LPS.

The type II receptor appears to act as "decoy" molecule, particularly for IL-1β. The receptor binds IL-1β tightly, thus preventing binding to the signal transducing type I receptor. It is the lack of a signal transducing cytosolic domain that makes the type II receptor a functionally negative receptor. For example, when the extracellular portion of the type II receptor is fused to the cytoplasmic domain of the type I receptor, a biologic signal occurs. The extracellular portion of the type II receptor is found in body fluids, where it is termed IL-1 soluble receptor type II (IL-1sRII). It is assumed that a proteolytic cleavage of the extracellular domain of the IL-1RII from the cell surface is the source of the IL-1sRII.

Regulation of IL-1RII. Just as downregulation of IL-1RI surface expression reduces the biologic response to IL-1, upregulation of IL-1RII can also reduce the activity of IL-1. Gene expression of IL-1RII is under the control of two promoters, each of which control the usage of a divided first exon ( exon A or B). Early studies using B cells, monocytes, or bone marrow cells (type II receptor-bearing cells) showed that hematopoietic growth factors, dexamethasone, and PGE2 increase the number of IL-1 binding sites. Surface expression of IL-1RII is upregulated on neutrophils exposed to dexamethasone and IL-4 and on monocytes or B-cell lines exposed to dexamethasone. These observations have been confirmed using gene expression in different cell lines. A transcription factor called PU.1 that is present in cells of hematopoietic origin is required for expression of IL-1RII. In patients with bacterial sepsis, elevated IL-1RII expression has been observed on neutrophils. Although IL-1 itself downregulates gene and surface expression of IL-1RI, IL-1 upregulates gene and surface expression of the IL-1RII on an insulinoma cell line.

Soluble IL-1 receptors. It is likely that, as cell-bound IL-1RII increases, there is a comparable increase in soluble forms. Similar to soluble receptors for TNF, the extracellular domain of the type I and type II IL-1R are found as "soluble" molecules in the circulation and urine of healthy subjects and in inflammatory synovial and other pathologic body fluids. In healthy humans, the circulating levels of IL-1sRII are 100 to 200 pmol/L whereas levels of IL-1sRI are 10-fold less. The rank of affinities for the two soluble receptors are remarkably different for each of the three IL-1 molecules. The rank for the three IL-1 ligands binding to IL-1sRI is IL-1Ra > IL-1α > IL-1β, whereas for IL-1sRII, the rank is IL-1β > IL-1α > IL-1Ra. Elevated levels of IL-1sRII are found in the circulation of patients with sepsis and in the synovial fluid of patients with active rheumatoid arthritis, whereas the elevations of soluble type I receptor in these fluids are 10-fold lower.

High-dose IL-2 therapy induces IL-1sRI and IL-1sRII.
Differences in binding affinities between cell-bound and soluble forms of IL-1R. Differences exist in the binding affinity and association and dissociation rates of the mature forms of each member of the IL-1 family to cell-bound and soluble IL-1R receptors.\textsuperscript{194,215,224,225,229} In some cells, there is a discrepancy between the dissociation constant of either form of IL-1 (usually 200 to 300 pmol/L) and concentrations of IL-1 that can elicit a biologic response (10 to 100 pmol/L). In cells expressing large amounts of IL-1R-AcP, the high-affinity binding of the IL-1R/IL-1R-AcP complex may explain which two classes of binding have been observed. Human, recombinant 17-kD IL-1α binds to cell surface and soluble type I receptors with approximately the same affinity (200 to 300 pmol/L); however, binding to surface and soluble type II receptors is nearly 100-fold less (30 and 10 nmol/L, respectively). If one examines the binding of IL-1Ra, the affinity is even higher than that of IL-1α. IL-1Ra avidly binds to the surface type I receptor (50 to 100 pmol/L). Although IL-1Ra binds less to the soluble form of this receptor, it is, nevertheless, a high-affinity binding. One can conclude from the in vitro binding studies that, once IL-1Ra binds to the cell surface type I receptor, it is very effective as a blocker of IL-1 activity. However, during disease, the effectiveness of IL-1Ra is less apparent.

Although the type I receptor binds IL-1α or IL-1β and transmits a signal upon complex formation with the IL-1R-AcP, of the three members of the IL-1 family, IL-1β has the lowest affinity for the cell bound form of IL-1RI (500 pmol/L to 1 nmol/L). By comparison, IL-1β binds more avidly to the nonsignal transducing type II receptor (100 pmol/L). IL-1β binding to the soluble form of the IL-1RI is lower compared with the cell bound receptor. However, the most dramatic differences in IL-1β binding can be seen at the level of the soluble form of the type II receptor. Of the three ligands, the most avid binding is that of mature IL-1β (500 pmol/L). By comparison, IL-1α and IL-1Ra bind with 50-fold or lower affinities. In addition to the highest affinity, IL-1β binding to IL-1sRII is nearly irreversible due to a long dissociation rate (2 hours).\textsuperscript{194,212} Moreover, proIL-1β also preferentially binds to IL-1sRII.\textsuperscript{223,224} Unlike soluble TNF receptors,\textsuperscript{225} it is unknown whether the IL-1sRII protects IL-1β and prolongs its half-life in the circulation.

Anti-IL-1α autoantibodies. Neutralizing autoantibodies directed against IL-1α may function as "natural buffers" for IL-1α just as soluble type II receptors do for IL-1β. Autoantibodies to IL-1α have been detected in 20% to 25% of healthy subjects.\textsuperscript{251,252} These are neutralizing IgG antibodies and bind both natural proIL-1α as well as 17-kD recombinant IL-1α.\textsuperscript{253} The incidence of these antibodies in the population is increased in patients with autoimmune diseases.\textsuperscript{254,255} When injected into rats, these autoantibodies prolong the half-life of IL-1α.\textsuperscript{254} Commercial preparations of intravenous IgG contain these antibodies and it has been calculated that patients infused with therapeutic concentrations of intravenous IgG receive about 2 μg of anti-IL-1α.\textsuperscript{255} Two antibody molecules bind to one IL-1α molecule, suggesting that these antibodies recognize two epitopes on IL-1α.\textsuperscript{256} In a competitive receptor binding assay, these antibodies completely prevent the binding of IL-1α to type I cell surface receptors.\textsuperscript{256}

Commentary on the balance of two IL-1 receptors. Unlike other cytokine receptors, in cells expressing both IL-1 type I and type II receptors, there is competition to bind IL-1 first. This competition between signaling and non-signaling receptors for the same ligand appears unique to cytokine receptors, although it exists for atrial natriuretic factor receptors.\textsuperscript{257} Because the type II receptor is more likely to bind to IL-1β than IL-1α, this can result in a diminished response to IL-1β. The soluble form of IL-1sRII circulates in healthy humans at mole concentrations that are 10-fold greater than those of IL-1β measured in septic patients and 100-fold greater than the concentration of IL-1β after intravenous administration.\textsuperscript{155} Why do humans have a systemic response to an infusion of IL-1β? One concludes that binding of IL-1β to the soluble form of IL-1R type II exhibits a slow "on" rate compared with the cell bound IL-1RI.

In addition to naturally occurring conditions that reduce a biologic response to IL-1β, neutralizing antibodies to IL-1α are present in many subjects and likely reduce the activity of IL-1α. Vaccinia and cowpox virus genes encode for a protein with a high amino acid homology to the type II receptor and this protein binds IL-1β.\textsuperscript{238,239} Despite the portfolio of soluble receptors and naturally occurring antibodies, IL-1 produced during disease does, in fact, trigger the type I receptor because, in animals and humans, blocking receptors or neutralizing IL-1 ameliorates disease. These findings underscore the high functional level of only a few IL-1 type I receptors. They also imply that the postreceptor triggering events are greatly amplified. It seems reasonable to conclude that treating disease based on blocking IL-1R needs to take into account the efficiency of so few type I receptors initiating a biologic event.

SIGNAL TRANSDUCTION

Early events in IL-1 signal transduction. The topic of IL-1 signal transduction has been reviewed in detail.\textsuperscript{201,246,247} Within a few minutes after binding to cells, IL-1 induces several biochemical events. It remains unclear which is the most "upstream" triggering event or whether several occur at the same time. No sequential order or cascade has been identified, but several signaling events appear to be taking place during the first 2 to 5 minutes. Some of the biochemical changes associated with signal transduction are likely to be cell specific. Within 2 minutes, hydrolysis GTP,\textsuperscript{247} phosphotidylinositol, phosphotidylserine, or phosphotidylethanolamine\textsuperscript{188,244} and release of ceramide by neutral,\textsuperscript{245} not acidic, sphingomyelinase\textsuperscript{246} have been reported. In general, multiple protein phosphorylations and activation of phosphatases can be observed within 5 minutes\textsuperscript{247} and some are thought to be initiated by the release of lipid mediators. The release of ceramide has attracted attention as a possible early signaling event.\textsuperscript{248} Phosphorylation of PLA\textsubscript{2} activating protein also occurs in the first few minutes,\textsuperscript{249} which would lead to a rapid release of arachidonic acid. Multiple and similar signaling events have also been reported for TNF.

Of special consideration to IL-1 signal transduction is the unusual discrepancy between the low number of receptors (<10 in some cells) and the low concentrations of IL-1 that can induce a biologic response.\textsuperscript{250} However, this latter
observation may be clarified in studies on high-affinity binding with the IL-1R-AcP complex. A rather extensive "amplification" step(s) takes place after the initial postreceptor binding event. The most likely mechanism for signal amplification is multiple and sequential phosphorylations (or dephosphorylations) of kinases that result in nuclear translocation of transcription factors and activation of proteins participating in translation of mRNA. IL-1RI is phosphorylated after IL-1 binding. It is unknown whether the IL-1R-AcP is phosphorylated during receptor complex formation. In primary cells, the number of IL-1RI type I is very low (<100 per cell) and a biologic response occurs when only as few as 2% to 3% of IL-1RI receptors are occupied. In IL-1-responsive cells, one assumes that there is constitutive expression of the IL-1R-AcP.

With few exceptions, there is general agreement that IL-1 does not stimulate hydrolysis of phosphatidylinositol or an increase in intracellular calcium. Without a clear increase in intracellular calcium, early postreceptor binding events nevertheless include hydrolysis of a GTP with no associated increase in adenyl cyclase,242,243 activation of adenyl cyclase,251,252 hydrolysis of phospholipids,188,253 release of ceramide,248 and release of arachidonic acid from phospholipids via cytosolic phospholipase A₂ (PLA₂) after its activation by PLA₂ activating protein.249,250 In addition, tyrosine phosphorylations have been reported.256 Each of the above-mentioned mechanisms occurs within the first few minutes after the addition of IL-1 to cultured cells.

Although few comparative studies have been reported, it appears that some IL-1 signaling events are prominent in different cells. Postreceptor signaling mechanisms may therefore provide cellular specificity. For example, in some cells, IL-1 is a growth factor and signaling is associated with serine/threonine phosphorylation of the MAP kinase p42/44 in mesangial cells.257 The MAP p38 kinase, another member of the MAP kinase family, is phosphorylated in fibroblasts,80 as is the p54 MAP kinase in hepatocytes.81 These somewhat different phosphorylations may distinguish the phenotypic response in various cells stimulated with IL-1.

Characteristics of the cytoplasmic domain of the IL-1RI

The cytoplasmic domain of the IL-1RI does not contain a consensus sequence for intrinsic tyrosine phosphorylation but deletion mutants of the receptor show specific functions of some domains. There are four nuclear localization sequences that share homology with the glucocorticoid receptor. Three amino acids (Arg-518, Lys-515, and Arg-518) also found in the Toll protein are essential for IL-1-induced IL-2 production.200 However, deletion of a segment containing these amino acids did not affect IL-1-induced IL-8.200 If this discrepancy is due to different signaling pathways for IL-1-induced IL-2 or for induction of IL-8, then the cytoplasmic domain of IL-1RI can induce more than one biochemical event. There are also two cytoplasmic domains in the IL-1RI that share homology with the IL-6-signaling gp130 receptor. When these regions are deleted, there is a loss of IL-1-induced IL-8 production.202

The C-terminal 30 amino acids of the IL-1RI can be deleted without affecting biologic activity.258 Two independent studies have focused on the area between amino acids 513-529. Amino acids 508-521 contain sites required for the activation of NFκB. In one study, deletion of this segment abolished IL-1-induced IL-8 expression,200 and in another study, specific mutations of amino acids 513 and 520 to alanine prevented IL-1-driven E-selectin promoter activity.258 This area is also present in the Toll protein domain associated with NFκB translocation and previously shown to be part of the IL-1 signaling mechanism.258 This area (513-520) is also responsible for activating a kinase that associates with the receptor. This kinase, termed "IL-1RI associated kinase" phosphorylates a 100-kD substrate.256 Others have reported a serine/threonine kinase that complexates with the IL-1RI.259 Amino acid sequence comparisons of the cytosolic domain of the IL-1RI have shown similarities with a PKC acceptor site. Because PKC activators usually do not mimic IL-1--induced responses, the significance of this observation is unclear.

Activation of MAP kinases after IL-1 receptor binding

Multiple phosphorylations take place during the first 15 minutes after IL-1 receptor binding. A comparison of IL-1--induced phosphorylations with those induced by phorbol esters via PKC shows some similarities. However, unique, non-PKC activated kinases are also stimulated by IL-1, possibly through release of diacylglycerol188 with activation on non-PKC kinases.247 Most consistently, IL-1 activates protein kinases that phosphorylate serine and threonine residues, which are the targets of the MAP kinase family. An early study reported an IL-1--induced serine/threonine phosphorylation of a 65-kD protein clearly unrelated to those phosphorylated via PKC.201 Other studies have implicated a role for PKC.252 As reviewed by O'Neill,242 before IL-1 activation of serine/threonine kinases, IL-1 receptor binding results in the phosphorylation of tyrosine residues.80,81 Tyrosine phosphorylation induced by IL-1 is likely due to activation of MAP kinase that then phosphorylates tyrosine and threonine on MAP kinases.
After activation of MAP kinases, there are phosphorylations on serine and threonine residues of the epidermal growth factor receptor, heat-shock protein p27, myelin basic protein and serine 56 and 156 of β-casein, each of which has been observed in IL-1-stimulated cells. TNF also activates these kinases. There are at least three families of MAP kinases. The p42/44 MAP kinase family is associated with signal transduction by growth factors including ras-raf-1 signal pathways. In rat mesangial cells, IL-1 activates the p42/44 MAP kinase within 10 minutes and also increases de novo synthesis of p42. In addition to p42/44, two members of the MAP kinase family (p38 and p54) have been identified as part of an IL-1 phosphorylation pathway and are responsible for phosphorylating hsp27. These MAP kinases are highly conserved homologs to the HOG-1 stress gene in yeasts. In fact, when HOG-1 is deleted, yeasts fail to grow in hyperosmotic conditions; however, the mammalian gene coding for the IL-1-inducible p38 MAP kinase can reconstitute the ability of the yeast to grow in hyperosmotic conditions. In cells stimulated with hyperosmolar NaCl, LPS, IL-1, or TNF, indistinguishable phosphorylation of the p38 MAP kinase takes place. In human monocytes exposed to hyperosmolar NaCl (375 to 425 milliosmol/L), IL-8 gene expression and synthesis takes place that is indistinguishable from that induced by LPS or IL-1. Thus, the MAP kinase pathways involved in IL-1, TNF, and LPS signal transductions share certain elements that are related to the primitive stress-induced pathway. The dependency of Rho members of the Rho family (p38 and p54) have been identified as part of an IL-1R-Acp can reconstitute the IL-1 activation of these transcription factors. The multiple postreceptor phosphorylations may explain why IL-1 induces several genes in the same cell at the same time. LPS and TNF share with IL-1 many of these same MAP kinase pathways, most of them related to the HOG-1 "stress" gene family of MAP kinases. These observations support the concept that IL-1 signal transducing events mimic the evolutionary benefit of cell stress.

### IL-1 Production in Health and in Disease

**Age- and gender-related differences in IL-1 production.** No age-related changes were observed in PBMC production of IL-1β, proIL-1β, or TNFα in 420 elderly subjects (78 ± 5 years) compared with 50 younger controls (40 ± 11 years). However, in the elderly group, there was a 10-fold increase in production of IL-1Ra in the same samples. Elevated urine levels of IL-1β have been reported in older individuals and, using mitogen stimulation, there was an age-related increase in IL-1β, TNFα, and IL-6 production in 13 elderly subjects (80.8 ± 2.1 years) compared with a younger group. Compared with age-matched males, females produce IL-1 in association with their menstrual cycle when IL-1 is measured in the circulation, spontaneously released in cultures of PBMC, or excreted into the urine. In another study, human ovarian cells and PBMC increase their production of IL-1α and IL-1α mRNA during the luteal phase.

