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REVIEW ARTICLE

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The role of interleukin-1 receptor antagonist in the prevention and treatment of disease

Abstract Interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) play key proinflammatory roles in a variety of human diseases, including rheumatoid arthritis (RA). IL-1 receptor antagonist (IL-1Ra) is a naturally occurring structural variant of IL-1 that competitively inhibits receptor binding of IL-1. Four forms of IL-1Ra have been described: secretory IL-1Ra (sIL-1Ra) and three intracellular molecules (icIL-1Ra1, 2, and 3). Excess amounts of IL-1Ra are necessary to inhibit the biological effects of IL-1. The endogenous production of IL-1Ra plays an anti-inflammatory role, but the level of production of IL-1Ra in inflamed tissues may not be adequate to block IL-1 effectively. An allelic polymorphism in the IL-1Ra gene is associated with a variety of human diseases, largely of epithelial or endothelial cell origin. The disease associated allele IL1RN*2 may lead to a decreased production of icIL-1Ra1 by these cells, predisposing the patient to an imbalance in the IL-1 system. The therapeutic administration of IL-1Ra was found to be safe and efficacious in the treatment of RA. Intraarticular delivery of the IL-1Ra cDNA by ex vivo gene therapy in patients with RA was effective in enhancing local IL-1Ra production. This unique form of therapy is under further evaluation.

Key words Cytokines · Interleukin-1 (IL-1) · IL-1 receptor antagonist · Rheumatoid arthritis (RA)

Introduction

Considerable evidence has accumulated over the past 10 years to indicate that the cytokines interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) mediate inflammation and tissue destruction in a variety of human diseases. The most studied diseases are rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), but the list may also include forms of glomerulonephritis, pulmonary disease, and heart disease. The biological effects of these cytokines are multiple. In disease states, IL-1 and TNF-α function primarily by stimulating the migration of inflammatory and immune cells from the blood into tissues, and by inducing the production of tissue-injuring enzymes in fibroblasts and other cells. Both IL-1 and TNF-α also play important roles in normal physiology, including in host defense against microorganisms that reside within cells, and in stimulation of the turnover of extracellular matrix.[1]

Control mechanisms exist to limit the extent of cytokine activation and to avoid potential tissue injury. The IL-1 system is unique in having a natural inhibitor, known as the IL-1 receptor antagonist (IL-1Ra) molecule. IL-1Ra is a structural derivative of IL-1 that binds to IL-1 receptors (IL-1R), but does not activate target cells.[2–4] It is now known that IL-1Ra consists of a family of molecules (Table 1). The isoform of IL-1Ra originally described (sIL-1Ra) is secreted, and released from monocytes and macrophages after stimulation by adherent IgG, bacterial lipopolysaccharides (LPS), cytokines, or other substances. Three additional intracellular structural variants of IL-1Ra (icIL-1Ra1, 2, and 3) are formed by alternate transcriptional splice mechanisms. These isoforms of IL-1Ra do not possess leader sequences, and thus are synthesized in the cytoplasm and are not usually secreted from cells.

The members of the IL-1Ra family may carry out different functions. The role of sIL-1Ra is to block the effects of IL-1 in the immediate cell environment by competing for binding to cell-surface receptors. sIL-1Ra is also produced by the liver as an acute-phase protein, and is present in the circulation in large amounts in patients with inflammatory or immune diseases, or after surgery.[5] The major intracellular isoform of IL-1Ra (icIL-1Ra1) is produced primarily by epithelial cells, binds to IL-1R with equal avidity as sIL-1Ra, and can be secreted from cells under certain conditions. icIL-1Ra2 has been described in cells only at the mRNA level and may not normally exist as a protein. icIL-

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Table 1. Isoforms of IL-1Ra

Secretory IL-1Ra (sIL-1Ra)
Synthesized as a 17-kDa molecule in monocytes, macrophages, and other cells
Secreted as variably glycosylated molecules of 22–25 kDa
Competitively inhibits the binding of IL-1 to types I and II IL-1 receptors
Intracellular IL-1Ra type 1 (icIL-1Ra1)
Created by a transcriptional splice from an alternative upstream exon
Synthesized in the cytoplasm of epithelial cells, endothelial cells, monocytes, and macrophages as an 18-kDa molecule
Possesses additional 7 NH_2-terminal residues compared with sIL-1Ra
Binds to IL-1 receptors equally as well as sIL-1Ra
May be secreted from keratinocytes and other epithelial cells under certain conditions
May carry out additional functions inside cells not involving receptor binding
Intracellular IL-1Ra type 2 (icIL-1Ra2)
mRNA created by a transcriptional splice from an alternative upstream exon
May not be translated in vivo as the predicted protein of 26 kDa has not been identified in tissues
Intracellular IL-1Ra type 3 (icIL-1Ra3)
Created both by a transcriptional splice from an alternative upstream exon and by alternative initiation of translation
Synthesized in cytoplasm of neutrophils, hepatocytes, monocytes, and macrophages as a 16-kDa molecule
Lacks 9 NH_2-terminal residues compared with sIL-1Ra
Binds weakly to the IL-1 receptor

