Evidence for the biological modulation of IL-1 activity: The role of IL-1Ra

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ABSTRACT
Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are key mediators of inflammation and are produced by monocyte-macrophages (M) following the activation of soluble factors and contact with stimulated Th1 lymphocytes. The contact between lymphocytes and M is regulated by ligand and counterligands (i.e. -integrins, CD40-CD40L, CD69) and plasma lipoproteins (i.e. HDL-associated Apo-AI). IL-1 and TNF are potent inducers of matrix metalloproteinases (MMPs), eicosanoids, nitric oxide oxidase (iNOS), receptor activator of NF- B ligand (RANKL), products involved in the destruction of the extracellular matrix, the cartilage and in bone resorption. IL-1 – particularly important at the local level – is more potent than TNF in stimulating MMPs and specifically in impeding cartilage repair. However, IL-1 and TNF strongly synergize in multiple biological functions. Blockade of IL-1 by IL-1 receptor antagonist (IL-1Ra, sIL-1RII) in combination with the soluble IL-1 accessory protein (IL-1R AcP) result in a long-term beneficial effect in chronic inflammatory diseases. The association with anti-TNF therapy may also represent a logical approach, considering the number of patients that do not respond to either compound alone. An altogether new challenge would be to accomplish the blockade at a more proximal level (i.e. lymphocyte/M interaction).

1. Role of interleukin-1 in tissue destruction and repair
There is considerable evidence that interleukin-1 (IL-1) and tumor necrosis factor (TNF) are key mediators of inflammation and tissue degradation in rheumatoid arthritis (RA) (1). Both cytokines may influence local and systemic disease manifestations, as well as the mechanisms that result in the destruction of cartilage, tendons and bone matrix. In particular, the effects of IL-1 on the induction of proteolytic enzymes such as neutral matrix metalloproteinases and aggrecanases appear to be critical in the pathophysiology of structural joint damage. IL-1 and TNF are produced primarily by macrophages and, to a lesser extent, by fibroblasts, synoviocytes and neutrophils. IL-1 shares many biological activities with TNF, causing an increased expression of cyclo-oxygenase type 2 (COX-2), platelet activation factor (PAF) and inducible nitric oxide synthase (iNOS). This in turn leads to increased levels of prostaglandin E2 (PGE2) and nitric oxide. IL-1 and TNF also cause increased expression of adhesion molecules on endothelial cells, allowing migration of inflammatory cells into the extravascular space (2). Histological observation strongly suggests that the close vicinity of T lymphocytes (TL) – mainly CD4+ – with monocyte-macrophages (M) in the inflamed synovial tissue is primordial. However, B lymphocytes and complement components leading to the formation of immune complexes (rheumatoid factors) also play a role in stimulating M.

Based on in vitro experiments we proposed 10 years ago that direct contact between TL and M is a major pathway inducing the production of IL-1 and TNF in monocytes, and it appears to be more potent by far than the effect of TL soluble cytokines (3). In vitro experiments on human synoviocytes (4), as well investigations using experimental animal models, support the theory that it could be advantageous to block both IL-1 and TNF. Besides, the therapeutic use of either anti-TNF or anti-IL-1 agents has shown that the percentage of non-responding patients is not negligible. Therefore, by impeding this cell-cell contact, it will be possible to inhibit the production of both cytokines.
The claim that IL-1 is associated with osteoclast activation, consequently increasing bone resorption, is based on the observation that: (a) IL-1 induces bone resorption in vitro; (b) elevated IL-1 levels in gingival fluid correlate with the severity of bone resorption; (c) osteoporosis in elderly patients is reduced with the spontaneous expression of high IL-1Ra levels, while it increases in the presence of low IL-1Ra levels; and (d) blocking of IL-1 receptor expression on chondrocytes.

IL-1 acts on osteoblasts and bone resorption by inducing the expression of RANKL (receptor activator of the NF-kB ligand), also known as ODF (osteoclast differentiation factor). In turn, RANKL stimulates the differentiation of the osteoclast precursor into mature osteoclasts responsible for bone resorption (5). RANKL and IL-1 also stimulate directly mature osteoclasts. Fortunately, this system is not without an inhibitory molecule: osteoprotegerin (OPG), a RANKL antagonist that inhibits osteoclast differentiation.