**IL-1 production in various disease states.** The production of IL-1 in various diseases has been examined in many studies. In general, there is increased gene expression and synthesis of IL-1α, IL-1β, or IL-1Ra when determined by in situ hybridization, steady state mRNA levels, antibody staining of tissues, circulating levels, or ex vivo production from cultured cells. Increased IL-1 production has been reported in patients with various viral, bacterial, fungal, and parasitic infections; intravascular coagulation; high-dose IL-2 therapy; solid tumors; leukemias; Alzheimer's disease; HIV-1 infection; autoimmune disorders; trauma (surgery); hemodialysis; ischemic diseases (myocardial infarction); noninfectious hepatitis; asthma; UV radiation; closed head...
injury; pancreatitis; peridontitis; graft-versus-host disease; transplant rejection; and in healthy subjects after strenuous exercise. There is an association of increased IL-1β production in patients with Alzheimer's disease and a possible role for IL-1 in the release of the amyloid precursor protein.279 However, in most conditions, IL-1 is not the only cytokine exhibiting increased production and hence the specificity of the IL-1 findings as related to the pathogenesis of any particular disease is lacking.

Circulating IL-1 in humans. In various disease states, IL-1β but not IL-1α is detected in the circulation. Detection of IL-1α is rare because this cytokine is primarily cytosolic and is released only under unusual in vitro280 or in vivo conditions281 and probably due to cell death. The presence of autoantibodies to IL-1α in about 25% of healthy humans (more frequently in humans with autoimmune diseases) most likely interferes with detecting circulating IL-1α.231,235 Significant elevations in plasma IL-1β have been detected in healthy humans injected with LP5280,281 and in patients with septic shock and burns,282 but circulating IL-1β levels are relatively low compared with levels of IL-6, IL-8, and TNFα. Correlations have been shown for plasma IL-1β levels and severity of acute attacks of rheumatoid arthritis,283 thermal burns,284 and mortality in septic shock.285 Circulating levels of IL-6 or IL-1Ra are frequently correlated with severity of sepsis, inflammation, trauma, graft rejection, and autoimmune diseases and measurement of IL-6 as a surrogate marker for IL-1β is supported by recent studies in IL-1β-deficient mice.16

Why are IL-1β levels low and difficult to detect? Unlike TNFα, IL-6, or IL-1Ra, a significant amount of proIL-1β remains inside the cell. IL-1β also binds to large proteins such as α-2-macroglobulin, complement,286 and the soluble type II IL-1 receptor. Plasma normally contains approximately 100 pmol/L of IL-1αRII,274,276 which preferentially binds IL-1β compared with IL-1α or IL-1Ra.231,234,235,236 Concentrations of IL-1β in severe sepsis are rarely greater than 500 pg/mL (30 pmol/L)287 and are usually in the range of 10 pmol/L.280 The presence of IL-1αRII at 100 pmol/L reduces the detection of IL-1β in clinical samples by 50%.225

Production of IL-1 from cells in vitro and ex vivo. In general, IL-1 is not present in PBMC when taken from healthy subjects; however, when incubated for several hours, PBMC from patients with rheumatoid arthritis,291 osteomalacia, sepsis, hemodialysis, myocardial infarction, and some vasculitides produced elevated amounts of IL-1 compared with control subjects. Extreme caution needs to be exercised so that contaminating endotoxins in the tissue culture medium do not artifactually stimulate “spontaneous” IL-1 production during the culture period. IL-1 and other cytokines are also produced in whole blood cultures, thus eliminating the need to separate the mononuclear population. There is a positive correlation between production of IL-1 in whole blood and in PBMC cultures (r = .746, P = .005).288

Genetic linkage of disease and IL-1. In humans, IL-1α, IL-1β, and IL-1Ra are found on the long arm of chromosome 2,290 distributed over 430 kb.187 In addition, IL-1RI and IL-1RII genes also localize to chromosome 2,183 although in mice IL-1RI is found on chromosome 1. Some autoimmune diseases appear to be linked, in part, to IL-1 gene polymorphisms. For example, there is a TaqI polymorphism in the 5th exon of the human IL-1β gene that is a T to C transition. This mutation at amino acid 105 of proIL-1β is associated with a significantly greater portion of IL-1β secreted from the monocytes of homozygous diabetic individuals compared with monocytes from control subjects.292 Polymorphisms in IL-1α have also been reported but their link to disease has not been studied in detail.293,294

Several autoimmune diseases are associated with a polymorphism in the IL-1Ra gene.295 There are three intron alleles for IL-1Ra and a significantly higher incidence of allele 2 in humans with psoriasis, inflammatory bowel disease, autoimmune diabetes mellitus, diabetic nephropathy, and skin manifestations of autoimmune diseases compared with unaffected controls.296,297 In patients with autoimmune diabetes, allele 2 is associated with lower levels of plasma IL-1Ra compared with nondiabetic humans with allele 2.298 Polymorphism has also been found in the promoter region of IL-1RI in patients with autoimmune diabetes that is not present in controls.299 The polymorphism is due to a single basepair transition from C to T in the second promoter region (P2) of exon 1B, but this mutation did not seem to affect any known transcription recognition factors.300 In the nonobese diabetic mouse, an additional intron has been inserted in the coding regions of the type I receptor.301

Commentary on the association of disease severity and IL-1 production. The literature on IL-1 contains well over several hundred citations on the production of IL-1 in animals and humans in association with different diseases. If IL-1 production from cells or IL-1 levels in a body fluid are perfectly correlated with a particular disease, the relevance of IL-1 to that disease is still unproven. Only specific IL-1 neutralization or receptor blockade can establish causation or contribution. This restriction is not limited to IL-1 but exists for each cytokine. Nevertheless, studying IL-1 production in disease states can be useful as a marker of disease progression or therapeutic efficacy. Increasing evidence shows that the circulating level of IL-6 rather than IL-1 is the best assessment for an association of elevated production of biologically active IL-1 with the severity of disease. IL-6 levels appear to reflect synergistic induction by IL-1 and TNF during most disease states.

REDUCING PRODUCTION OF IL-1

Corticosteroids. Of the drugs that suppress the production of IL-1, the most studied are corticosteroids. Corticosteroids inhibit the transcription of IL-1, TNF, and nearly all cytokines; hence, they are not specific in this regard. There is also some indication that steroids reduce the secretion of IL-1. In human volunteers injected with corticosteroids just before an intravenous injection of endotoxin, there are reduced levels of circulating IL-1β, TNF, and IL-6.302,303 These reductions in IL-1β, TNF, and IL-6 take place without suppressing IL-1Ra production.304 Increased synthesis of 1αB with decreased translocation of NFκB is thought to account for the suppressive effect of glucocorticoids on cytokine synthesis.304,305

Inhibition of lipooxygenase. Earlier studies had implicated leukotriene B4 (LTB4) as the lipooxygenase product
triggering IL-1 and TNF synthesis. However, using specific inhibitors of 5-lipoxygenase, the importance of LTB4 in the production of cytokines is unlikely. When ω-3 fatty acids are incorporated into cell membranes, the cyclooxygenase and lipoxygenase products after phospholipase-mediated hydrolysis of membrane phospholipids are not PGE2 and LTB4, but rather PGF2 and LTB4. This change alters signal transduction pathway induced by exogenous stimulants and results in an attenuation in the synthesis of proinflammatory cytokines.

Inhibition by dietary ω-3 fatty acids. In controlled studies, a 50% to 60% decrease in IL-1, IL-6, and TNF production has been consistently observed in PBMC or subjects ingesting ω-3 fatty acid supplements compared with PBMC taken before this dietary intervention. The phenomenon can also be shown by measuring cytokine production in whole blood or circulating levels of IL-1β.

The source of the ω-3 fatty acid does not have to be from high-dose dietary supplementation. In fact, a significant suppression of in vitro IL-1 and TNF production was observed in volunteers consuming low-fat diets using fish as the primary source of protein. Although not the topic of this review, many studies have shown a reduction in inflammatory disease associated with increased ω-3 fatty acid content in membrane phospholipids. IL-4, IL-10, IL-13, and transforming growth factor-β (TGFβ). IL-4 and IL-10 have growing promoting effects on lymphocytes. These cytokines are often grouped together and called T-helper-2 (Th2) cytokines because they primarily promote the expansion of antibody-producing B cells and suppress Th1-mediated responses such as cellular immunity (delayed hypersensitivity) and generation of cytotoxic T cells. Although TGFβ suppresses lymphocyte proliferation, this ubiquitous cytokine promotes bone and fibroblast growth. In addition to their effects on cell growth, IL-4, IL-10, IL-13, and TGFβ suppress gene expression and synthesis of IL-1, TNF, and other cytokines. As such, they are potentially useful in some clinical situations. Mice deficient in IL-10 or TGFβ1 spontaneously develop multiple autoimmune diseases.

A randomized, double-blind, placebo controlled trial (phase I) in healthy human volunteers studied the effect of a single intravenous injection of IL-10 on cytokine production. Blood was removed before and 3, 6, 24, and 48 hours after the injection and incubated in vitro with endotoxin and the amounts of IL-1β, TNFα, IL-6, IL-8, IL-1Ra, and TNF soluble receptor p55 (TNFsp55) were measured. At doses of 10 or 25 μg/kg, there was a 90% reduction in LPS-induced IL-1β, TNFα, and IL-6 production in blood taken 3 and 6 hours after the injection; at 25 μg/kg, a 50% reduction in IL-1β, TNFα, and IL-6 production was present at 24 and 48 hours. In contrast, there was no suppression of IL-1Ra or TNFsp55. Mitogen-induced production of IFNγ was reduced in subjects receiving IL-10.

IL-4 and IL-13 suppress LPS-induced IL-1 and TNF gene expression and synthesis. In addition, they increase IL-1Ra production. IL-4 and IL-13 share the same receptor complex on monocytes; hence, similar biologic effects for both cytokines are often observed. However, there are few if any receptors for IL-13 on T lymphocytes; hence, the immunologic suppressive effects of IL-4 and IL-10 are not observed for IL-13. Similar to IL-4, IL-10, and IL-13, TGFβ suppresses gene expression and synthesis of IL-1 and TNF and also increases IL-1Ra production. However, TGFβ has profound immunosuppressive effects and also is a growth factor for normal and neoplastic cells.

IL-6 and other ligands for the gp130 signal transducer. Although IL-6 is found in a variety of inflammatory, hematologic, and infectious diseases, IL-6 does not cause hypotension or inflammatory symptoms when infused into humans at doses 10,000-fold greater than those of IL-1 or TNF. Instead, IL-6 induces high levels of IL-1Ra and TNFsp55 in humans. IL-6 does not induce PGE2, but rather suppresses IL-1–inducible cyclooxygenase, in addition, IL-6 suppresses gene expression and synthesis of inflammatory cytokines. In mice deficient in the IL-6 gene, there are higher levels of TNF compared with the control mice. A specific receptor for IL-6 (IL-6Ra) binds the ligand and the IL-6/IL-6Ra complex triggers homodimerization of gp130, a common signal transducer on many cells. Other cytokines that use gp130 for signaling are leukemia inhibitory factor (LIF), IL-11, oncostatin-M, cardioprotein-1, and CNTF. Similarly to IL-6, CNTF binds to its specific soluble receptor and, when incubated with human blood PBMC, the CNTF/soluble receptor complex suppresses LPS-induced PGE2 and IL-1α synthesis. In addition, the CNTF/soluble receptor complex suppresses IL-1α–induced IL-8 and PGE2 synthesis. The above studies suggest that signaling of gp130 inhibits IL-1–mediated cellular events.

IFNs. In some clinical situations, IFNs are thought to exert their effects by acting as anti-inflammatory agents. Early studies showed that IFNγ suppressed LPS or IL-1–induced PGE2 production in human monocytes. Although it is well-known that IFNγ suppresses IL-1 and TNF production induced by LPS, using IL-1 as a stimulant, IFNγ suppresses IL-1–induced IL-1. Subsequently, it was shown that IFNα also suppressed IL-1–induced IL-1 synthesis due to reduced transcription. Like CNTF, suppression of IL-1–induced IL-8 transcription by IFNγ has been reported to be mediated by NFKB. Of considerable importance is the finding that tyrosine phosphorylations after triggering of IFN receptors result in a kinase cascade similar to that described for gp130. Thus, depending on the signal, the phosphorylation of p91 can lead to suppression of IL-1–induced gene expression.

Effect of cyclooxygenase inhibitors on the production of IL-1. In general, adding cyclooxygenase inhibitors to LPS-stimulated PBMC in vitro can suppress, augment, or have no effect on IL-1 production. On the other hand, under the same culture conditions, LPS–induced TNF gene expression and synthesis is exquisitely sensitive to suppression by PGE2 and PGF2. In humans injected with LPS and treated with oral cyclooxygenase inhibitors, the circulating levels of TNF and IL-6 are higher compared with controls not receiving cyclooxygenase inhibitors. The mechanism of PGI2/PGE2–induced TNF suppression is via elevation in cAMP and explains why drugs such as pentoxiphylline suppress TNF production. An increase in cAMP can also be accomplished by triggering the H2 receptor on monocytes with histamine without the intermediate production of PGE2.
In the absence of an external stimulus, increasing intracellular levels of cAMP has a minimal or no effect on IL-1β gene expression. In contrast to PBMC stimulated with LPS, when these cells are stimulated with IL-1, cyclooxygenase inhibitors suppress IL-1 and IL-6 production. To confirm this observation, the addition of either exogenous PGE₂ or histamine to increase intracellular cAMP results in enhanced IL-1-induced IL-1, IL-6, and IL-8 synthesis. In terms of noninfectious diseases, cyclooxygenase inhibitors likely reduce IL-1-induced cytokine production. One can conclude that IL-1-induced cytokine synthesis is positively influenced by prostanooid-induced cAMP, but in the same cells, LPS-induced cytokines are either unaffected or minimally reduced.

Commentary on drugs and cytokines reducing IL-1 production. Several orally active “anti-inflammatory” drugs presently used clinically affect the production or activity of IL-1 (eg, corticosteroids and cyclooxygenase inhibitors). Corticosteroids suppress IL-1 gene expression and secretion and increase type II IL-1R expression. Cyclooxygenase inhibitors reduce IL-1-induced IL-1, IL-6, and IL-8 synthesis. In addition, cyclooxygenase suppress PGE₂-induced increased IL-1RII surface expression, reducing a biologic response to IL-1. IFNs, which are the most widely used cytokines in humans, also reduce IL-1-induced IL-1 production and increase IL-1Ra production. In a similar manner, most of the cytokines using gp130 suppress IL-1-induced genes and augment the production of IL-1Ra. Suppressing IL-1 production or activity by endogenously produced cytokines such as IL-4, IL-10, IL-13, TGFβ, or cytokines triggering gp130 suggests that an anti-inflammatory cytokine network contributes to disease outcome by affecting the production or activities of IL-1 and TNF.