1Ra3 is a lower molecular weight protein that is produced in large amounts by neutrophils and hepatocyes, but binds poorly to IL-1 receptors. The role in biology of the intracellular isoforms of IL-1, particularly icIL-1Ra1, may be to inhibit the binding of IL-1 to extracellular receptors in specific situations. However, these unique molecules may carry out additional, as yet unclear, functions inside cells that do not involve receptor binding.

This review summarizes information on the role of endogenous IL-1Ra in the prevention of disease, the association of IL-1Ra polymorphisms with the predisposition to, or severity of, disease, and the efficacy of the administration of exogenous IL-1Ra in the treatment of disease.

IL-1Ra in the prevention of disease

An important concept that has emerged in recent years is that a balance between the amounts of IL-1 and IL-1Ra present in a particular organ or tissue may influence the development of disease. Although IL-1Ra binds to IL-1R with equal avidity as IL-1, a 100-fold or greater excess of IL-1Ra over IL-1 is necessary to inhibit the biological effects of IL-1.[6] This requirement is because cells are extraordinarily sensitive to IL-1, exhibiting full responses to the presence of a few molecules of IL-1 per cell bound to the type I IL-1R (IL-1RI). Because more than 1000 IL-1RI per cell are present in most cells, a great excess of IL-1Ra is required to prevent the binding of only a few molecules of IL-1.

An anti-inflammatory role for endogenous IL-1Ra has been investigated in experimental animal models of disease and also in human disease. LPS injection into the knee joints of rabbits induced acute arthritis.[7] The molar ratio of IL-1Ra over IL-1 was 160- to 400-fold in the synovial fluids of these animals. The coadministration of a neutralizing antibody to IL-1Ra along with LPS into the joints enhanced both the peak infiltration of leukocytes into the synovial fluid and the local production of IL-1β.[7] This finding illustrates the point that IL-1 is capable of stimulating its own production, and this positive feedback loop is inhibited by endogenous IL-1Ra. The balance between IL-1 and endogenous IL-1Ra is also important in human arthritis. The synovial fluids of patients with Lyme disease and acute knee arthritis were examined for the concentrations of these two cytokines in relation to the duration of the attack. Those patients with high synovial fluid levels of IL-1Ra and low levels of IL-1β experienced a more rapid resolution of arthritis than did patients with the opposite cytokine pattern.[8] Thus, endogenous IL-1Ra produced in the joints of animals and humans with inflammatory arthritis plays a role in reducing local inflammation.

Although it exhibits anti-inflammatory effects, the amounts of IL-1Ra produced locally in a disease may not be sufficient to fully inhibit IL-1. This principle was illustrated in studies on cultured synovial cells from patients with active RA where the amount of IL-1Ra produced was insufficient to block the stimulatory effects of the IL-1 synthesized by these cells in vitro.[9] A lack of adequate tissue levels of IL-1Ra has also been described in other diseases, including in the intestinal wall in patients with IBD. However, in collagen-induced arthritis (CIA) in mice, an experimental animal model closely resembling RA, the resolution of acute arthritis and the development of fibrosis was accompanied by a decrease in the synovial production of IL-1 and an increase in the production of icIL-1Ra1.[10] Thus, this intracellular isoform of IL-1Ra may play a role in the healing phase of this animal model of inflammatory arthritis. We have observed that synovial tissues from patients with long-term active RA entirely lack the mRNA for icIL-1Ra1, suggesting that an imbalance between IL-1 and IL-1Ra may help to perpetuate this disease (C. Gabay and W.P. Arend, unpublished observations).