RANKL also induces the production of pro-inflammatory cytokines such as IL-1 and of others that stimulate and induce differentiation of T cells (e.g. IL-12 and IL-15 produced by APC). To some degree, IL-1 and TNF also act on marrow stromal cells and osteoclasts to secrete OPG. However, in RA the concentration of RANKL is probably higher than that of OPG.

As far as tissue destruction is concerned, at equal molar concentrations IL-1 is more potent than TNF in that it induces matrix metalloproteinases (MMP) and aggrecanases. However, once more the synergism with TNF is significant. The respective roles of IL-1 and TNF in terms of tissue destruction remain to be clearly defined in the different human diseases since they may vary. For the time being, the relevance of IL-1 as a crucial destructive mediator and propagator of joint inflammation is highlighted by the absence of chronic arthritis and joint erosions in IL-1-deficient mice (6). On chondrocytes IL-1 is clearly a more potent catabolic factor than TNF. This may partly be due to the differential level of receptor expression on chondrocytes.

In chronic inflammation and joint destruction one of the main differences between IL-1 and TNF is probably related to their effect on the repair process since IL-1 strongly suppresses aggrecan synthesis, type II collagen synthesis and chondrocyte proliferation. The disparate effects of IL-1 and TNF are illustrated in chronic relapsing SCW arthritis. Indeed, repeat injections of SCW activate macrophages and give rise to destructive arthritis, characterized by loss of proteoglycans, erosion of the surface and pronounced infiltration of the synovial tissue. These characteristic features of arthritis can be induced in normal mice but are severe in a TNF-deficient background, which illustrates that erosion and inflammation occur even in the absence of TNF. In contrast, proteoglycan depletion is obvious in control litter-mates but not in IL-1-deficient mice. In various in vitro models of human cartilage culture, explants or cell cultures (Table I) (see review in 7) IL-1 strongly decreases the synthesis of proteoglycans, whereas the effect of TNF is less pronounced (8).

In summary, IL-1 is a key mediator of rheumatic diseases because of: (a) its greater capacity (greater than that of TNF) of increasing matrix degradation by inducing the production of MMPs and PGE$_2$ in synovial cells; (b) its role as mediator in bone resorption and cartilage destruction; (c) its property of decreasing the repair process by suppressing matrix synthesis; (d) its strong synergism with TNF in inducing many inflammatory genes at both local and systemic levels; and (e) its possible genetic link to highly erosive RA. Generally speaking, it appears that TNF is more involved in systemic manifestations and inflammation, endothelial cells being exquisitely sensitive to TNF. IL-1 is mainly involved at the local level, bone destruction and the lack of a repair process. Finally, in innate immunity, IL-1 shares with TNF and IL-6 many biological activities, the most important being the hepatic acute-phase response, the response of the central nervous system (fever, altered sleep behavior, anorexia), increased muscle proteolysis and adipocty lipoysis, macrophage activation, release and activation of neutrophils (2, 9).

### II. The discovery of IL-1 receptor antagonist

As early as 1983/1984, before the cloning of IL-1, we suspected the presence of a potential inhibitor to IL-1. Indeed, while pursuing the goal of isolating large amounts of IL-1 using the bioassay of stimulation of collagenase and prostaglandin on synovial cells, our attention was drawn to diseases associated with large amounts of monocytes, i.e. monocytic leukemia, or diseases associated with a high temperature or chronic debilitating diseases such as RA and juvenile rheumatoid arthritis. To our surprise, considering that the screening for IL-1 was only possible by bioassay (no immunoassays were available at that time), we failed to detect IL-1 biological activities in the serum or urine of seriously ill patients suffering from the above diseases. This prompted the hypothesis that IL-1 might be masked by inhibitory molecules, and after biochemical purification a factor of ~17 kDa was isolated from the urine of patients with monocytic leukemia (10, 11). This factor specifically blocked the biological activities of IL-1, without affecting those of TNF (12). This was the seminal identification of IL-1 receptor antagonist in vivo.