MECHANISMS OF IL-1-INDUCED BIOLOGIC EFFECTS

Multiple biologic effects of IL-1. In vitro and in vivo experiments have shown multiple biologic effects of either IL-1α or IL-1β without much species specificity. Most studies have used recombinant human IL-1 injected into a wide variety of animals ranging from grasshoppers and fishes to primates and humans. There are some notable exceptions in the hypothalamic responses of rats in which human IL-1β is more likely to have an effect than human IL-1α. Table 3 depicts commonly studied biologic effects of IL-1. These effects have been reviewed in detail, particularly in the areas of IL-1 effects on pancreatic endocrine tissue, thyroid gland, hypothalamic-pituitary-adrenal axis, fever, bone metabolism, destruction of cartilage in the pathogenesis of rheumatoid arthritis, uterine implantation, and loss of lean body mass. Effect of IL-1 on gene expression. The basis for the varied biologic properties of IL-1 is the effects of this single cytokine on the expression of various genes and/or expression of surface receptors. In general, IL-1 either initiates transcription or stabilizes mRNA for a variety of genes. This is particularly the case for many inducible genes that are responsive to exogenous or endogenous challenges (Table 4). These include the ability of IL-1α and IL-1β to increase the expression of the IL-1 family of genes. In a similar fashion, other inflammatory cytokines, lymphocyte growth factors, colony-stimulating factors, and mesenchymal growth factor genes are upregulated by IL-1. For example, concentrations of 5 pmol/L IL-1 increase transcription and synthesis of hepatocyte growth factor. In contrast, some constitutively expressed genes are suppressed by IL-1 (Table 5). Of these, the expression of housekeeping genes such as albumin and cytochrome p450 are suppressed by IL-1 at the transcriptional level and the half-life of mRNA is not affected by IL-1. These effects of IL-1 on gene expression for cytokines or albumin are also observed in vivo.
Table 4. Increased Expression of Various Genes by IL-1

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Tissue remodeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1; IL-1Ra; TNF*; IL-2*; IL-3*; IL-6*; IL-12; GM-CSF; TGF-β-3</td>
<td>Stromelysin; gelatinases; elastase; collagenases*†</td>
</tr>
<tr>
<td>G-CSF; M-CSF; stem cell factor; leukemia inhibitory factor; IFNα, β, γ</td>
<td>Tissue inhibitor of metalloproteinases-1; transin</td>
</tr>
<tr>
<td>IL-8* and other chemokines</td>
<td></td>
</tr>
<tr>
<td>Macrophage inflammatory protein-1α</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cytokine receptors</th>
<th>Neuropeptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2 (p55)*†</td>
<td>Pro-opiomelanocorticotropin, corticotropin releasing factor*†</td>
</tr>
<tr>
<td>IL-2; IL-3; IL-5; GM-CSF (β-c receptor chain); c-kit</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Proinflammatory mediators</th>
<th>Lipid synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclooxygenase, type-2*†</td>
<td>Triglyceride increasing Apo CIII*</td>
</tr>
<tr>
<td>Cytosolic and secretory phospholipase A, type-2*</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>Inducible nitric oxide synthase*</td>
<td></td>
</tr>
<tr>
<td>Endothelin-1</td>
<td></td>
</tr>
<tr>
<td>Gamma glutamyl transferase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hepatic acute phase reactants</th>
<th>Oncogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn superoxide dismutase*</td>
<td>c-jun*; c-abl*; c-fms*; c-myc*; c-fos*</td>
</tr>
<tr>
<td>C-reactive protein; serum amyloid A*</td>
<td></td>
</tr>
<tr>
<td>Complement C2; C3; factor B</td>
<td></td>
</tr>
<tr>
<td>Metallothioneins; ceruloplasmin; lysozyme</td>
<td></td>
</tr>
<tr>
<td>Xanthine dehydrogenase; xanthine oxidase</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Growth factors</th>
<th>Adhesion molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF A chain; fibroblast growth factor; keratinocyte growth factor</td>
<td>ICAM-1*; ELAM*1; VCAM-1; lymphocyte L-selectin</td>
</tr>
<tr>
<td>Hepatocyte growth factor</td>
<td></td>
</tr>
<tr>
<td>Nerve growth factor*</td>
<td></td>
</tr>
<tr>
<td>Melanoma growth stimulatory activity (pro-α, β, γ)*†</td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor-1*†</td>
<td></td>
</tr>
<tr>
<td>Activin-A</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clotting factors</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen; tissue factor</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>Urokinase plasminogen activator†</td>
<td>FGF</td>
</tr>
<tr>
<td>Type 1 and 2 plasminogen activator inhibitor*†</td>
<td>IL-1R type II</td>
</tr>
<tr>
<td>Protease nexin-1†</td>
<td></td>
</tr>
</tbody>
</table>

* Increased transcription.
† Stabilization/increased mRNA half-life.

The differential effect on gene expression is consistent with the requirement of the host undergoing an exogenous or endogenous challenge to boost its natural defenses while at the same time to conserve energy by suppressing those genes not needed for the response. The best example is infection and injury in which IL-1 increases gene expression for each of the colony-stimulating factors resulting in increased bone marrow neutrophil release. On the other hand, IL-1 induces the transcription or increases the steady-state mRNA levels for genes associated with inflammation. Three genes that code for specialized enzymes appear to be exquisitely sensitive to IL-1: iNOS, type-2 cyclooxygenase (COX-2), and type-2 phospholipase A2 (PLA2). Their products, NO, PGs, leukotrienes, and platelet-activating factor, are potent proinflammatory mediators. By increasing gene expression and synthesis for these enzymes, the effects of IL-1 are prolonged several hours after triggering the cell. In some cells, IL-1 induction of PGE2 increases cAMP levels that, together with IL-1, enhance the response. In the case of induction of NO synthase gene expression, IL-1 inhibits iNOS via the protein kinase C epsilon isoenzyme, suggesting the existence of a negative feedback for IL-1-induced NO synthase.

Effect of IL-1 on receptor expression. Whether at the level of gene or surface expression, an important biologic effect of IL-1 is to regulate cytokine receptors, noncytokine receptors, and cellular adhesion molecules. IL-1-induced changes in the binding of ligands to their respective receptors are not due to altered affinities but rather to a change in the number of surface receptors. As shown in Table 6, the change in surface expression may be reflected at the level of receptor gene expression. IL-1-mediated upregulation of adhesion molecule expression on cultured endothelial cells is a commonly used laboratory method in several studies. Low concentrations (1 to 10 pmol/L) of IL-1 alone increases gene and surface receptor expression for ELAM and ICAM-1. In contrast, an increase in IL-2 (p55) receptors by IL-1 requires a costimulant. The p55 TNF receptor is downregulated by IL-1; on closer examination, this effect of IL-1 is due to shedding of the receptor as well as suppression of gene expression.

IL-1 effects mediated by prostanoids. Many IL-1-in-
proliferation of these cells; when cyclooxygenase inhibitors are incorporated into the culture, IL-1 stimulates cell proliferation. When cyclooxygenase inhibitors for a variety of inflammatory conditions is often a therapeutic strategy to reduce IL-1-induced PGE₂. Humans injected with IL-1 experience fever, headache, myalgias, and arthralgias, each of which is reduced by coadministration of cyclooxygenase inhibitors. One of the more universal activities of IL-1 is the induction of gene expression for type-2 phospholipase A₂ and COX-2. IL-1 induces transcription of COX-2 and seems to have little effect on increased production of COX-1. Moreover, once triggered, COX-2 production is elevated for several hours and large amounts of PGE₂ are produced in cells stimulated with IL-1. Therefore, it comes as no surprise that many biologic activities of IL-1 are actually due to increased PGE₂ production. Table 7 depicts cyclooxygenase-dependent and independent effects of IL-1 in vitro and in vivo.

There appears to be selectivity in cyclooxygenase inhibitors in that some nonsteroidal anti-inflammatory agents are better inhibitors of COX-2 rather than COX-1. Similar to COX-2 induction, IL-1 preferentially stimulates new transcripts for the inducible type-2 form of PLA₂, which cleaves the fatty acid in the number 2 position of cell membrane phospholipids. In most cases, this is arachidonic acid. The release of arachidonic acid is the rate-limiting step in the synthesis of prostaglandins and leukotrienes. IL-1 also stimulates increased leukotriene synthesis in many cells.

One must consider that, when cells are stimulated with IL-1 in vitro, a significant release of PGE₂ takes place and that, in the absence of cyclooxygenase inhibitors, PGE₂ accumulates in the culture vessel and exerts its own effects. For example, human smooth muscle cells incubated with IL-1 produce large amounts of PGE₂, which suppresses the proliferation of these cells; when cyclooxygenase inhibitors are incorporated into the culture, IL-1 stimulates cell proliferation. Most genes induced by IL-1 are not affected by inhibiting PGE₂ synthesis. Gene expression of other cytokines, collagenases, colony-stimulating factors, and hepatic acute-phase proteins are unaffected by cyclooxygenase inhibitors added to cultured cells or administered in vivo. On the other hand, many activities of IL-1 in the central nervous system are mediated by the formation of prostaglandins. IL-1 induction of sleep is an exception.

<table>
<thead>
<tr>
<th>Table 5. Decreased Expression of Genes by IL-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Housekeeping genes</strong></td>
</tr>
<tr>
<td>Albumin*; alkaline phosphatase</td>
</tr>
<tr>
<td>Cytochrome p450c17; p450 IID; II D</td>
</tr>
<tr>
<td><strong>Receptors</strong></td>
</tr>
<tr>
<td>TNFRp55t</td>
</tr>
<tr>
<td>IL-1R type 1</td>
</tr>
<tr>
<td>PDGF or R</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
</tr>
<tr>
<td>TGFβ-1; insulin-like growth factor 1 (in Leydig cells)</td>
</tr>
<tr>
<td><strong>Extracellular matrix proteins</strong></td>
</tr>
<tr>
<td>Fibronectin* and thrombospondin*</td>
</tr>
<tr>
<td>Proteoglycans (chondroitin sulphate)</td>
</tr>
<tr>
<td>Type II collagen†</td>
</tr>
<tr>
<td><strong>Others</strong></td>
</tr>
<tr>
<td>CD34 on endothelialism</td>
</tr>
<tr>
<td>Thyroid peroxidase</td>
</tr>
<tr>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>Glutaminase in fibroblasts</td>
</tr>
</tbody>
</table>

* Decreased constitutive transcription.  
† Decreased mRNA half-life.

<table>
<thead>
<tr>
<th>Table 6. Effect of IL-1 on Surface Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased surface receptors or receptor binding</td>
</tr>
<tr>
<td>LDL binding to hepatocytes*</td>
</tr>
<tr>
<td>Cell adhesion molecules*</td>
</tr>
<tr>
<td>Decay accelerating factor (CD55) on endothelial cells</td>
</tr>
<tr>
<td>Shiga toxin (globotriaosylceramide) and vertoxin on endothelial cells</td>
</tr>
<tr>
<td>Betal subunit of IL-3, IL-5, and GM-CSF*</td>
</tr>
<tr>
<td>c-Kit on bone marrow cells*</td>
</tr>
<tr>
<td>Nuclear receptors for dexamethasone</td>
</tr>
<tr>
<td>IgG binding to endothelial cells</td>
</tr>
<tr>
<td>Complement C1q receptors on fibroblasts</td>
</tr>
<tr>
<td>High endothelial lymphocyte receptors</td>
</tr>
<tr>
<td>Transferrin receptors on fibroblasts*</td>
</tr>
<tr>
<td>la expression on dermal dendritic cells</td>
</tr>
<tr>
<td>IFNγ receptor on macrophages*</td>
</tr>
<tr>
<td>β-glucan receptor on macrophages</td>
</tr>
<tr>
<td>Fc receptor on microglia, macrophages, and leukemic cells</td>
</tr>
<tr>
<td>Complement C3b receptor on neutrophils</td>
</tr>
<tr>
<td>γ-aminobutyric acid receptor†</td>
</tr>
<tr>
<td>IL-2 and IL-4 receptors on leukemic cell lines</td>
</tr>
<tr>
<td>TGFβ receptors on T cells</td>
</tr>
<tr>
<td>Opioid peptide receptor in rat brain and lymphocytes</td>
</tr>
<tr>
<td>TNF receptor (p75)*</td>
</tr>
<tr>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>Decreased surface receptors or receptor binding</td>
</tr>
<tr>
<td>IL-1RI on murine Th2 cells</td>
</tr>
<tr>
<td>PGE₂ binding to macrophage cell lines</td>
</tr>
<tr>
<td>CD68 on endothelial cells</td>
</tr>
<tr>
<td>N-formyl-methionyl-leucyl-phenylalanine receptors on neutrophils</td>
</tr>
<tr>
<td>FGF receptors on endothelial cells</td>
</tr>
<tr>
<td>FcyRII on macrophages but not FcγRI or FcγRII§</td>
</tr>
<tr>
<td>Platelet-derived growth factor-α and -β receptors*</td>
</tr>
<tr>
<td>Luteinizing hormone receptor in granulosa cells</td>
</tr>
<tr>
<td>Peripheral type benzodiazepine receptors</td>
</tr>
<tr>
<td>TNF receptor (p55)*</td>
</tr>
<tr>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>Decreased surface receptors or receptor binding</td>
</tr>
</tbody>
</table>

* Associated with change in receptor gene expression.  
† Facilitates chloride channels.  
‡ Through increased IFNγ production.  
§ Associated with decreased phagocytosis.  
¶ Associated with increased receptor shedding.  
‖ Transient reduction in affinity due to IL-1-mediated phosphorylation.
## Table 7. Cyclooxygenase-Dependent and Cyclooxygenase-Independent Activities of IL-1

<table>
<thead>
<tr>
<th>Cyclooxygenase-dependent</th>
<th>Cyclooxygenase-independent</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo</td>
<td>In vivo</td>
</tr>
<tr>
<td>Fever</td>
<td>Slow wave sleep</td>
</tr>
<tr>
<td>Natriuresis</td>
<td>Non-rapid eye movement sleep</td>
</tr>
<tr>
<td>ACTH and growth hormone release</td>
<td>Neutrophilia</td>
</tr>
<tr>
<td>Suppression of norepinephrine release</td>
<td>Lethality in adrenalectomized mice</td>
</tr>
<tr>
<td>Enhancement of capsaicin hyperemia</td>
<td>Decreased mast cell degranulation</td>
</tr>
<tr>
<td>Suppression of T-cell mitogenesis</td>
<td>Production of CSF</td>
</tr>
<tr>
<td>Increased mucosal ion transport</td>
<td>Hypoferremia; hypozincemia</td>
</tr>
<tr>
<td>Colonic hypersecretion</td>
<td>Hepatic acute phase protein synthesis</td>
</tr>
<tr>
<td>c-fos expression in brain</td>
<td>Hypoglycemia</td>
</tr>
<tr>
<td>Gastroparesis</td>
<td>IL-6 production†</td>
</tr>
<tr>
<td>Decreased pain threshold</td>
<td>TNF production†</td>
</tr>
<tr>
<td>Suppression of insulin release</td>
<td>Increased ovulation</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>Migration of neutrophils into tissues</td>
</tr>
<tr>
<td>Rapid arterial relaxation</td>
<td>Urinary calcium excretion*</td>
</tr>
<tr>
<td>Hypotension in rabbits</td>
<td>Monocyte IL-1 synthesis in adjuvant arthritis</td>
</tr>
<tr>
<td>Intra-articular edema, leucocyte infiltration, and substance P</td>
<td>Protection against</td>
</tr>
<tr>
<td>Increased melanoma bone marrow metastases</td>
<td>Lethal radiation</td>
</tr>
<tr>
<td>Increased cerebrospinal calcium levels</td>
<td>Lethal infection</td>
</tr>
<tr>
<td>Decreased water intake</td>
<td>Acetaminophen-induced hepatotoxicity</td>
</tr>
<tr>
<td>Protection against</td>
<td>In vitro</td>
</tr>
<tr>
<td>Hypoxic lung damage</td>
<td>Gene expression and synthesis of cytokines†</td>
</tr>
<tr>
<td>Skin hypersensitivity</td>
<td>Destruction of islet β cells</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Increased glucose uptake</td>
</tr>
<tr>
<td>In vitro</td>
<td>Augmentation of T-cell responses†</td>
</tr>
<tr>
<td>Facilitation of ion-transport</td>
<td>Corticosterone synthesis (direct)</td>
</tr>
<tr>
<td>Suppression of smooth muscle cell proliferation</td>
<td>Collagenase/stromelysin gene expression</td>
</tr>
<tr>
<td>Inhibition of HLA-DR expression</td>
<td>Induction of inducible NO synthase†</td>
</tr>
<tr>
<td>Increased corticotrop releasing factor</td>
<td>Suppression of gastric secretion</td>
</tr>
<tr>
<td>Formation of osteoclasts</td>
<td>Increased progesterone synthesis</td>
</tr>
<tr>
<td>Expression of heme oxygenase-1 gene expression</td>
<td>Increased nerve growth factor in astrocytes†</td>
</tr>
<tr>
<td>Collagenase and stromelysin*</td>
<td>Increased GM-CSF production†</td>
</tr>
<tr>
<td>Increased relaxation of arterial vessels</td>
<td>Increased IGF-I production in bone cultures*</td>
</tr>
<tr>
<td>Osteoblast production of IGF-II</td>
<td>Induction of LIF in fibroblasts†</td>
</tr>
<tr>
<td>Inhibition of osteoclasts</td>
<td>Induction of LIF in fibroblasts†</td>
</tr>
<tr>
<td>Increased IGF-I production in bone cultures*</td>
<td>Induction of LIF in fibroblasts†</td>
</tr>
<tr>
<td>Induction of LIF in fibroblasts†</td>
<td>Induction of LIF in fibroblasts†</td>
</tr>
</tbody>
</table>

* IL-1-induced PGE effect is via increased cAMP formation.
† Addition of cyclooxygenase inhibitors augment the response.
‡ IL-1-induced PGE2 may augment gene expression and/or synthesis of some IL-1-induced genes, for example, other cytokines and iNOS via increase cAMP (see text).