The importance of maintaining a balance between the local production of IL-1 and IL-1Ra is further illustrated by the results of studies on mice that are genetically engineered to overproduce sIL-1Ra (transgenic mice) or those that lack the capacity to make any isoforms of IL-1Ra (knockout mice). Collagen-induced arthritis in mice was reduced in incidence and severity in IL-1Ra transgenic mice, whereas the opposite pattern was observed in IL-1Ra knockout mice.[11] Furthermore, mice which were genetically deficient in the production of IL-1Ra, when crossed into the BALB/cA background, spontaneously developed an inflammatory arthritis which exhibited many features of RA.[12] Other investigators observed that a backcross of IL-1Ra knockout mice into the MF1 strain led to the spontaneous appearance of inflammatory arterial disease with features of the human disease polyarteritis nodosa.[13] These

observations suggest that the presence of IL-1Ra in both the joint and the arterial wall is necessary to oppose the inflammatory effects of IL-1 and to maintain homeostasis. It would be of great interest to know which are the other genes in these two inbred strains of mice that predispose them to the spontaneous development of disease in the absence of IL-1Ra.

IL-1Ra polymorphisms and disease

There is increasing evidence that the ratio of IL-1Ra to IL-1 may be under genetic influence, and may influence the development or severity of certain diseases.[14] The hypothesis is that in a sub-population of individuals, excess production of IL-1 or inadequate production of IL-1Ra would be associated with a disease process. An allelic polymorphism is present in intron 2 of the sIL-1Ra gene (IL1RN), or in intron 3 of the extended gene containing the additional 5′ exon encoding the NH_2-terminal portion of icIL-1Ra1. This polymorphism consists of two to six numbers of an 86-bp tandem repeat; allele 1 (IL1RN*1) is present in 73.6% of the normal Caucasian population, and allele 2 (IL1RN*2) is found in 21.4%.[15] IL1RN*2 has been associated with a variety of human diseases, primarily of epithelial and endothelial cells (Table 2). Depending on the disease, the presence of IL1RN*2 may be associated with severity rather than with an increased predisposition to acquire the disease.

A possible association between polymorphisms in the IL-1Ra gene and various rheumatic diseases has been studied. Because the production of IL-1Ra by cultured synovial tissue from patients with RA is deficient relative to IL-1, allelic polymorphisms in the IL-1Ra and IL-1 genes have been examined in this disease. In initial studies, the frequency of IL1RN*2 was not increased in Caucasian patients with RA in comparison to healthy controls.[16,17] However, the combination of certain HLA-DR alleles containing the shared epitope with allele E2 in IL-1β exon 5, which predisposes to high levels of IL-1β production, is associated with a high risk of developing erosive RA (odds ratio of 8.20).[17,18] In another European study, the more rare −511 allele in the IL-1β promoter was associated with a less severe course in RA of long duration, although the influence of this allele on the production of IL-1β is not known.[19] Thus, the possibility exists that an excess production of IL-1 over IL-1Ra may lead to more tissue destruction in RA.

Patients with RA are known to have an enhanced development of cardiovascular disease, contributing to increased mortality. The combination of allele A1 of IL-1β with allele 2 of IL-1Ra is associated with an apparent protection against cardiovascular complications of RA.[20] However, single-vessel coronary artery disease in the general population is significantly associated with the carriage of IL-1Ra allele 2.[21] The suggestion was offered in this study that perhaps the presence of IL1RN*2 slowed progression to multiple-vessel disease. Furthermore, IL1RN*2 appeared to be associated with protection against restenosis after coronary artery angioplasty.[22] Only icIL-1Ra1 is produced by human umbilical vein and coronary artery endothelial cells in vitro and in vivo, with no sL-1Ra mRNA or protein having been detected in these cells.[23] A decreased production of icIL-1Ra1 was demonstrated in cultured umbilical vein endothelial cells from IL1RN*2 individuals. One interpretation of these observations is that the presence of lower IL-1Ra production by endothelial cells in ILRN*2 individuals, relative to that of IL-1β, decreases the development of coronary artery narrowing under conditions of chronic inflammation and fibrosis, such as may occur in RA or after coronary artery angioplasty. Perhaps IL-1 is proinflammatory in acute conditions, but protective against scarring in chronic situations.