In 1985 an independent observation was made by W. Arend of an inhibitor to chondrocyte and thymocyte responsiveness to IL-1 in cultured human monocytes (13), at which stage the mechanism of action had not yet been identified. In 1987 we identified by the ligand-binding assay the mechanism of action that justified the nomenclature...
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of ‘receptor antagonist’ (IL-1Ra), which consisted in natural purified IL-1Ra interfering with the binding of IL-1 to lymphocytes (14). The same year, our group in collaboration with A.-M. Prieur made the first clinical observation of the variation in IL-1Ra levels in disease, high IL-1Ra levels being observed during the afebrile phase, and low levels in the febrile phase of patients with systemic juvenile rheumatoid arthritis (15).

Pursuant to our observation that natural IL-1Ra blocked the binding of IL-1 to the cells, IL-1Ra was cloned at Synergen in 1990 (16, 17). Having thus recombinant IL-1Ra at our disposal, we established that natural IL-1Ra, purified from urine, and recombinant IL-1Ra were similar in that both inhibited IL-1-mediated bone resorption and PGE2 production (18). Recombinant IL-1Ra was introduced in clinical trials involving patients with RA in 1991, almost eight years after its initial isolation (19, 20).

III. IL-1 and IL-1 receptor family
(Fig. 1)
The IL-1 family is growing and consists amongst others of two proinflammatory molecules, IL-1 and IL-1, and the specific receptor antagonist, IL-1Ra. There are two types of IL-1 receptors (IL-1R): IL-1R type I possesses a long cytoplasmic tail and is responsible for the induction of the intracellular response after binding to IL-1. IL-1 or IL-1 binding to IL-1RI results in the formation of a heterodimer with the IL-1 receptor accessory protein (IL-1AcP) (Fig. 2). The receptor contains a Toll domain, important for signal transduction. The heterodimer complex recruits IL-1 receptor-activating kinase (IRAK), and a signal is transduced to the cell nucleus through different pathways, including TRAF 6 and P1,3 kinase to IκB kinases, but also through MEKK and p38 MAPK. Thus, IL-1Ra inhibits competitively IL-1 binding to the receptor, preventing the formation of the heterodimer and consequently signal transduction. It must be noted, however, that the binding affinity of IL-1Ra to IL-1RI is as strong as the binding of IL-1 or . However, between 70 and 80% of the binding of IL-1 or IL-1 has to be blocked for the adequate inhibition of their biological activities.

IL-1R type II has a short cytoplasmic domain and functions as a ‘decoy’ receptor, leading to a distinct decrease in the amount of IL-1 available for bind-
ing to IL-1R type I. IL-1R type II is cleaved from the cell surface in soluble form and by binding to IL-1 in solution it prevents the cytokine from reaching the target cells. We demonstrated that in vivo soluble IL-1R type II in association with IL-1Ra is highly efficacious in blocking the IL-1-induced production of collagenase and PGE\(\_2\) on synoviocytes, whereas soluble IL-1R type I reverses this beneficial synergistic effect of soluble IL-1R type II and IL-1Ra by binding to IL-1Ra (3).

IV. Balance and imbalance between IL-1 and IL-1Ra

The relative production of IL-1 to IL-1Ra is important in biology and disease. It is likely that in patients with RA the concentration of endogenous IL-1Ra is not sufficient to regulate the effects caused by increased IL-1 levels. It has also been shown that in patients with Lyme arthritis, the ratio of IL-1 to IL-1Ra in synovial fluid is indicative of the severity of disease. Patients with the shortest time to resolution of the attack had the lowest levels of IL-1 and the highest levels of IL-1Ra. In contrast, patients with the longest time to resolution of the attack had the highest levels of IL-1 and the lowest levels of IL-1Ra (21). This ratio also proved crucial to the regulation of the temperature and the systemic manifestations in juvenile rheumatoid arthritis.