### Effects of IL-1 mediated by NO.

The generation of NO in disease appears to be a fundamental event (reviewed in Moncada et al34). Several studies have shown that IL-1 induces NOS and specifically the inducible form of NOS (iNOS). These include induction from a variety of cells in vitro and in vivo; eg, IL-1 induces NO from osteoclasts, murine macrophages, pituitary cells, mast cells, osteoblasts, glial cells, insulin producing β cells in the pancreas, smooth muscle cells, chondrocytes, myocytes, and mesangial cells. In mesangial cells, IL-1β-induced NOS is augmented by elevated levels of cAMP.344 Like induction of COX-2 and type-2 PLA2, IL-1 induction of NO likely accounts for a considerable number of biologic effects. For example, in experimental septic shock, the decrease in mean arterial pressure and the decrease in systemic vascular resistance are thought to be mediated by the induction of NO from smooth muscle cells as shown in vitro.345 LPS injection into animals increases NO in several tissues and, when treated with IL-1Ra, there is a 70% decrease in NO.346 In vitro, very low concentrations of IL-1 (1 pg/mL) are sufficient to induce NO synthase. Table 2 depicts some biologic effects of IL-1 that are mediated by IL-1-induced NOS.

In rabbits, blockade of constitutive and inducible NOS with N-nitro-L-arginine methyl ester (L-NAME) resulted in inhibition of IL-1-induced slow wave sleep but not fever.351 However, in the rat, IL-1-induced fever was inhibited,352 as were effects of IL-1 on the endocrine pancreas. In the rat, blocking the generation of NO augmented IL-1-induced release of ACTH.353 In vitro, blocking NO production reduces IL-1-induced inhibition of PAF release from mast cells,
The mechanism for synergism may also involve receptor modulation; however, in the case of IL-1 and TNF synergism, receptors for TNF are downregulated by IL-1.346.7

IL-1 plus TNF
- Hemodynamic shock and lactic acidosis in rabbits
- Radioprotection
- Generation of Schwartzman reaction
- Luteal cell PGE$_2$ synthesis
- PGE$_2$ synthesis in fibroblasts
- Galactosamine-induced hepatotoxicity
- Sickness behavior in mice
- Circulating nitric oxide and hypoglycemia in malaria
- Nerve growth factor synthesis from fibroblasts
- Insulin release and beta islet cell death
- Insulin resistance
- Loss of lean body mass
- IL-8 synthesis by mesenchelial cells

IL-1 plus IL-6 or CSF
- Antigen-induced T-cell IL-2 production
- Hepatic synthesis of acid glycoprotein and C3
- Hepatic synthesis of LPS binding protein
- Endothelial cell synthesis of Mn superoxide dismutase
- Stem cell protection; hematopoiesis; progenitor cell proliferation

Table 8. Synergistic Activities of IL-1

<table>
<thead>
<tr>
<th>IL-1 plus Bradykinin</th>
<th>Angiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE$_2$ synthesis in gingival fibroblasts</td>
<td>Arachidonic acid release from synoviocytes</td>
</tr>
<tr>
<td>PGE$_2$ synthesis in synoviocytes</td>
<td>Degradation of articular cartilage</td>
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<tr>
<td>IL-6 production from hepatoma cells and fibroblasts</td>
<td>PGE$_2$ synthesis in osteoblastic cells</td>
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Inhibition of proteoglycan synthesis by chondrocytes, and apoptosis of the insulin-producing $\beta$-islet cell. In a murine macrophage line, the induction of COX-2 by LPS was blocked by the NOS inhibitor N$\omega$-monomethyl-L-arginine and thus has been linked to a requirement for NO generation for PGE$_2$ synthesis.346 IL-1$\beta$-induced PGE$_2$ in fibroblasts was enhanced by the NO donor, nitroprusside, and its enhancement was inhibited by hemoglobin.354 IL-1$\beta$-induced NOS is not inhibited by indomethacin.355

Synergistic actions of IL-1. As shown in Table 8, IL-1 acts synergistically with bradykinin, other cytokines, or growth factors. The synergism between IL-1 and TNF is highly consistent and a frequently reported phenomenon. In addition, the synergism between IL-1 and TNF is also observed in vivo, whereas the synergism between IL-1 and IL-6, IL-1 and bradykinin, or IL-1 and the various growth factors is mostly on prostanoid synthesis and is primarily an in vitro finding. The mechanism for IL-1 synergism in the synthesis for PGE$_2$ may be the initial release arachidonate followed by IL-1’s ability to stimulate COX-2 synthesis. The mechanism for synergism may also involve receptor modulation; however, in the case of IL-1 and TNF synergism, receptors for TNF are downregulated by IL-1.346,347 Could the synergism be explained at the level of signal transduction? Because IL-1 and TNF signal mechanisms appear similar, additive rather than synergistic effects should be observed. A reviewer of this manuscript suggested that the synergism might be due to each cytokine sustaining the “on” mode of the signal emanating from each receptor. This remains an attractive hypothesis.

Commentary on IL-1 effector mechanisms. Understanding how IL-1 manifests so many different biologic properties can be focused on relatively few mechanistic pathways, mostly those involving changes in constitutive and inducible gene expression or numbers of surface receptors for biologically active molecules. For example, the genes controlling increased synthesis of inflammatory leukotrienes and prostaglandins (PLA$_2$-type 2 and COX-2) are highly relevant to understanding the multiple effects of IL-1. Another gene is inducible NO synthase. Therefore, many of the pleiotropic effects of IL-1 are reduced by cyclooxygenase or nitric oxide synthase inhibitors (Tables 2 and 7). However, some biologic effects of IL-1 are not mediated by intermediate production of eicosanoids (Table 7) or NO but rather by regulation of various cellular receptors (Table 6). Synergism between IL-1 and other cytokines (Table 8), particularly TNF, should be considered in the context that, in most diseases, both cytokines are produced and act nearly at the same time on gene expression and receptor regulation. Therefore, blocking one cytokine may only reduce disease severity by 50%, which is often the case.

IL-1 IN HEMATOPOIESIS DURING DISEASE

A role for IL-1 in normal hematopoiesis? At present, there is no evidence that IL-1$\beta$ has a role in normal hematopoiesis. IL-1$\beta$-deficient mice have procreated over 10 generations and, in the absence of external challenges, is without evidence of hematologic impairment (H. Zheng and A. Shaw, personal communication, August 1995). Preliminary data of an IL-1RI-deficient mouse also support the conclusion that normal (unchallenged) hematopoiesis is independent of IL-1 activity (M. Labow, personal communication, June 1995). However, during a localized inflammatory process, IL-1$\beta$-deficient mice do not produce IL-6.15 In this same inflammatory model, blocking IL-1RI with a neutralizing antibody suppresses the production of IL-6.356 Because IL-1-induced IL-6 is a mechanism by which IL-1 increases platelets in animals and humans after irradiation or myelo-suppressive drugs,357 these animal studies support a role for IL-1$\beta$ during bone marrow responses to acute infection or inflammation. In healthy humans, sustaining a plasma level of 20 to 30 µg/mL of IL-1Ra for several hours is not associated with hematologic changes,357 an observation consistent with the IL-1$\beta$-deficient mice. However, there was a 50% decrease in the neutrophilia and circulating granulocyte colony-stimulating factor (G-CSF) levels induced by endotoxin in these volunteers.358

Effects of IL-1 on hematopoiesis. The best characterized role for IL-1 in hematopoiesis is its ability to increase the production of CSFs and stem cell factors, either by increasing their transcription or by stabilization of mRNA.359 In models of infection and inflammation, IL-1 mediates, in part, the induction of circulating levels of CSFs such as G-CSF,
granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-3, as shown by injecting IL-1 or by blocking IL-1R. There is also a well-described protective effect of IL-1 in mice after irradiation or cytotoxic drugs. A single, low dose of IL-1 can protect up to 90% of mice exposed to lethal radiation.

Several mechanisms may explain the ability of IL-1 to protect bone marrow cells; these include a protective effect on the pluripotent stem cell, the myeloid stem cell, and the early progenitor cells. In addition, arresting cell cycling or increases in Mn superoxide dismutase and other antioxidants may be involved. IL-1 increases gene expression and synthesis of c-kit on bone marrow cells and this is thought to explain the synergy of IL-1 and stem cell factor in protecting against lethal radiation. Using IL-1- treated male marrow cells before irradiation and transplantation into female mice, administration of IL-1 protected both short and long-term repopulating stem cells and accounted for the reconstitution of myeloid and lymphoid organs.

Animal models of myelosuppression often include IL-1 as part of the recovery protocol and studies in marrow transplantation have shown that a single, low-dose injection of IL-1 accelerates multilineage recovery. At higher doses of IL-1, PGE2, which is a potent suppressor of myeloid stem cell proliferation, may be induced. Higher doses also induce TNF, which can suppress colony formation. In a mouse model of zidovudine (AZT)-induced myelosuppression, a low dose of 5 U per day per mouse of IL-1α increased peripheral blood indices and progenitor stem cells, whereas TNFα can suppress hematopoiesis.

**IL-1 synergizes with CSFs for ex vivo expansion of bone marrow.** A typical biologic effect of IL-1 is its ability to synergize with a variety of cytokines, including CSFs. In fact, “hemopoietin-1,” a factor that synergized with CSF, was due to IL-1. The synergism is most apparent on the ex vivo culture enriched with CD34+ cells. In ex vivo expansion of enriched peripheral blood CD34+ cells, IL-1 is often added to the cultures together with IL-3 and other CSFs. Treatment of bone marrow endothelial cells with IL-1 increases the adherence of CD34+ progenitor cells, which may play a role in regulating the trafficking of pluripotent stem cells. Purified mouse stem cells require IL-3, IL-6, and IL-1 for proliferation in vitro, which suggests that primitive stem cells require multiple signals for growth.

**Effect of IL-1 on erythropoiesis.** The pathogenesis of the anemia of chronic disease has recently been reviewed, but a role for IL-1 in this syndrome remains unclear. Although studies show that the severity of the anemia in chronic inflammatory diseases, eg, rheumatoid arthritis, correlates with circulating levels of IL-1β, this association per se does not define a direct role for IL-1 in the anemia. Decreased erythropoietin production is observed in diseases associated with overproduction of IL-1, especially chronic renal failure and hemodialysis. IL-1 suppresses the production of erythropoietin from HepG2 cells, a hepatocyte cell line, and antagonizes erythropoietin-stimulated proliferation of mouse bone marrow erythroid progenitor cells. IL-1 also inhibits human colony forming units-erythrocyte (CFU-E) in the presence of IFNγ. The extent that these effects take place in vivo is unknown.

Chronic administration of IL-1 to mice induces anemia associated with a decrease in peripheral reticulocytes and suppression of mature CFU-E. However, the effect of IL-1 appears to be due to IL-1-induced TNF. This is not surprising because IL-1 induces TNF in vivo and in vitro and blockade of IL-1 receptors in vivo is associated with decreased levels of circulating TNF. In rats, TNF, but not IL-1, induces anemia that is due to both decreased red blood cell numbers and half-life. Why is there a discrepancy between the effects of chronic IL-1 in mice and rats? Part of the conflicting issue of IL-1’s role in anemia is that, in addition to inducing suppressing factors such as TNF and IFN, IL-1 has a positive effect on the primitive progenitor cells. In fact, while suppressing mature CFU-E, IL-1 increases colony formation of more primitive erythroid progenitors.

**Commentary on the role of IL-1 in the hematologic changes associated with disease states.** Evidence in mice with specifically targeted gene deletions indicates that normal hematopoiesis takes place in the absence of ICE, IL-1β, or IL-1RI. Nevertheless, there is likely a role for IL-1 in mediating hematologic responses to disease. Although positive correlations of IL-1 levels and hematologic changes have been made in various diseases, the most convincing data are derived from patients treated with intravenous infusions of IL-1Ra during sepsis or in healthy volunteers receiving a coinjection of IL-1Ra during experimental endotoxemia. In septic patients receiving IL-1Ra, there was a dose-dependent decrease in circulating IL-6, and in volunteers, a 50% reduction in LPS-induced neutrophilia. The latter observation was associated with a 50% reduction in circulating levels of IL-6 and G-CSF. These results are consistent with human hematologic responses to intravenous infusions of IL-1. It can be concluded that IL-1 contributes to the neutrophilia and production of CSFs during disease but is unlikely in health.

**IL-1 and leukemia.** Numerous studies have shown spontaneous IL-1 gene expression and synthesis in a variety of peripheral blood or bone marrow-derived leukemic cells. These include AML, chronic myelogenous leukemia (CML), adult T-cell leukemia, acute lymphocytic leukemia (ALL), and chronic lymphocytic leukemia. Of these, AML, CML, and ALL are the leukemias most consistently found to implicate spontaneous production of IL-1 in its pathogenesis. In some reports, AML blasts from more than 70% of patients exhibit autonomous growth in vitro and many of these are associated with spontaneous gene expression of IL-1β. It should be noted that, using a sensitive polymerase chain reaction (PCR) method, the gene for IL-1β is not expressed in peripheral mononuclear cells of healthy subjects, but AML blasts in the peripheral circulation show expression of IL-1β using the less sensitive method of hybridization to polyadenylated RNA. In addition to spontaneous production, nearly all studies have shown that exogenous IL-1 added to cultured leukemic cells induces or increases proliferation. In cells from AML patients, the addition of IL-1 to the cultures also results...
in increased production of GM-CSF. In some, IL-1 drives GM-CSF production, which, in turn, acts as an intracellular autocrine growth factor, because antisense GM-CSF oligonucleotides but not antibody to GM-CSF reduced the proliferation.\(^{405}\) IL-1 also increases the expression of stem cell factor and c-kit, which contributes to the growth of these cells.\(^{406}\)

Before initial treatment, steady state levels of IL-1\(\beta\) mRNA in marrow cells were quantitated in 22 high-risk, newly diagnosed patients with AML. Although there was no correlation between the expression of IL-1\(\beta\) and the outcome of a remission, there was a strong inverse relationship between the level of spontaneously expressed, steady state IL-1\(\beta\) mRNA before treatment and the duration of the remission.\(^{406}\) Blasts from 7 of 10 AML patients expressing IL-1\(\beta\) have the subtype M4 and M5 classification.\(^{399}\) A cell line derived from biphenotypic leukemic blasts with features of early B-cell and myeloid lineages spontaneously express the IL-1\(\beta\) gene, secrete IL-1\(\beta\), possess IL-1\(R\), and proliferate in response to IL-1\(\beta\).\(^{407}\) The addition of anti-IL-1\(\beta\) antibodies prevents growth in vitro. Despite the ability of exogenous IL-1\(\alpha\) to stimulate the proliferation of AML in vitro, AML cells do not spontaneously express the gene for IL-1\(\alpha\),\(^{406}\) and neutralizing antibodies to IL-1\(\alpha\) do not reduce spontaneous proliferation of AML blasts.\(^{387}\)

The most convincing experiment for the importance of IL-1\(\beta\) in the autonomous growth of AML blasts is the ability of specific blockade of IL-1 to reduce proliferation. This has been shown using neutralizing antibodies to IL-1\(\beta\),\(^{399,407}\) IL-1Ra,\(^{397,398}\) or soluble IL-1RI.\(^{398}\) An antisense oligonucleotide to ICE results in a significant reduction in spontaneous proliferation of peripheral and marrow-derived AML cells and also in the level of GM-CSF.\(^{158}\) Using a competitive inhibitor of ICE activity, there was a significant reduction in colony proliferation in bone marrow blasts from 19 AML patients.\(^{389}\) Other cytokines that inhibit the spontaneous proliferation of AML cells in vitro include TGF\(\beta\),\(^{409,410}\) macrophage inflammatory protein-1\(\alpha\),\(^{411}\) TNFr\(\alpha\),\(^{407,411}\) and IFN\(\gamma\),\(^{388,399,407,411}\)

**CML.** IL-1 is also spontaneously produced by CML and juvenile type CML blasts in vitro.\(^{396,402,404}\) IL-1\(\beta\) gene expression was found in cells of 5 of 5 juvenile type CML patients during blast crisis\(^{405}\) and 5 of 6 CML blasts.\(^{402}\) IL-1\(\beta\) protein can be detected in most of these cultures. In contrast, cells from 11 patients in the chronic phases of their disease or in patients with lymphoid blast crisis did not express IL-1\(\beta\).\(^{402}\) Specific blockade of IL-1 using IL-1Ra or IL-1sRI reduced the spontaneous proliferation of these cells in vitro.\(^{401,405}\) In the juvenile type of CML, the importance of IL-1 was originally shown by Bagby et al\(^{406}\) using neutralizing antibodies to human IL-1, suggesting that IL-1 was regulating the spontaneous production of CSF in this leukemia. In the presence of IL-1Ra, clonal expansion of juvenile-type CML colonies was dramatically reduced.\(^{404}\) A role for TNFr in the juvenile CML cell cultures has been studied\(^{412}\) and it is possible that TNFr provides a growth signal by inducing IL-1.