IL-1Ra gene polymorphisms have also been examined in other rheumatic diseases. No association of IL-1β or IL-1Ra allelic polymorphisms with spondyloarthropathies were found in a European transmission disequilibrium study of 37 families representing 217 subjects, 99 of whom had a form of spondyloarthropathy (62 ankylosing spondylitis, 37 other diagnoses).[24] However, in a case-controlled study from Scotland of 187 HLA-B27-positive patients with ankylosing spondylitis, there was a significant increase in the carriage of IL1RN*2 (odds ratio 2.3).[25] No associations were detected between this disease and polymorphisms in the IL-1α and IL-1β genes. Identical results were obtained in a Dutch study.[26] A genotyping study in 61 patients with osteoarthritis indicated an association with allele E2 in IL-1β exon 5 and allele A2 of IL-1Ra; these alleles lead to an increased production of IL-1β and decreased production of IL-1Ra.[27] In a recent study from the United Kingdom, knee osteoarthritis in 136 patients was associated with the IL-1Ra locus, but with the opposite alleles.[28] Further work will be necessary to reconcile these apparently contradictory findings. The IL-1Ra gene allele A2 has also been positively associated with Sjögren's syndrome,[16] skin disease in systemic lupus erythematosus (SLE),[29–31] and oligoarthritis in juvenile idiopathic arthritis.[32] In contrast, IL1RN*1 has been described as being associated with juvenile idiopathic

Table 2. Diseases associated with IL-1Ra allelic polymorphism (IL1RN*2)

Lichen sclerosis
Alopecia areata
Early-onset psoriasis
Ulcerative colitis in certain population groups
Multiple sclerosis in certain population groups
Severe forms of Sjögren's syndrome
Skin disease in systemic lupus erythematosus
Juvenile chronic arthritis (oligoarticular type)
Henoch–Schönlein purpura
IgA nephropathy
Gastric cancer
Diabetic nephropathy
Severe sepsis
Early-onset periodontitis
Nonatopic asthma
Fibrosing alveolitis
Silicosis in coal minors
Severity of GVHD
Idiopathic recurrent miscarriage

Adapted from ref. 14

inflammatory myopathies; the effect of this allele on IL-1Ra production is not known.[33]

Thus, the results of multiple studies from different ethnic groups suggest that genetic influences on IL-1β or IL-1Ra production may influence the development or severity of a variety of rheumatic diseases. These are all likely to be polygenic diseases. Therefore, the increased or decreased production of IL-1β and IL-1Ra are acting within the environment of other, as yet poorly described, genetic predisposing factors.

IL-1Ra in the treatment of disease

Studies in numerous animal models of chronic inflammatory diseases have indicated that the exogenous administration of IL-1Ra is therapeutically beneficial. This treatment has been evaluated in patients with sepsis syndrome or RA. The symptoms induced by the infusion of small amounts of endotoxin into human volunteers were reduced by the concomitant administration of IL-1Ra; however, the hemodynamic, immunological, and metabolic responses were not blunted.[34] An initial clinical trial of an IL-1Ra infusion for 72 h in 99 clinically heterogeneous patients with sepsis syndrome indicated a possible dose-dependent improvement in mortality at 28 days.[35] However, no overall decrease in 28-day mortality was observed in a subsequent phase III trial in 893 patients with sepsis syndrome.[36] A retrospective analysis indicated a possible benefit in those sepsis patients who had organ failure and/or a predicted mortality of 24% or greater. However, no further clinical trials with IL-1Ra in this disease have been carried out.

Clinical trials in RA examining the administration of recombinant human IL-1Ra by subcutaneous injection have yielded more positive results, and this therapy has now been approved by the regulatory agencies in the USA and Europe (Table 3). In an initial randomized, double-blind phase I clinical trial in 175 patients with active RA, IL-1Ra was administered in varying doses and schedules for 3 weeks, followed by a once-weekly drug for a 4-week maintenance phase.[37] This therapy was well tolerated, although 62% of patients developed injection-site reactions, which required premature withdrawal from the treatment in 5% of cases. The efficacy could not be assessed, but a daily subcutaneous injection appeared to be more effective than once-weekly administration.

A total of 472 patients with active and severe RA were subsequently studied in a 24-week, double-blind, randomized, placebo-controlled, multicenter clinical trial.[38] All disease-modifying antirheumatic drugs were stopped at least 6 weeks prior to entering into the trial, with NSAIDs and oral prednisone (dose ≤10 mg daily) being continued at constant dosages. Patients were randomized into four treatment groups: placebo, or a single daily subcutaneous injection of IL-1Ra at 30, 75, or 150 mg. In patients treated with the highest dose of IL-1Ra, 43% achieved an ACR20 response, compared with 27% in the placebo group. No serious adverse events were observed, although injection-site reactions were again frequent, leading to a 5% withdrawal rate in the 150 mg/day treatment group. A continued clinical benefit was observed over a second 6 months in the patients who received IL-1Ra, and patients who initially received placebo also responded to IL-1Ra equally well in this extended study. The IL-1Ra-treated group overall demonstrated a lower rate of radiological progression over both 24 and 48 weeks.[39] Histological studies on serial synovial biopsies showed a reduction in mononuclear cell infiltration in four patients who showed a favorable clinical response.[40]