Monocyte-macrophages are by far the principal source of IL-1 and IL-1Ra. However, since neutrophils produce large amounts of IL-1Ra (22) it is possible that at certain stages of a disease (e.g., infection, crystal arthropathies) neutrophils actually have a protective role in the prevention of bone and cartilage production. A small subgroup of patients participating in a trial with IL-1Ra (23) underwent a synovial biopsy before and after treatment to determine the effects of IL-1Ra on inflamed synovial tissue. There was a notable reduction in intimal layer macrophages and subintimal layer macrophages and lymphocytes following administration of IL-1Ra 150 mg/day, which is an additional proof of pathogenetic effects being mediated by IL-1 (24).

V. Animal models as examples for the role of IL-1 and IL-1Ra

The role of IL-1Ra in the potential prevention and/or treatment of disease has been determined in various animal models of destructive arthritic diseases.

- Increased destruction of articular joints consequent to the administration of IL-1: Intra-articular injections of recombinant IL-1 in rodents induced transient synovial inflammation as well as leukocyte infiltration into the joint cavity and synovial lining. It also resulted in the depletion of proteoglycans. TNF caused less extensive damage in articular cartilage. The administration of soluble IL-1 receptor markedly reduced cartilage degradation and white-blood cell infiltration. The association of both soluble IL-1 and TNF receptors enhanced the inhibition of cartilage deterioration, white blood cell infiltration and synovitis. Similar observations were made in models of murine arthritis and rat adjuvant arthritis. In contrast, in models of streptococcal cell wall (SCW) arthritis the blocking of TNF proved more efficient than the blocking of IL-1 (7).
- IL-1Ra knockout animals: In collagen-induced arthritis (CIA), mice with an aberrant expression of IL-1 receptor antagonist exhibited an earlier onset with increased severity (25). In mice of BALB/c background, but not of C57 BL/6J background, the spontaneous, early development of inflammatory arthritis was observed (26), whereas other strains of IL-1Ra-deficient mice presented first signs of arthritis (27).
- IL-1Ra transgenic mice show a reduced incidence and severity of arthritis in CIA models (25%).
- The genetic factor observed in animal models may be of similar importance in humans: IL-1Ra allelic polymorphism was observed when an association exists between IL-1Ra allele A2 and diseases characterized by a decrease in IL-1Ra levels and an increase in IL-1, as in the case of ulcerative colitis, alopecia areata, psoriasis and susceptibility to severe sepsis.

VI. Cell-cell contact and induction of IL-1 and TNF

As already mentioned, the interaction between T cells and monocyte-macrophages is believed to play an important role in the production of IL-1 and TNF in the rheumatoid joint. According to our in vitro observations, T cells in direct contact with monocytes are part of a paramount mechanism inducing IL-1 and TNF production (28). The subtypes of CD4\(^+\) T cells in RA synovial tissue expressed CCR5 and CCR3 as ‘markers’ of Th1 and Th2, with the caveat usually applied to such markers.
Thus, Th1 cells were the predominant T cells found in the synovium, Th2 cells being much less detectable. Direct contact between human Th1 clones and monocytes leads to the production of large amounts of IL-1 and TNF, whereas contact with Th2 clones produces IL-1Ra and very little IL-1 and TNF (Fig. 3) (30). Consequently, at the level of rheumatoid synovial tissue, the production of pro-inflammatory cytokines appears to overrule that of IL-1Ra.

The interactions between T cells and macrophages leading to IL-1 and TNF production depend only slightly on cell-associated IL-1 and TNF, but to a marked extent on other molecules on the surface of stimulated T cells, including 2-integrins like CD11b and c (31). Blocking of these cellular adhesion molecules at the systemic level, however, may be detrimental to the normal immune response to infection. CD69, an early activation antigen on T cells, is also involved since antibodies to CD69 inhibited IL-1 production by 40%. However, the association of CD11b antibody and CD69 antibody did not block more than ~50% of the production of IL-1 or TNF (Fig. 4) (3, 31). In addition, CD11b and c engagement in human monocytes strongly induces IL-1 and chemokine production (32, 33). Other ligands and counter-ligands such as CD40-CD40L also play a role when T cells and monocytes are placed in co-culture, and their effect is potentiated by the presence of IL-2 and still more by that of IL-15 (34).