**IL-1 in ALL.** Using a bioassay, spontaneous production of IL-1 was detected in culture supernatants of myeloid antigen-positive ALL cells but not myeloid antigen-negative blasts.\(^{387}\) The spontaneous proliferation of ALL blasts in vitro was inhibited by a neutralizing antibody to IL-1\(\alpha\) and there was spontaneous expression of IL-1\(\alpha\) in these cells.\(^{385}\) Others have reported that, unlike AML cells, ALL cells do not express the IL-1\(\beta\) gene.\(^{390}\) IL-1 added to cultures of ALL cells stimulated proliferation that was not inhibited by neutralizing antibodies to GM-CSF, TNFr, IL-3, or IL-6.\(^{388}\) In contrast, IL-1sRI did prevent IL-1--induced growth.

**Chronic leukemias.** IL-1\(\alpha\) is constitutively produced by adult T-cell leukemic cells and also by HTLV-1--transformed cell lines.\(^{383,385,386}\) It is thought that HTLV-1 infection induces gene expression for IL-1\(\alpha\). To study such a mechanism, two plasmid constructs of the promoter regions of the human IL-1\(\alpha\) gene (−1437 to +19 and −1437 to +725) linked to a reporter gene were transfected into Jurkat cells. When these cells were cotransfected with a plasmid expressing the HTLV-1 Tax gene product, there was a 9.8-fold increase in IL-1\(\alpha\)--driven expression compared with the control plasmid vector not containing the Tax gene.\(^{413}\) These results suggest that the autocrine expression and growth promoting effect of IL-1\(\alpha\) in T-cell leukemia may be under the direct influence of HTLV-1. In T cells from a patient with T4 chronic lymphocytic leukemia, IL-1 increased proliferation and the expression of the \(\beta\) chain of the IL-2 receptor.\(^{386}\) When neutralizing antibodies to IL-1 were added, expression of the IL-2 receptor \(\beta\) chain was inhibited, suggesting that endogenous IL-1 was stimulating the expression of this receptor. In hairy cell leukemia, increased levels of circulating IL-1\(\beta\) and soluble IL-2 receptor correlate with increased disease activity.\(^{414}\) In patients in remission with IFN, these levels are reduced (V. Barak, personal communication, September 1995).

**IL-1 and multiple myeloma.** Human bone marrow myeloma cells spontaneously produce IL-1\(^{139,415,417}\); however, IL-1 produced by myeloma cells stimulates IL-6 production, which is thought to be the actual growth factor for myeloma cells.\(^{418,419}\) Deletion of the tumor-repressive retinoblastoma gene (Rb-1) is found in approximately 50% of patients with multiple myeloma and loss of Rb-1--induced suppression of IL-6 production may contribute to elevated IL-6 production in these patients.\(^{420}\) Human myeloma cells spontaneously express the genes for IL-1\(\beta\), IL-6, and G-CSF, and these genes were coexpressed in most of 36 tumor samples from myeloma patients.\(^{421}\) IL-1\(\alpha\) was coexpressed only in 32% of those expressing IL-1\(\beta\).\(^{421}\) Neutralizing antibodies to IL-1\(\beta\) but not IL-1\(\alpha\) partly inhibited spontaneous proliferation of myeloma cells in vitro.\(^{417}\)

Culture supernatants from myeloma cells possess strong osteoclast-activating factor activity\(^{416}\) and the levels IL-1\(\beta\) and bone resorbing activity content in these culture supernatants are highly correlated (\(r = .923\)).\(^{422}\) IL-1Ra completely abolished the bone resorbing activity of the myeloma supernatants.\(^{423}\) However, using immunomagnetic separation techniques, purified human myeloma cells were shown not to be the source of IL-1 or of IL-6.\(^{423}\) According to those data, production of IL-1 and IL-6 in myeloma tumors is derived from nonmyeloma cells such as endothelium or fibroblasts.

**Other leukemias and lymphomas.** In perhaps what is the first study of its kind, cultured lymph nodes from patients
with Hodgkin’s disease were shown to produce an endogenous pyrogen. 424  Subsequently, immunohistologic staining of Reed-Sternberg cells showed an association of IL-1β and IL-1α-positive cells with symptoms of fever and malaise. 425  In situ hybridization, gene expression for IL-1α and TNFα was observed in Reed-Sternberg cells of 12 of 19 tumor specimens; 426  a probe for IL-1β was not tested. There was no correlation between the presence of IL-1α or TNFα gene expression and clinical status.

In chronic B-lymphocytic leukemia, spontaneous gene expression for IL-1α, IL-1β, and IL-6 has been noted. 427  Both IL-1 proteins were found in permeabilized stained cells; in nonpermeabilized cells, membrane IL-1α was observed. Cells from patients with chronic B-cell hairy cell leukemia express IL-1β. 428  In a study of 25 patients with hairy cell leukemia, there was a correlation between disease severity and circulating IL-1β, both decreasing with treatment. 414

Commentary on IL-1 production and the proliferation of leukemic cells. Examining the data reported from studies in which spontaneous production of either isoform of IL-1 have been observed in various leukemic cells, one concludes that IL-1 contributes to the expansion of leukemic blasts or myeloma cells. This is supported by in vitro studies in which blocking IL-1 activity with neutralizing antibodies, soluble receptors, IL-1Ra, or inhibition of ICE has reduced spontaneous proliferation of these cells or leukemic colony growth. However, the use of these specific anti-IL-1 strategies in patients is still untested. Preliminary results using IL-1Ra in patients with AML and CML showed a reduction in circulating blasts during infusions of IL-1Ra (Z. Estrov and M. Talpaz, personal communication, August 1994). If anti-IL-1 strategies such as IL-1Ra, soluble IL-1R, or inhibition of ICE do reduce circulating and marrow blasts, a nontoxic strategy to synchronize the proliferation of these leukemic cells exists. The extent these anti-IL-1 agents can be used in the induction of remissions remains to be tested.

IL-1 AND SOLID TUMORS

Spontaneous production of IL-1 by malignant cells. Constitutive production of IL-1α or IL-1β from human or animal tumor cell lines has been reported in numerous studies; these commonly include cells from melanoma, hepatoblastoma, and sarcoma as well as from squamous cell, transitional cell, and ovarian carcinomas. Spontaneous production of IL-1Ra and IL-1β from primary ovarian cells has also been reported. 429  In a study of several cell lines derived from human melanomas, the spontaneous production of IL-1 coincided with a mutation of glutamine 61 to arginine in the RAS protein. 430  A positive association was made between IL-1 production and metastatic melanoma in 29 surgical specimens. 431  Some but certainly not all tumor cell lines appear to use endogenous IL-1 as a growth factor. How does IL-1 affect tumor cell growth? In many cases, growth enhancement is due to IL-1 induced synthesis of other cytokines such as IL-6, 432  TGFβ, or CSFs, chemokines such as IL-8, 433  adhesion molecules, 434  or uncharacterized growth factors. IL-1-dependent growth factors can be derived from the malignant cells themselves or are induced by the malignant cells from fibroblasts or endothelial cells. 435  For example, in vitro growth of hepatoblastoma or melanoma cells cocultured with fibroblasts or endothelial cells is completely inhibited by the presence of IL-1Ra. 436  IL-1Ra. 436  In some animal models, in vitro melanoma cell production of IL-1 correlates with the ability to metastasize to the liver. The production of increasing amounts of IL-6 and cachexia in mice with sarcoma is also under the control of IL-1. 437  In cells derived from bladder transitional cell carcinomas, the autocrine growth factor effect of ICAM-1 is, in part, due to constitutive production of IL-1α. 437  Chemical carcinogens or UV radiation, which result in local tumor formation, induce gene expression and synthesis of IL-1 in normal keratinocytes. However, these stimuli are hardly specific for IL-1 because several cytokines are induced by carcinogens or UV radiation. 438

Effects of exogenous IL-1 on proliferation of malignant cells in vitro. Incubating certain malignant cells with IL-1 can increase the proliferation rate of these cells in vitro. 439  Similar to endogenous production of IL-1 by neoplastic cells, a “growth factor effect” is usually attributed to IL-1 induction of other growth factors. Some have been identified: TNFα, IL-6, IL-8, and TGFβ. For example, IL-1 stimulates the growth of human epithelial ovarian carcinoma cells, but antisense TNFα RNA prevents the growth effects of exogenous IL-1. 440  The addition of IL-1 to murine B16 melanoma cells in vitro increases the expression of adhesion molecules, 434  tissue factor, and mannosylation of cell surface proteins; 441  these effects, in turn, contribute to increased growth factor production on contact with endothelial cells. However, in many cases, tumor growth factors or combination of growth factors have not been identified.

Inhibition of malignant cell growth by IL-1 in vitro. In sharp contrast to the growth-promoting effects of exogenous IL-1, the addition of IL-1 to cultures of malignant cells can inhibit tumor cell growth. The addition of IL-1 reduces the spontaneous proliferation of tumor cells by cytokidal 442 443  or cytostatic effects. 444  IL-1-induced growth inhibition has been observed in cultured melanoma, glioma, meningioma, breast, cervical, thyroid, and ovarian carcinoma cells. 445  The growth inhibitory effect of IL-1 is clonally dependent. In a variety of primary tumors, the addition of IL-1 results in reduced colony formation in only 9 of 56 (16%) of tumors. 446  The combination of IL-1 together with doxorubicin, cisplatin, inhibitors of thymidine phosphorylase, or other cytokines can be synergistic in reducing malignant cell growth in vitro. 447

The mechanisms proposed for the inhibitory effect of IL-1 on certain tumor cells include induction of other cytokines, particularly TNFα and IL-6, increased release of oxygen radicals, 448  decreased polyamine synthesis, 449  increased differentiation and polymerization of F-actin, 450  arrest of cell cycle, 451  and NO generation. 452  The importance of oxygen radicals was shown by increasing the endogenous synthesis of oxygen scavenging molecules in which case tumor cells were protected from the cytotoxic effects of IL-1. 448  Both normal and malignant cells, inhibition of NO generation can be associated with inhibition of IL-1-induced cytotoxicity (Table 2). Using a human melanoma cell line sensitive to growth inhibition by IL-1, the expression of several early genes associated with growth arrest (gro-α, gro-β, and oth-
ers) has been described, including a novel IL-1–inducible
gene termed IL-1 response gene-9.452 In addition, the lack
of induction of c-myec and Erg-1 are associated with growth
inhibition of IL-1.453

**IL-1 in metastasis and growth of tumors in vivo.** The
use of neutralizing antibodies to IL-1 or IL-1 receptor block-
ade has shown a role for endogenous IL-1 in contributing
to growth and metastasis of some malignant cells. A dose-
dependent reduction in the size of subcutaneous B16 murine
melanoma tumors was observed in mice treated with IL-
1Ra.453 Using either an intravenous or intrapleural injection
of tumor cells, IL-1Ra reduced the number and size of lung
and liver metastatic melanomas, respectively.436–454 In addi-
tion, the survival of mice injected with IL-1Ra showed a
dose-dependent improvement even when the receptor antag-
onist was administered after the injection of tumor cells.436
These data support a role for endogenous IL-1 in metastasis,
whether produced from the tumor cells or induced by the
tumor.436,441

Additional evidence exists for IL-1 promoting a prometa-
static environment. Administration of IL-1 to mice with a
subcutaneous melanoma increases the size fivefold, which is
accompanied by increased vascularity and local invasion.438
Other studies show that “priming” of the animal by adminis-
tration of IL-1 before the injection of tumor cells increases
the number and size of pulmonary metastases.455,456 LPS also
can “prime” an animal for increased metastasis but this
effect appears to require the endogenous mediation by IL-
1.454 IL-1 priming of mice before a left intracardiac injection
of tumor cells doubled the number of bone marrow meta-
static colonies.457 Similar to IL-1, priming with TNFα in-
creases the number of pulmonary metastases of fibrosarcoma
cells.458

What are the mechanisms for endogenous IL-1 in promot-
ing metastasis and tumor growth? The well-established abil-
ity of IL-1 to upregulate endothelial adhesion molecules459
is of primary consideration (reviewed in Bevilacqua and
Nelson460). The organ-specific microenvironment for tumor-
endothelial cell interaction greatly influences metastasis.461
IL-1 upregulated tumor cell adherence to umbilical vein en-
dotheilial cells.460,462 Incubation with IL-1 increases the
expression of ICAM-1 on melanoma cells and spontaneous
expression of ICAM-1 by human melanoma cells is a para-
crine effect of IL-1.434 IL-1–induced ELAM-1 is thought to be
the major if not the only receptor for colon carcinoma
self to endothelium.463 Neutralizing anti–IL-1 antibo-
dies reduce the adherence of melanoma cells to themselves
as well as to endothelial cells.464 In addition to upregulation
of endothelial cell adhesion molecules, IL-1 has increased
the activity of mannose receptors on hepatic endothelial cells
facilitating binding of melanoma cells to this specialized
endothelium.464 Another likely mechanism for IL-1–en-
hanced metastasis and tumor cell growth is via IL-1–induced
angiogenesis.465–466

**Non-specific effect of exogenous IL-1 on tumor regression.**
Treatment of animals with exogenous IL-1 can reduce tumor
size either when administered systemically or intratumorally.
In some studies, tumor regression is probably secondary to
vascular collapse, particularly when large amounts of IL-1
are administered (100 to 400 µg/kg).467 However, in other
experiments, administration of low doses of IL-1 enhances
non-specific host antitumor defense mechanisms or boosts
the effect of known antitumor adjuvants. For example, in
methylcholanthrene-induced tumors, IL-1 induces peritoneal
leukocytes to release a tumoricidal substance that mediates
tumor rejection.468 Because IL-1 itself has no direct antipro-
iferative effect on this tumor, induction of NO from leuko-
cytes may be responsible. In fact, IL-1 enhanced macrophage
tumoridal activity is often mediated by induction of NO and
other non-specific antitumor products.453 IL-1 also acts
synergistically with IFNγ to increase tumor killing by mac-
rophages.469 In addition to macrophages, increased neutrophil
infiltration is thought to mediate the reduction of melano-
ma tumors after an intratumoral injection of IL-1.469
Synergism for antitumor effects have been reported for com-
binations of IL-1 plus cis-platin, cyclophosphamide, or hy-
perthermia.467,470,471

**IL-1 immunoadjuvant therapy against tumors.** In immu-
noologically mediated tumor regression, administration of
IL-1 has reduced tumors via an “adjuvant effect.” The
mechanisms underlying the adjuvant effect of IL-1 include
increased cytotoxic T-lymphocyte activity,472 influx of lympho-
cytes and inflammatory cells into the tumor,473 and in-
creased tumor antigen presentation.474 Synergistic IL-1 protects
animals against rechallenge with tumor cells 180 days later,
and this protection is mediated by a tumor-specific lympho-
proliferative response.474 IL-1 treatment increases the gener-
ation of lymphokine activated killer T cells in models of
adoptive immunotherapy.475 Transfecting murine fibroblasts
with IL-1α increases their immunogenicity during induction
of specific cytotoxic T lymphocytes. This results in reduced
tumor enlargement and survival compared with non-IL-1α
producing tumor cells that progressively grow and result in
death.476–478

**Commentary on IL-1 and tumor biology.** Various cyto-
kines have been administered to humans with cancer as part
of immunotherapeutic strategies. In some cases, decreased
production of IL-1 and other cytokines has been reported.479
Administration of IL-1 to animals has resulted in tumor re-
gression by either non-specific or tumor-specific immune-
mediated mechanisms. Based on these findings, humans with
a variety of solid tumors have been injected with IL-1α or
IL-1β in phase I/II trials. These include melanoma, breast
carcinoma, colon adenocarcinoma, renal cell carcinoma, gli-
omas, and cutaneous T-cell lymphoma. Regressions of some
tumors have been reported480,481 but there were no consistent
effects.153,482 It remains unclear whether giving IL-1 will be
of significant value in upregulating immune parameters in
the treatment of solid tumors. However, it is unclear whether
an antiproliferative effect of IL-1 on a particular tumor in
vitro can be achieved in vivo without considerable toxicity.
On the other hand, strategies to block IL-1 activity, which
is without toxicity, may be therapeutically useful in treating
those tumors in which the metastatic process appears to be
enhanced by endogenous IL-1 or in those tumors in which IL-1
induces growth factors.