Combination therapy with IL-1Ra and methotrexate was then studied in 419 patients with continuing active RA despite receiving methotrexate for 6 months or longer.[41] The patients were randomized into six groups: placebo, or 0.04, 0.1, 0.4, 1.0, or 2.0 mg/kg IL-1Ra administered as a single daily subcutaneous injection. All patients continued to receive methotrexate at their previously stable dose, averaging ca.17 mg/week. Patients treated with methotrexate and IL-1Ra showed a dose–response improvement in ACR20 responses at 12 weeks. Patients receiving 1.0 or 2.0 mg/kg IL-1Ra showed ACR20 responses at 12 weeks of 46% and 38%, respectively, compared with 19% in the placebo group. At 24 weeks, ACR20, 50, and 70 responses were present in 42%, 24%, and 10%, respectively, of patients receiving 1.0 mg/kg IL-1Ra plus methotrexate, compared

Table 3. Clinical trials with recombinant human IL-1Ra in RA

Initial phase I trial, IL-1Ra alone[37]
3-week randomized, double-blind trial in 175 patients with active RA
Varying doses and schedules
Daily subcutaneous injection more efficacious than weekly injection
Injection-site reactions in 62%, with premature withdrawal in 5% of patients
Phase II-III trial, IL-1Ra alone[38]
24- and 48-week double-blind, randomized, placebo-controlled trial in 472 RA patients
Four treatment groups: placebo, or 30, 75, or 150 mg IL-1Ra by daily subcutaneous injection
At 24 weeks, 43% ACR20 response in 150-mg group compared with 27% in placebo group
Benefit maintained through 48 weeks
Injection-site reactions led to premature withdrawal in 5% of patients treated with 150 mg IL-1Ra
Lower rate of radiological progression in IL-1Ra-treated patients over 24 and 48 weeks
Phase III trial, IL-1Ra with methotrexate[41]
419 patients with active RA despite treatment with methotrexate for 6 months or longer
Maintained on methotrexate at an average dose of ~17 mg per week
Six treatment groups: placebo (methotrexate alone), or 0.04, 0.1, 0.4, 1.0, or 2.0 mg/kg IL-1Ra
At 24 weeks, ACR20, 50, and 70 responses were observed in 42%, 24%, and 10%, respectively, of patients treated with 1.0 mg/kg IL-1Ra compared with 23%, 4%, and 0%, respectively, in the placebo group
Injection-site reactions in 56% of patients treated with 1.0 mg/kg IL-1Ra, with withdrawal in 7%
Four patients developed transient leukopenia
No increased incidence of severe infections in IL-1Ra-treated patients

with values of 23%, 4%, and 0%, respectively, in the placebo group treated with methotrexate alone. Adverse events included injection-site reactions in 56% of patients receiving 1.0 mg/kg IL-1Ra, and these led to premature withdrawal in 7%. Four patients withdrew from the study because of transient leukopenia, but there was no increased incidence of infections in the IL-1Ra-treated patients.

Thus, the results of three major clinical trials with recombinant human IL-1Ra administered to patients with RA indicated that this treatment was safe and efficacious. The most common adverse events were injection-site reactions. Additional studies are in progress to assess the safety of treatment with IL-1Ra over longer periods of time, with particular attention being paid to infections and the possible development of malignancies.

Gene therapy with IL-1Ra has been effective in animal models of inflammatory arthritis, with the interesting observation that the delivery of the cDNA for IL-1Ra into one joint led to an improvement in adjacent joints.[42] This more systemic benefit is probably due to the trafficking of cells between joints carrying the IL-1Ra gene. An initial clinical trial of ex vivo gene therapy with IL-1Ra in RA has been completed in nine patients (Evans C.H. et al., unpublished observation). These patients were scheduled to receive silastic implants into the 2nd–5th metacarpal joints of one hand. Autologous synovial fibroblasts were obtained from surgery on another joint in each patient, and half of the cells were transduced in vitro with a retrovirus containing the IL-1Ra cDNA. Two metacarpophalangeal (MCP) joints were injected with transduced cells, and the other two MCP joints in each patient received control cells. After 1 week, the injected joints were resected and examined for evidence of IL-1Ra mRNA and protein. All patients tolerated the procedure well, with no complications. The joints receiving the transduced cells showed IL-1Ra mRNA and protein, and synovia obtained from these joints produced elevated levels of IL-1Ra in vitro. These results indicate that gene therapy with IL-1Ra in RA is feasible and safe. Additional clinical trials are planned.

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