We found adult human serum - but not fetal calf serum - and to a lesser extent human cord blood serum to inhibit the production of IL-1 and TNF resulting from interactions between stimulated T cells in contact with monocytes (35). The factor found in high-density lipoprotein (HDL) preparations from human serum, that blocked contact-dependent cytokine production, was identified after sequencing as apolipoprotein A-I (apo A-I), the main protein of HDL. Apo A-I associated with HDL binds to stimulated T cells, thus preventing contact-mediated activation of monocytes and subsequent IL-1 and TNF production. It is well established that IL-1, TNF and IL-6 stimulate the production of acute-phase reactants from hepatocytes, including C-reactive protein, complement C3, and fibrinogen. Simultaneously several hepatocyte-derived proteins – one of which, apo A-I – also decrease during systemic inflammation. It could be speculated that the decrease in apo A-I during the inflammatory process may aggravate the clinical situation by allowing greater contact between T cells and monocytes, ultimately resulting in higher levels of IL-1 and TNF production. In contrast, SAA-HDL present in chronic diseases has been shown to stimulate cytokine production in monocytes.

In summary, the present consensus is that IL-1 in synergism with TNF is the principal pro-inflammatory and 'pro-destructive' cytokine in RA and probably in many other chronic inflammatory diseases as well. In addition to the complex balance between pro-inflammatory and anti-inflammatory cytokines, the imbalance between IL-1 and IL-1Ra or IL-1sRII, or between TNF and TNF-sRI or TNF-sRII may be an important factor leading to chronic inflammation. Other examples of such an imbalance are [Th1]/[Th2]; [MMP]/[TIMP]; [RANK]/[OPG]; [HDL-Apo A-I]/[HDL-SAA]. However, this has to be seen in the context of the whole network of pro- and anti-inflammatory cytokines (Table II).

IL-1, TNF- and their inhibitory molecules, such as IL-1Ra and TNF-sRI, are naturally occurring molecules, discovered by bioassays in vitro. Their use as therapeutic biologics was based on both rational concept and hypothesis when studying the pathogenesis of human diseases. This fully justifies the considerable effort made by numerous investigators to bring to light new therapeutic approaches by studying the mechanisms of disease.

The role of IL-1 and TNF in the pathogenesis of RA and other chronic inflammatory diseases that lead to tissue destruction is well established at present. However, despite the strong synergism between the two molecules, some quantitative and qualitative differ-

![Fig. 4: Pathogenetic pathways in RA.](image-url)
rences may be important, and their respective inhibitors may be linked to the genetic background, subtypes of patients, stage of disease, or organ-specific symptoms.

Taking into account the usual caveat, TNF is overall more important in the systemic level and IL-1 at the local level. The repair process tends to be counteracted by IL-1 rather than TNF. Due to a difference in the mechanism of post-receptor transduction, the biological effect may not only differ from a quantitative point of view, but also with regard to side effects such as infection, neoplasia and immune surveillance. In this regard, a combination of the two cytokine inhibitors (blocking IL-1 and TNF) – implying the decrease in concentration of each inhibitor – may possibly reduce long-term side effects. A major difference between the inhibition of IL-1 and that of TNF at the mechanistic level is that the inhibition of the former occurs at the receptor level (IL-1Ra) and that of the latter at the ligand level, whether using antibody or a soluble receptor. It would be of interest to find out how IL-1 soluble receptor type II compares to IL-1Ra. According to data obtained in vitro, the association of IL-1Ra and IL-1sRIL yields a strongly cumulative inhibitory signal.

At the present time, anti-cytokine therapy offers new hope to individuals suffering from RA and many other severe inflammatory diseases. The prospect of specifically targeting and modulating the effects of key pro-inflammatory cytokines or destructive mediators in a complex pathogenetic network heralds a new therapeutic era (36-38). The next step will consist in going upstream in the network, regulating the production of IL-1 and TNF, while bearing in mind that the use of TNF and IL-1 precludes relapses.

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