**IL-1 AND ATHEROSCLEROTIC VASCULAR DISEASE**

**Production of IL-1 from normal and diseased blood ves-
sels.** Similar to cultured fibroblasts, cultured human endo-
thelial and smooth muscle cells derived from healthy blood vessels produce IL-1 when stimulated by a variety of agents, including endotoxin, TNF, or IL-1 itself.\textsuperscript{483,486} Primary endothelial cells grown on an inert surface release IL-1 when subjected to laminar flow-induced shear stress (6 dyne \cdot cm\(^{-2}\)).\textsuperscript{487} Using in situ hybridization, IL-1\(\alpha\) and IL-1\(\beta\) mRNA have been detected in endothelial cells of various organs taken from apparently healthy animals. However, the function of constitutive IL-1\(\beta\) expression in endothelial cells is unclear because mice deficient in IL-1\(\beta\) have normal tissue phenotypes.\textsuperscript{488} Using specific cDNA probes for rabbit IL-1\(\alpha\) and IL-1\(\beta\),\textsuperscript{489} freshly homogenized or cultured aortic tissue did not show spontaneous expression of either IL-1 gene.\textsuperscript{490} However, 1 and 3 hours after an intravenous injection of LPS, both IL-1 genes were expressed in aortic tissues. Using specific radioimmunoassays for rabbit IL-1\(\alpha\) and IL-1\(\beta\),\textsuperscript{491} the IL-1 proteins were present in homogenates 3 hours after an injection of LPS.\textsuperscript{495}

Thoracic and abdominal aortic segments taken from hypercholesterolemic monkeys spontaneously express the gene for IL-1\(\beta\). This expression was associated with an increase in platelet-derived growth factor B-chain synthesis in lipid-filled macrophages (foam cells) that had invaded the atheromatous lesions.\textsuperscript{492} Using immunohistochemical staining, each of 55 sclerotic vein coronary artery bypass grafts showed the presence of IL-1\(\alpha\), whereas nonoccluded internal mammary artery grafts did not.\textsuperscript{493} The affected vessels also showed myointimal proliferation, reduced luminal patency, and macrophage infiltration. Cultured tissue minces from human abdominal aneurysms release significantly more IL-1\(\beta\) compared with control aortic tissue from cadavers.\textsuperscript{494} The presence of IL-1 in atheromatous tissues is thought to be due to infiltrating foam cells that synthesize IL-1 in response to oxidized lipoproteins (LDL). Experimental data have shown that LDL of varying oxidation states and associated linoleate oxidation products stimulate normal human blood monocytes to produce IL-1\(\beta\).\textsuperscript{495,496} Reducing lipoprotein peroxidation by antioxidants or adding antioxidants to LPS-stimulated IL-1 production has reduced IL-1 synthesis.\textsuperscript{497}

\textbf{Effects of IL-1 on endothelial, smooth muscle, and myocardial cells.} The proinflammatory properties of IL-1 are relevant to the development of the atheromatous lesion, tissue destruction after ischemia, restenosis after coronary artery angioplasty, and myocardial function in diseased hearts. As shown in Table 4, IL-1 increases gene expression for clotting factors and inhibitors of fibrinolysis. In cultured endothelial cells, IL-1 induces transendothelial passage of neutrophils through junctional gaps.\textsuperscript{498} In the atheromatous lesion, IL-1 production from lipid-laden foam cells can increase the surface expression of endothelial adhesion molecules, which facilitates the attachment of inflammatory and lymphocytic cells onto a damaged endothelium. In addition, IL-1 produced from either foam cells or the endothelium induces the chemokines IL-8 and monocyte chemotactic protein-1, which facilitate the transendothelial migration of inflammatory cells and activate these cells. IL-1 induction of hematopoietic growth factors GM-CSF and M-CSF has been implicated in the progression of the inflammatory process in the atheromatous lesion.\textsuperscript{499}

IL-1 can also act as growth factor for smooth muscle cell proliferation in the absence of inhibitory prostaglandins.\textsuperscript{134} Early intimal thickening of coronary arteries in transplanted pig cardiac allografts was associated with IL-1-induced fibrinectin deposition.\textsuperscript{500} It is unlikely that IL-1 is acting directly as a growth factor in proliferative vascular lesions but rather to increase the expression of traditional vascular cell mitogens such as platelet-derived growth factor and fibroblast growth factor and their respective receptors (Table 4). Furthermore, IL-1 often acts synergistically with these vascular mitogens. Long-term blockade of IL-1 receptors or decreasing IL-1 synthesis or release in the vascular lesion would help identify an essential role for IL-1 in the pathogenesis of vascular lesions. Slow local release of IL-1Ra from a polymer sleeve surrounding a damaged sciatic nerve in the mouse reduced neovascularization of the damaged area.\textsuperscript{501} Angiotensin converting enzyme inhibitors appear to reduce the restenosis of grafted vessels and in vitro these inhibitors reduce gene expression and synthesis of IL-1 and TNF.\textsuperscript{502}

Ischemia-reperfusion in liver, brain, lung and myocardium results in the synthesis of IL-1, TNF, and other cytokines. Because of their importance to infiltration of inflammatory cells, IL-8 and other chemokines released after ischemia-reperfusion contributes to local tissue damage. Moreover, production of IL-8 is often under the control of IL-1.\textsuperscript{503} In the case of ischemic injury due to coronary occlusion, myocardial muscle damage correlates with neutrophil infiltration. In dogs, acute coronary occlusion results in accumulation of myocardial neutrophils and infarction; however, IL-1 receptor blockade with IL-1Ra reduced neutrophil infiltration and infarct size by 50%. Other studies have shown a beneficial effect of IL-1Ra on reducing tissue injury after ischemia.\textsuperscript{504} A 24-hour pretreatment with a low dose of IL-1 will protect animals from an acute injurious event. This protective role for IL-1 has been used in reducing the damage induced by ischemic perfusion in isolated rat hearts.\textsuperscript{505}

In addition to its effects on coronary arteries, IL-1 also directly affects myocardial function. IL-1\(\beta\) suppresses the spontaneous rhythmic beating of cultured neonatal rat myocytes, an effect inhibited by the addition of TGF\(\beta\).\textsuperscript{506} Exposure of excised papillary muscle to IL-1 in vitro prolonged the duration of action potential and refractory period, and both effects were reversed by cyclooxygenase inhibitors.\textsuperscript{507} Similar suppressive effects of IL-1 have been reported using isolated myocytes stimulated with \(\beta\) adrenergic agonists.\textsuperscript{508} Because IL-1 induces NOS, some effects of IL-1 on isolated hearts may be mediated by NO. Although no specific IL-1 receptor blocking studies have been reported, IL-1 produced during myocarditis may affect myocardial function. For example, IL-1\(\beta\) mRNA was found in endomyocardial biopsies from patients with myocarditis and elevated IL-1RI gene expression was detected in samples from dilated cardiomyopathies.\textsuperscript{509} Treating mice with IL-1 or TNF induced autoimmune myocarditis in mice infected with Coxsackie B3.\textsuperscript{310}

\textbf{Role of macrophage membrane and platelet IL-1.} Nearly all working hypotheses of the pathogenesis of atherosclerotic vascular disease implicate a role for the lipid-filled invading macrophage and the activated platelet.\textsuperscript{511,512} Several labora-
tories have shown biologically active membrane bound IL-1α (see above section on membrane IL-1α) and immunohis- 
tochemical staining in diseased blood vessels also shows the 
presence of IL-1α. Human monocytes that have been fixed 
after LPS activation express IL-1α on their surface and when 
incubated with cultured human endothelial cells induced IL- 
8 production. Moreover, nearly all the IL-8-inducing ac-
tivity was blocked by either an antibody to IL-1α or IL-1Ra, 
suggesting that no other membrane cytokine, e.g., TNF, was 
invololved. IL-1α production by a cultured monocyte line is 
enhanced by coinubcation with trombhin-activated but not 
nonactivated platelets. Inhibiting platelet-activating factor 
reduces the enhancing effect of activated platelets on mono-
cyte IL-1α synthesis.

In a similar fashion, epinephrine-activated human platelets 
induce IL-8 synthesis from endothelial cells and nearly all 
this activity was blocked by IL-1Ra. However, the IL-1 
biologic activity of activated platelets appears to be due 
to IL-1β rather than to IL-1α and IL-1 activity was 
associated with platelet membranes rather than cytosolic 
extracts. Thrombin-activated platelets induce ICAM-1 and 
ELAM-1 expression as well as synthesis of GM-CSF and 
IL-6 from cultured endothelial cells and these activities are 
neutralized by an antibody to IL-1β, not IL-1α. The 
increased expression of adhesion molecules by either soluble 
or platelet-associated IL-1 likely contributes to the role of 
the platelet in the inflammatory phase of atherosclerotic le-

disturbance. A recent study shows IL-1 receptors on approxi-

mately 5% of circulating platelets from healthy donors but 10% 
from patients with inflammatory bowel disease. In addi-
tion, IL-1 activates normal platelets as assessed by a dose-
dependent increase in the expression of the leukocyte ad-
hesion molecule p-selectin (CD62) and the fibrinogen 
receptor. Taken together, IL-1β activity on activated plate-

Commentary on the role of IL-1 in atherosclerotic vascular 
disease. Disruption of endothelial surface is not completely 
understood, but the subintimal location of activated mono-
cytes and platelets expressing surface IL-1 activity likely 
contribute to the early events of the atherosclerotic lesion. 
Hyperlipidemia and oxidized LDL activate IL-1 production 
by macrophages and this event may be part of the initiation 
process. Once a pathologic process is associated with the 
presence of IL-1-producing cells or IL-1 activity on cell 
membranes, the multiple biologic effects of IL-1 as an in-
flammatory mediator implicate this cytokine. However, there 
are no long-term studies in atherosclerotic-prone hypercho-


IL-1 ADMINISTERED TO HUMANS

Toxicity of IL-1. IL-1α or IL-1β has been injected in 
patients with various solid tumors. In general, the acute tox-
icities of either isoform of IL-1 were greater after intravenous 
compared with subcutaneous injection. Subcutaneous injec-
tion was associated with significant local pain, erythema, 
and swelling. Most patients have received 15 to 30 
minutes of infusion with doses from 1 ng/kg to 1.0 μg/kg. 
Chills and fever were observed in nearly all patients, and 
even the 1 ng/kg dose group experienced fever. The febrile 
response increased in magnitude with increasing doses. 
Fever began within 30 minutes of starting the infusion and 
reached peak elevations 1.5 to 3 hours later. When adminis-
tered subcutaneously, IL-1β-induced chills and fever were 
abated with indomethacin treatment. In several studies, a 
transient increase in blood pressure and heart rate was ob-
erved soon after the initiation of the infusion and at the 
onset of the chill. After this transient increase in vascular 
tone, a progressive, dose-dependent decrease in systolic 
blood pressure was observed.

In a trial of 28 patients receiving IL-1α and trials of 
IL-1β in 19 and 17 patients, acute toxicities of intrave-

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receiving IL-1β, there was a fall in serum glucose within the first hour of administration and, in 11 patients, glucose levels decreased to 70 mg/100 mL or less.55 In addition, there were increases in ACTH and thyroid stimulating hormone but a decrease in testosterone.492 No changes were observed in coagulation parameters such as prothrombin time, partial thromboplastin, or fibrinogen degradation products.492 This latter finding is to be contrasted to TNFa infusion into healthy humans that results in a distinct coagulopathy syndrome.522

Not unexpectedly, IL-1 infusion into humans significantly increased circulating IL-6 levels in a dose-dependent fashion.482 At a dose of 30 ng/kg, mean IL-6 levels were 300 pg/mL 4 hours after IL-1 (baseline <50 pg/mL) and 8,000 pg/mL after a dose of 300 ng/kg. In another study, infusion of 30 ng/kg of IL-1α induced increased IL-6 levels within 2 hours.516 These elevations in IL-6 are associated with an increase in C-reactive protein and a decrease in albumin.492 Serum GM-CSF were less than 50 pg/mL. In two studies, one with IL-1α522 and one with IL-1β,493 a rapid increase in circulating IL-1Ra and TNF soluble receptors (p55 and p75) was observed after a 30-minute intravenous infusion. The increase in the circulating levels of both naturally occurring antagonists is greater than those measured in human volunteers injected with LPS.291,526

Hematologic changes in patients with normal marrow reserves. Even at doses of 1 or 2 ng/kg, IL-1 increased the peripheral WBC.155,177,428 At the higher doses (30 and 100 ng/kg), the leukocytosis was preceded by a transient leukopenia due mostly to a decrease in monocytes and lymphocytes.155,482 After 2 hours, the increase in WBC was due to an absolute neutrophilia with a greater percentage of band forms (25%) occurring in the high-dose groups. The rapid increase in neutrophils was associated with an increase in cortisol and with circulating G-CSF.527 The neutrophilia was sustained for several weeks with increasing frequency and higher doses of IL-1.452 Daily doses of IL-1α also increased the numbers of neutrophils entering the oral cavity and paralleled the increase in peripheral neutrophils.528 The subcutaneous route also resulted in a several-fold increase in neutrophils.480 The lymphopenia and neutrophilia after a single injection of IL-1 was remarkably similar to those observed after a bolus injection of endotoxin into humans.155,529

Increases in platelets were also observed in patients with normal marrow reserves after either 1, 2, or 7 injections of IL-1.155,177,482 The increase was slow, taking several days, and was preceded by a thrombocytopenia during daily injections.482 After two injections of IL-1β into patients, mean platelet counts doubled 14 to 22 days after IL-1.155 A 50% increase occurred 24 days after a single injection of 10 ng/kg IL-1β.155 In patients injected with 7 daily doses of 100 ng/kg IL-1α, there was a mean increase of 73% in platelet counts after 15 days.452 These findings were also associated with increased marrow cellularity and increased numbers of megakaryocytes.155,482 Despite increased marrow cellularity, there were no increases in CD34+ cells in the periphery155,482 and there was either no change155 or a decrease in the formation of colonies in response to IL-3.482

In vivo IL-1 enhances ex vivo cellular functions. After 5 days of IL-1 therapy, neutrophils stimulated with phorbol esters produced less H2O2 in vitro compared with baseline levels.500 The receptors for G-CSF on neutrophils decreased by 40% in patients treated with two injections of IL-1β in a dose- and time-dependent fashion, whereas these same neutrophils exhibited a twofold to sixfold increase in binding IL-1β.527 These changes were associated with a dramatic increase in neutrophil alkaline phosphatase compared with baseline levels.156 The increase in IL-1β binding was due to increased numbers of receptors. These in vivo and in vitro observations are consistent with the ability of corticosteroids to upregulate IL-1RII on neutrophils, monocytes, and B cells.506

Monocyte functions were also altered during IL-1 therapy. After 4 days of continuous IL-1α therapy, the peripheral monocyte count was sixfold greater than baseline numbers and there was an increase in phorbol-induced superoxide production.531 When stimulated with IL-1 itself in vitro, these monocytes exhibited increased gene expression for IL-1α and IL-1β but not when stimulated with LPS.531 The increased responsiveness of monocytes from IL-1-treated patients may reflect increased numbers of receptors. In PBMC from patients receiving four weekly injections of IL-1β, IL-2–stimulated lymphokine activated killer cell activity was significantly enhanced compared with baseline activity.532

Effects of IL-1 on chemotherapy-induced myelosuppression. Contrary to IL-1–associated myelostimulation in patients with normal marrow reserves, patients with aplastic anemia treated with 5 daily doses of IL-1α (30 to 100 ng/kg) had no increases in peripheral blood counts or bone marrow cellularity.533 IL-1 has been administered to patients undergoing various regimes of chemotherapy to reduce the nadir of neutropenia and thrombocytopenia. These studies are based on well-established models of myeloprotection by prior treatment with IL-1 in rodents562 or in primates.534 In neutropenic patients (<500 neutrophils/µL) receiving chemotherapy, two doses of IL-1β significantly shortened the duration of neutropenia compared with the duration of neutropenia in the same patients during a previous cycle of chemotherapy.521 Two doses of IL-1β 28 days before and together with 3 days of 5-fluorouracil (5-FU) were associated with fewer days of neutropenia compared with 5-FU alone.155 Using this regimen, there was no consistent effect on thrombocytopenia. Patients treated with high-dose ACNU had a higher nadir of neutrophils and platelets compared with historical controls.535

In other studies, treatment with IL-1α for 5 days significantly reduced chemotherapy-induced thrombocytopenia. There was a significant and earlier recovery of platelet counts (>100,000 µL) in IL-1–treated patients after high-dose carboplatin compared with carboplatin alone.591 This benefit was dose-dependent and was not observed when IL-1 was administered before the carboplatin. Similar findings were reported in a study using 4 days of continuous IL-1α therapy.591 In both studies, there was a clear benefit of using IL-1, but the effect was best seen at the higher and more toxic doses of IL-1.

IL-1 therapy during autologous bone marrow transplantation. Daily treatment with 40 ng/kg IL-1α from day 0 to
day 13 of autologous bone marrow or stem cells resulted in an earlier recovery of neutropenia (median, 12 days) compared with controls or lower doses of IL-1 (median, 27 days; \( P < .001 \)). After 14 days of treatment, the bone marrow was significantly enriched with committed myeloid progenitor cells. IL-1–treated patients were discharged from the hospital earlier than controls (median, 25 vs 37 days). Lower doses of IL-1 together with other hematopoietic growth factors may achieve the same benefit without the toxicity. Similar results were reported in patients with AML receiving 50 ng/kg/d of IL-1/\( \beta \) for 5 days starting at the time of transplantation with purged or nonpurged bone marrow. In that study, an IL-1 treatment–associated decrease in infection rate and improved survival was observed. Using subcutaneously administered IL-1/\( \beta \), there were greater numbers of CFU-GM at day 21 compared with historical controls.

**Commentary on the toxicity and efficacy of IL-1 treatment in humans.** Injecting humans with low doses of either IL-1\( \alpha \) or IL-1/\( \beta \) confirms the impressive pyrogenic and hypersensitivity-inducing properties of the molecules. The human studies also confirm the effects of IL-1 on stimulating the hypothalamic-pituitary-adrenal axis and on increased cytokine production, particularly IL-6. In many ways, the signs and symptoms after IL-1 injection into humans are indistinguishable from those of low doses of endotoxin. Similar to endotoxin, IL-1 induces a general enhancement of hematopoiesis, particularly in increased neutrophil, monocyte, and platelet counts. In patients receiving marrow-suppressing chemotherapy, cotreatment with IL-1 decreases the nadir and the duration of the marrow suppression. However, the benefits of IL-1 therapy in these patients are clouded by its formidable toxicity. Low doses of IL-1 may be useful in combination with other hematopoietic growth factors for reducing myelosuppression during chemotherapy or bone marrow transplantation.

### IL-1Ra and amelioration of disease

**Effects of IL-1Ra in animal models of disease.** Table 9 lists several in vitro experiments in which blocking IL-1 receptors by IL-1Ra has provided a basis for animal and human studies. Because there is little species specificity, human IL-1Ra has been used in animals. Blocking IL-1 receptors with IL-1Ra has increased our understanding of IL-1 as a mediator of disease. Other methods such as neutralizing anti–IL-1 antibodies, antibodies to IL-1RI, and soluble IL-1 receptors, although equally effective, are limited by their animal specificity. As listed in Table 10, a reduction in the severity of various disease models has been reported. In most cases, other cytokines are produced in addition to IL-1. Therefore, the data depicted in Table 10 show that IL-1 plays an important role in the pathogenesis of inflammatory and immunologically mediated disease. In these studies, a reduction of at least 50% is observed, but the amelioration of pathologic changes can be complete in many. One consistent observation is the reduction in the number of infiltrating neutrophils associated with local inflammation and this effect of IL-1Ra may be due to preventing IL-1–induced synthesis of IL-8 and related chemokines.

However, there is one important caveat. In the majority of these studies, IL-1Ra has been administered just before the challenging event. This is particularly the case in models of infection in which injecting IL-1Ra before a lethal challenge has significantly reduced mortality, but when injected shortly after the challenge, IL-1Ra had little or no effect on reducing death. On the other hand, in acute pancreatitis, a dose-dependent administration of IL-1Ra late in the disease model, the number of organisms injected intravenously ranges from 10⁷ to 10¹⁰ per kilogram. Although systemic levels of IL-1 can be low, tissue production of IL-1 can be high due to membrane IL-1Ra and IL-1Ra from activated platelets. In addition to these sources of IL-1, there is rapid excretion of IL-1Ra, a slow receptor on-rate, increased IL-1RI expression, IL-1Ra binding to the soluble type 1 receptor, and poor tissue penetration of IL-1Ra. Each can also contribute to a requirement for high doses of IL-1Ra. It is

<table>
<thead>
<tr>
<th>Table 9. Effect of IL-1Ra In Vitro</th>
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<tbody>
<tr>
<td><strong>Leukemic cells</strong></td>
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<tr>
<td>Reduced spontaneous proliferation of AML and CML cells</td>
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<tr>
<td>Reduced colony formation in CML (juvenile type) leukemia cells</td>
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<tr>
<td>Reduced spontaneous cytokine production from human AML cells</td>
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<tr>
<td>Reduced spontaneous osteoclast activating factor activity from multiple myeloma cells</td>
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<tr>
<td><strong>Immune-mediated responses</strong></td>
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<tr>
<td>No effect on mitogen- or antigen-induced T-cell proliferation</td>
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<tr>
<td>Decreased IL-2-induced LAK cell generation</td>
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<tr>
<td>Decreased IL-2-induced LAK cell TNF production</td>
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<tr>
<td>Decreased phorbol ester-induced B-cell proliferation and IgG synthesis</td>
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<tr>
<td><strong>Production of cytokines and other molecules</strong></td>
</tr>
<tr>
<td>Reduced LPS-induced monocyte production of IL-1/( \beta ), IL-1( \alpha ), and IL-6</td>
</tr>
<tr>
<td>Decreased asbestos-stimulated IL-6 from mesothelial cells</td>
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<tr>
<td>Reduced spontaneous production of substance P by cultured neurons</td>
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<tr>
<td>Enhanced natural killer cell activity*</td>
</tr>
<tr>
<td>Increased spontaneous PGE(_2) production by cultured decidual cells*</td>
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<tr>
<td>Increased smooth muscle cell proliferation*</td>
</tr>
<tr>
<td><strong>Other effects</strong></td>
</tr>
<tr>
<td>Reduction in <em>Actinobacillus</em> LPS-induced bone resorption</td>
</tr>
<tr>
<td>Reduction in spleen cell colony forming units after radiation</td>
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</tbody>
</table>

*These effects may be due to a reduction in the suppressive effects of PGE\(_2\) induced by IL-1 in these cells.
Models of infection

- Improved survival in endotoxin shock in mice, rats, and rabbits
- Improved survival in Klebsiella pneumoniae infection in newborn rats
- Reduction in shock and mortality in rabbits and baboons from E. coli or Staphylococcus epidermidis bacteremia
- Amelioration of shock and reduction in death after cecal ligation and puncture
- Attenuation of LPS-induced lung nitric oxide activity
- Decreased hypoglycemia, production of CSF, and early tolerance in mice after administration of endotoxin
- Reduction in LPS-induced hyperalgesia
- Protection against TNF-induced lethality in D-galactosamine-treated mice
- Reduction in nematode-induced intestinal nerve dysfunction
- Decreased circulating or cellular TNF production in models of sepsis
- Decreased IL-6 production after LPS
- Protection from Bacillus anthracis toxin-induced lethality in mice
- Decreased intestinal inflammation and bacterial invasion in shigellosis

Models of local inflammation

- Decreased neutrophil accumulation in inflammatory peritonitis in mice
- Reduction in immune complex-induced neutrophil infiltration, eicosanoid production, and tissue necrosis in rabbit colitis
- Reduction in acid-induced neutrophil infiltration and enterocolitis in rats
- Decreased endotoxin-induced intestinal secretory diarrhea in mice
- Reduction in ischemia and excitotoxic-induced brain damage in rats
- Decrease in number of necrotic neurons in cerebral artery occlusion
- Inhibition of permanganate-induced granulomas in rats
- Inhibition of LPS-induced intra-articular neutrophil infiltration
- Decreased IL-1-induced synovitis and loss of cartilage proteoglycan
- Reduced myocardial neutrophil accumulation after coronary occlusions in dogs
- Reduced inflammation and mortality in acute pancreatitis
- Decreased hepatic inflammation following hemorrhage shock

Models of acute or chronic lung injury

- Decreased local LPS-induced neutrophil infiltration in rats
- Inhibition of antigen-induced pulmonary eosinophil accumulation and airway hyperactivity in guinea pigs
- Prevention of bleomycin or silica-induced pulmonary fibrosis
- Reduction in hypoxia-induced pulmonary hypertension
- Reduction in carrageenan-induced pleurisy in rats
- Decreased intratracheal IL-1-induced fluid leak (systemic administration)
- Decreased albumin leak after systemic LPS
- Inhibition of antigen-induced eosinophil accumulation in guinea pigs

Models of metabolic dysfunction

- Reduction in hepatocellular damage after ischemia-reperfusion
- Improved survival after hemorrhagic shock in mice
- Inhibition of SAA gene expression and synthesis in high-dose IL-2 toxicity
- Decreased muscle protein breakdown in rats with peritonitis due to cecal ligation
- Reduced muscle protein breakdown in rats with chronic septic peritonitis
- Inhibition of weight loss after muscle tissue injury
- Decrease in bone loss in ovariectomized rats
- Reversal of LPS-induced CRF gene expression in the hypothalamus
- Prevention of LPS-induced ACTH release

Models of autoimmune disease

- Diminution of Staphylococcus well-induced arthritis in rats
- Reduction in collagen arthritis in mice
- Suppression of anti-basement membrane glomerulonephritis
- Delayed hyperglycemia in the diabetic BB rat
- Reduction in streptozotocin-induced diabetes

Models of immune-mediated disease

- Prevention of graft-versus-host disease in mice
- Prolongation of islet allograft survival
- Reduction in autoimmune encephalomyelitis
- Reduction in skin contact hypersensitivity
- Decrease in coronary artery fibronectin deposition in heterotopic cardiac transplant

Models of malignant disease

- Reduction in the number and size of metastatic melanoma
- Reduction in growth of Mycobacterium avium in organs
- Reduction in tumor-mediated cachexia (intratumoral injection)
- Prevention of graft-versus-host disease in mice
- Prolongation of islet allograft survival
- Reduction in autoimmune encephalomyelitis
- Reduction in skin contact hypersensitivity
- Decrease in coronary artery fibronectin deposition in heterotopic cardiac transplant

Miscellaneous

- Inhibition of TNF-induced social depression in mice
- Prevention of stress-induced hypothalamic monoamine release
- Reduction in LPS-induced sickness behavior in rats
- Suppression of crescentic glomerulonephritis in rats
- Attenuation of muramyl dipeptide-induced sleep in rabbits

Impairment of host responses

- Decreased sciatric nerve regeneration in mice
- Increased mortality to Klebsiella pneumoniae in newborn rats (high dose)
- Increased mortality to Listeria infection
- Enhanced growth of Mycobacterium avium in organs
- Worsening of infectious arthritis (late administration)
- Increased vascular leak in mice given high-dose IL-2

Studies without an effect of IL-1Ra

- Antigen-induced arthritis in rabbits
- LPS- and Staphylococcus epidermidis bacteremia-induced fever in rabbits
- Fever after LPS injection into the brain
- Leukopenia in rats after LPS
- Hypertriglyceridemia after LPS in mice
- LPS-induced increase in skin blood flow

Table 10. Effects of IL-1Rα on Severity of Disease

<table>
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<td></td>
<td>Toxicity</td>
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<td>Decreased muscle protein breakdown in rats with peritonitis due to cecal</td>
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<td>Inhibition of weight loss after muscle tissue injury</td>
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<td>Decrease in bone loss in ovariectomized rats</td>
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<td>Reversal of LPS-induced CRF gene expression in the hypothalamus</td>
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<td>Reduction in streptozotocin-induced diabetes</td>
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<td>Models of immune-mediated disease</td>
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<td>Decrease in coronary artery fibronectin deposition in heterotopic</td>
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<td>Models of malignant disease</td>
<td>Reduction in the number and size of metastatic melanoma</td>
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<td>Suppression of crescentic glomerulonephritis in rats</td>
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<td>Attenuation of muramyl dipeptide-induced sleep in rabbits</td>
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<td>Impairment of host responses</td>
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<td>Increased mortality to Klebsiella pneumoniae in newborn rats (high dose)</td>
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<td>Increased mortality to Listeria infection</td>
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<td>Enhanced growth of Mycobacterium avium in organs</td>
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<td>Worsening of infectious arthritis (late administration)</td>
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<td>Increased vascular leak in mice given high-dose IL-2</td>
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<td>Studies without an effect of IL-1Ra</td>
<td>Antigen-induced arthritis in rabbits</td>
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<td>LPS- and Staphylococcus epidermidis bacteremia-induced fever in rabbits</td>
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also possible that the amount of IL-1β produced during disease has been underestimated because binding of IL-1β to the soluble (and cell-bound) type II receptor has prevented accurate measurements.228

Because triggering so few IL-1 receptors results in a biologic response, it is necessary to sustain a high level of IL-1Ra to block unoccupied receptors. When exogenous IL-1 is injected intravenously into animals, pretreatment with a 100-fold molar excess of IL-1Ra prevents the response to IL-1. For example, injecting rabbits with 100 ng/kg of IL-1β produces fever; a preinjection of 10 μg/kg of IL-1Ra prevents the fever. However, under natural conditions in which endogenous IL-1 and other cytokines are released, an IL-1Ra plasma level of 20 to 30 μg/mL is needed before one observes a reduction in disease.779 In humans, similar levels of IL-1Ra are needed to block the hematologic response to LPS. In vitro, considerably lower concentrations of IL-1Ra are needed.80 For example, a 1 to 1 molar ratio of IL-1Ra to IL-1 blocks 50% of the IL-1–induced response in blood monocytes541 and a concentration of 100 ng/mL of IL-1Ra reduces the spontaneous proliferation, colony formation, and cytokine production of AML or CML cells.397,401,404

Levels of IL-1Ra in human body fluids. Nearly all studies measuring IL-1Ra in human body fluids detect the secreted form of IL-1Ra (sIL-1Ra). It is not unusual to measure high and more sustained levels of IL-1Ra than IL-1β in a variety of human disease states. For example, in healthy volunteers injected intravenously with a low dose of E coli endotoxin, circulating IL-1Ra levels are at a 100-fold molar excess (peak level of 6,000 to 7,000 pg/mL) to those of IL-1β (70 to 80 pg/mL) and are significantly elevated above the baseline levels for over 24 hours.281 In patients with septic shock, juvenile rheumatoid arthritis, or inflammatory bowel disease, a similar ratio can be observed62 and elevated IL-1Ra levels can correlate with the severity of disease.223 In patients with thermal burns, levels of IL-1Ra correlated with the burn surface area and the highest levels of IL-1Ra were measured in nonsurvivors.544

In some clinical situations, markedly elevated levels of IL-1Ra are present in the circulation without elevated IL-1β. High levels of circulating IL-1Ra have been observed after myocardial infarction, after general surgery, and in asymptomatic persons infected with HIV-1. In addition, levels of IL-1Ra are often a better indicator of disease severity than are levels of IL-1β in various liver, autoimmune, and infectious diseases. Under control conditions, healthy IL-1Ra levels increase rapidly after injection of IL-4,454 TNF,546 IFNo or γ,547 IL-6,546 IL-2,548 or IL-1.524 In fact, intravenous injection of 30 ng/kg of IL-1α into humans induces 25 to 30 ng/mL of IL-1Ra,224 which is fourfold higher than that induced by LPS. Injection of IL-1β into humans results in an 86-fold increase in plasma IL-1Ra after 1 hour.525 Under pathologic conditions, IL-1Ra is present in amniotic, cerebrospinal, peritoneal, and joint fluid.

Dysregulation of IL-1Ra production in disease. The molar "ratio" of endogenous IL-1Ra to IL-1β levels in body fluids from patients with infectious, inflammatory, or autoimmune disease is often 10- to 100-fold more IL-1Ra than IL-1β. In some selected clinical conditions, that ratio is far less. If the molar ratio of endogenous IL-1Ra to IL-1 decreases, does this affect disease outcome? Some data provide important findings regarding this question. In AML cells in which IL-1β is spontaneously expressed, IL-1Ra gene expression is suppressed even when stimulated with GM-CSF.379 In 81 patients with CML, cell lysates contained more IL-1β than cells from healthy subjects, whereas the levels of IL-1Ra were the same for both groups.549 In addition, the survival of 44 patients with elevated IL-1β was lower compared with patients with low IL-1β levels. During accelerated blast crisis, IL-1Ra levels were lower compared with patients in a chronic phase.549 Stomal cultures established from bone marrow of patients with aplastic anemia produced less spontaneous as well as induced IL-1Ra compared with stomal cells established from normal bone marrow.540 Recently, we have measured high levels of IL-1α526 in the circulation of 25 patients with hairy cell leukemia that correlate with high levels of IL-1β; however, there was no increase in IL-1Ra levels in these patients.

In patients with acute Lyme arthritis, the duration of joint inflammation is shortest in those patients with the highest joint fluid levels of IL-1Ra, whereas it is prolonged in those patients with low levels of IL-1Ra.551 The reciprocal relationship was found for synovial fluid levels of IL-1β in the same patients. Similar findings were found in the relative production of IL-1α and IL-1β in synovial tissue explants of patients with rheumatoid or osteoarthritis.552,554 In normal skin, icIL-1Ra is present in higher concentrations compared with IL-1α,523 but in psoriatic lesions, the balance is in favor of IL-1α.33,555,556 Alveolar macrophages from smokers produce less IL-1α than those from nonsmokers.557 Under experimental conditions, humans pretreated with corticosteroids before an injection of LPS produce lower circulating levels of TNF, IL-6, and IL-8, but IL-1Ra levels are unaffected by the steroids.503 Does endogenous production of IL-1Ra affect disease outcome? There is little question that elevated production of IL-1Ra is an excellent marker of disease and certainly a better indicator than IL-1 itself. In some clinical conditions, the elevation in IL-1Ra rather than IL-1 may indicate the presence of a pathologic condition. For example, spontaneous and inducible IL-1Ra production by PBMC is higher in patients undergoing chronic hemodialysis compared with age matched patients with renal failure.558 Detecting elevated IL-1Ra production could indicate a natural compensatory mechanism to counter the activity of IL-1, eg, in rheumatoid arthritis559 or HIV-1–infected persons.560 Is the amount of IL-1Ra produced in disease sufficient to dampen the response to IL-1? Using specific, neutralizing antibodies to mouse IL-1Ra, an increase in the formation of Schistosoma egg granulomas was observed when endogenous IL-1Ra was neutralized.561 In rabbits with immune complex colitis, infusion of a neutralizing antibody to rabbit IL-1Ra resulted in exacerbation and prolongation of the colitis.562 As of this writing, the phenotype of an IL-1Ra–deficient mouse is unknown, but neutralizing endogenous IL-1Ra appears to worsen inflammation.

Production of IL-1Ra in vitro. Gene expression and synthesis of sIL-1Ra and IL-1β are differently regulated,
but not IL-1p. Most notable are those cytokines (IL-4, IL-13, and TGFP) that stimulate IL-1Ra and suppress IL-1 production. Triggering the $\beta$-glucan receptor production of these two cytokines has been compared. Nearly all cytokines primarily if not exclusively stimulate IL-1Ra synthesis, both stimuli requiring cross-linking of the respective receptors.

Table 11. Inducers of IL-1p and IL-1Ra

<table>
<thead>
<tr>
<th>Inducer</th>
<th>IL-1p</th>
<th>IL-1Ra</th>
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<tbody>
<tr>
<td>Agents inducing both cytokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viruses, bacteria, yeasts</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Soluble microbial products</td>
<td>+</td>
<td>+</td>
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<tr>
<td>IL-1</td>
<td>+</td>
<td>+</td>
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<tr>
<td>TNF</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Agents inducing primarily IL-1Ra, not IL-1p</td>
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<td></td>
</tr>
<tr>
<td>Immobilized IgG</td>
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<td>+</td>
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<tr>
<td>Immune complexes</td>
<td>-</td>
<td>+</td>
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<tr>
<td>$\beta$-glucans</td>
<td>-</td>
<td>+</td>
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<tr>
<td>IL-4/IL-13</td>
<td>-</td>
<td>+</td>
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<td>IL-10</td>
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<td>+/-</td>
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<td>PDGF BB</td>
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<td>+</td>
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<td>IFN$\gamma$</td>
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<td>IL-6</td>
<td>-</td>
<td>+</td>
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<tr>
<td>TGF$\beta$</td>
<td>+/-</td>
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<tr>
<td>G and GM-CSF</td>
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<td>C-reactive protein</td>
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<td>$\alpha$-antitrypsin and FVYLI</td>
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<td>$\alpha$-1-acid glycoprotein</td>
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<td>Agents inducing primarily IL-1p, not IL-1Ra</td>
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<td>Urate and Ca crystals</td>
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<td>Cs*</td>
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<tr>
<td>Mycobacterium bovis</td>
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<tr>
<td>Staphylococal enterotoxin A</td>
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<tr>
<td>Live Borrelia burgdorferi</td>
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<td>+/-</td>
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* The effect is augmented by the presence of a low concentration of LPS.

and the promoter regions for each cytokine are different. Moreover, the LPS-induced expression of IL-1Ra is controlled by multiple interacting elements. These findings provide additional explanations why circulating levels of these two members of the IL-1 family are often not correlated. Table 11 lists those agents or conditions for which the production of these two cytokines has been compared. Nearly all viruses, bacteria, yeasts, and their products induce both cytokines. Included in this list are IL-1 and TNF. Some inducers require the presence of a low concentration of LPS to stimulate translation of mRNA into the respective proteins.

IL-6 synthesis in the central nervous system is dramatically reversed because of the intense stimulation of IL-1p gene expression and synthesis by these spirochetes.

IL-1Ra administered to healthy humans. IL-1Ra administered intravenously to healthy volunteers is without side effects or changes in biochemical, hematologic, or endocrinologic parameters, even when peak blood levels reach 30 $\mu$g/mL and are sustained above 10 $\mu$g/mL for several hours. These studies support the concept that there is no role for IL-1 in the regulation of body temperature, blood pressure, or hematopoesis in health. It is also consistent with the failure to observe spontaneous expression of the IL-1p gene in circulating blood cells from healthy persons using sensitive PCR methods. Interestingly, PBMC taken from these volunteers after receiving IL-1Ra failed to produce IL-6 when stimulated ex vivo with LPS. A role for IL-1Ra in PBMC in LPS stimulated IL-6 in vitro has been reported.

To evaluate the effect of IL-1Ra on peripheral blood mononuclear cells in vitro. However, in volunteers injected with endotoxin plus IL-1Ra, there was no suppression of the response. Mitogen- and antigen-induced proliferation is a well-established parameter of immunocompetence and is associated with decreased production of IL-2. Similar to experimental endotoxin injection, this suppression is observed in patients with multiple trauma, sepsis, and cardiopulmonary bypass. In experimental endotoxemia and the above clinical conditions, treatment with cyclooxygenase inhibitors restores these cell-mediated immune responses. This effect of cyclooxygenase inhibitors is consistent with the well-known suppressive effects of PGE2 on IL-2 production and T-cell proliferation.

Endotoxin injection suppresses the mitogen-induced proliferative response of peripheral blood mononuclear cells in vitro. However, in volunteers injected with endotoxin plus IL-1Ra, there was no suppression of the response. Mitogen- and antigen-induced proliferation is a well-established parameter of immunocompetence and is associated with decreased production of IL-2. Similar to experimental endotoxin injection, this suppression is observed in patients with multiple trauma, sepsis, and cardiopulmonary bypass. In experimental endotoxemia and the above clinical conditions, treatment with cyclooxygenase inhibitors restores these cell-mediated immune responses. This effect of cyclooxygenase inhibitors is consistent with the well-known suppressive effects of PGE2 on IL-2 production and T-cell proliferation.

Because IL-1 is a potent inducer of COX-2, it is not surprising that blocking IL-1 receptors during endotoxemia would reduce IL-1-induced PGE2 production during endotoxemia. Thus, these studies establish that, under conditions of low-dose endotoxemia, it is possible to block IL-1-mediated responses with IL-1Ra. Those host response parameters that were unaffected by IL-1Ra are likely due to other cytokines

1/ than IL-1Ra. In the case of live Borrelia burgdorferi organisms, the usual balance of IL-1Ra over IL-1p production is dramatically reversed because of the intense stimulation of IL-1p gene expression and synthesis by these spirochetes.
such as TNF or IL-6 or the combination of these cytokines with IL-1.

**IL-1Ra in clinical trials.** IL-1Ra has been administered to patients with septic shock, rheumatoid arthritis, steroid-resistant graft-versus-host disease, AML, and CML. Only the trials in septic shock have used large numbers of patients. The initial (phase I) trial was a randomized, placebo-controlled, open-label study in 99 patients. Patients received either placebo or a loading bolus of 100 mg followed by a 3-day infusion of 17, 67, or 133 mg/h IL-1Ra. A dose-dependent improvement in 28-day mortality was observed; mortality was reduced from 44% in the placebo group to 16% in the group receiving the highest dose of IL-1Ra (P = .015). In that study, there was a dose-related decrease in the circulating levels of IL-6 24 hours after the initiation of IL-1Ra infusion. This decrease in IL-6 levels is consistent with the well-established control of circulating IL-6 levels by IL-1Ra infusion. This decrease in IL-6 levels is consistent with the well-established control of circulating IL-6 levels by IL-1Ra infusion.

A phase II trial in 893 patients showed a trend but without a statistically significant reduction in 28-day mortality. However, a retrospective analysis of 563 patients with a predicted risk of mortality of 24% or greater showed a significant reduction in 28-day mortality (45% in the placebo group and 35% in patients receiving 2 mg/kg/h for 72 hours; P = .005). Similar improvement was observed when patients were scored based on organ failure at entry. Circulating levels of thromboxane B2, PGL2, and leukotrienes C4, D4, and E4 were attenuated (P < .05) at 72 hours in patients receiving the highest dose of IL-1Ra, whereas, in patients receiving the placebo, these eicosanoids were increased at 72 hours. A second phase III trial using 10 g of IL-1Ra infused over 3 days was undertaken but terminated during an interim analysis because a reduction in overall 28-day mortality would not likely reach statistical significance. Similar to trials of monoclonal antibodies to TNF in septic shock using strategies of cytokine or endotoxin blockade, the efficacy of IL-1Ra in reducing 28-day all-cause mortality is not easily demonstrated despite the impressive results in animal models of septic shock and death. Patient heterogeneity is thought to contribute to a failure to bridge the gap between animal and clinical data in sepsis.

IL-1Ra was initially tested in a double-blind, placebo-controlled trial in 25 patients with rheumatoid arthritis. In the group receiving a single subcutaneous dose of 6 mg/kg, there was a decrease in the mean number of tender joints (P < .05). In patients receiving 4 mg/kg per day for 7 days, there was a reduction in the number of tender joints from 24 to 10, the erythrocyte sedimentation rate decreased from 48 to 31, and the C-reactive protein level decreased from 2.9 to 1.9 μg/mL. In this group, the mean plasma concentration of IL-1Ra was 660 ± 240 ng/mL. In an expanded trial, IL-1Ra was administered to 175 patients. Patients were enrolled into the study with active disease and taking nonsteroidal anti-inflammatory drugs and/or up to 10 mg/d of prednisone. There was an initial phase of 3 weeks of either 20, 70, or 200 mg 1, 3, or 7 times per week. Therafter, patients received the same dose once weekly for 4 weeks. Placebo was administered to patients once weekly for the entire 7-week study period. Four measurements of efficacy were used: number of swollen joints, number of painful joints, patient assessment of disease severity, and physician assessment of disease severity. A reduction of 50% or greater in these scores from baseline was considered significant in the analysis. A statistically significant reduction in the number of parameters was observed with the optimal improvement in patients receiving 70 mg per day. Antibodies to TNF in similar patients have reduced symptoms and biochemical markers of the disease and suggest that blocking TNF reduces IL-1 production in rheumatoid joints.

A phase III trial of escalating doses of IL-1Ra in 17 patients with steroid-resistant graft-versus-host disease has been completed. IL-1Ra (400 to 3,400 mg/d) was administered as a continuous intravenous infusion every 24 hours for 7 days. Using an organ-specific, acute disease scale, there was improvement in 16 of the 17 patients. Moreover, a decrease in the steady state mRNA for TNFα in peripheral blood mononuclear cells correlated with improvement (P = .001). These studies in humans are similar to the use of IL-1Ra in animal models of graft-versus-host disease.

In many patients with AML or CML, the spontaneous production of IL-1β appears to play an important, growth-promoting role. In an open-label trial, patients have been infused intravenously or received subcutaneous injections of IL-1Ra. A dose-dependent decrease in the peripheral blast count and bone marrow cellularity has been observed (M. Talpax, personal communication, August 1994). However, the amount of IL-1Ra administered to these patients was not as high as that administered to patients with septic shock or graft-versus-host disease; hence, these patients could benefit from a more aggressive use of IL-1Ra. The rationale for treating an individual patient with IL-1Ra should be based on the ability of that individual's peripheral mononuclear cells to respond to IL-1Ra in vitro by a decrease in spontaneous proliferation or a decrease in production of CSF.

**Commentary on IL-1Ra in treating human disease.** Sustaining a plasma level of IL-1Ra in humans of 30 μg/mL in healthy humans for several hours or in patients with the septic shock syndrome for 3 days is without untoward effects. These observations underscore the safety of IL-1Ra. For clinical efficacy, IL-1Ra in patients with septic shock, graft-versus-host disease, rheumatoid arthritis, or leukemia exhibit a dose-dependent response. Even the reduction of endotoxin-induced neutrophilia in healthy subjects is dose-dependent. Animal studies support these clinical observations. The requirement for such high plasma levels of IL-1Ra is not completely understood because IL-1Ra levels are already several logs higher than measurable IL-1 levels in the most severe cases of septic shock. Rapid renal clearance, binding to the soluble form of the type I receptor, and increased type I receptor expression may explain a need for these high levels. In addition, paracrine effects and IL-1 activity on platelets and monocytes may be binding to type I receptors on cells outside the vasculature before tissue levels of IL-1Ra match those in the circulation.
In the two phase III trials of IL-1Ra in septic shock, retrospective analysis showed decreased mortality in patient subgroups, particularly during the first 7 days after entry into the trials. These results suggest that not all patients with life-threatening septic shock benefit from IL-1Ra and that factors other than IL-1 contribute to the cause of death 28 days later. Similar conclusions have been made using antibodies to TNF in clinical trials of septic shock. Are there any clinical situations in which administration of IL-1Ra will be efficacious? Patients with acute pancreatitis, AML, CML, hairy cell leukemia, or multiple myeloma deserve to enter clinical trials using high-dose IL-1Ra similar to those used in septic shock and graft-versus-host disease. In addition, defining entry criteria in patients with septic shock will likely identify those patients who will benefit from IL-1Ra. One candidate for consideration is a high level of IL-6, which often reflects the amount of IL-1 produced. It is also likely that the benefit of IL-1Ra in acute life-threatening disease will be enhanced when administered together with anti-TNF therapy.

AMELIORATION OF DISEASE USING SOLUBLE IL-1 RECEPTORS

**Soluble IL-1R in animal studies.** The extracellular domain of the type I receptor has been used in several models of inflammatory and autoimmune disease. Administration of murine IL-1sRI to mice has increased the survival of heterotopic heart allografts and reduced the hyperplastic lymph node response to allogeneic cells. In a rat model of antigen-induced arthritis, local instillation of the murine IL-1sRI reduced joint swelling and tissue destruction. When a dose of soluble receptor (1 µg) was instilled into the contralateral, unaffected joint, a reduction in the degree of tissue damage was observed in the affected joint. These data suggest that the amount of IL-1sRI administered in the normal, contralateral joint was acting systemically. In a model of experimental autoimmune encephalitis, the IL-1sRI reduced the severity of this disease. Administration of IL-1sRI to animals has also been reported to reduce the physiologic response to LPS, acute lung injury, and delayed-type hypersensitivity. To date, there are no data on the efficacy of soluble type II receptors in animal models of disease.

**Soluble IL-1 receptor type I in human studies.** Recombinant human IL-1sRI has been administered intravenously to healthy humans in a phase I trial without side effects or changes in physiologic, hematologic, or endocrinologic parameters. Thus, similar to infusions of IL-1Ra, IL-1sRI appears safe and reinforces the conclusion that IL-1 does not have a role in homeostasis in humans. Administration of IL-1sRI in humans has been effective in reducing the delayed hypersensitivity skin reaction to recall antigens. Patients with known antigen hypersensitivity received an intradermal injection of a specific antigen and also an injection of either placebo or IL-1sRI near the site. In addition, IL-1sRI was injected locally at a contralateral site. As the amount of IL-1sRI increased, there was a progressive decrease in the inflammatory lesion due to the allergen. However, there was also a decrease in the lesion size as the amount of IL-1sRI injected at the contralateral site was increased to 10 to 100 µg. These findings are similar to those observations in rats receiving a contralateral instillation of IL-1sRI during antigen-induced arthritis.

**Commentary on the therapeutic use of soluble forms of IL-1 receptors.** The goal of any anti-IL-1 strategy is to prevent IL-1 binding to surface receptors. Using soluble receptors to block IL-1 activity in disease is similar to using neutralizing antibodies against IL-1 and distinct from using receptor blockade with IL-1Ra. Because the molar concentrations of circulating IL-1 in disease are relatively low, pharmacologic administration of IL-1sRI to reach a 100-fold molar excess of IL-1sR over that of IL-1 is feasible. The human trial of IL-1sRI in delayed hypersensitivity reactions supports the notion that low doses (100 µg/patient) can have anti-inflammatory effects. The fusion of two chains of extra-cellular domains of the type IL-1R to the Fc portion of Ig enhances the binding IL-1 over that of monomeric IL-1sRI and may have a greater plasma half-life compared with that of the monomeric form. In contrast to neutralizing IL-1, the goal of receptor blockade requires the condition of blocking all unoccupied IL-1 surface receptors because triggering only a few evokes a response. Receptor blockade is a formidable task and the large amounts of IL-1Ra required to reduce disease activity contributes to this conclusion. The potential disadvantage for using soluble IL-1 receptor therapy is the possibility that these receptors will prolong the clearance of IL-1 and risk the chance of a delayed response to IL-1. Although the soluble form of the type II receptor should perform better than the type I, there are no studies on the type II soluble receptor in animals or humans.

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CA Dinarello