

# The Pathogenesis of Rheumatoid Arthritis: Pivotal Cytokines Involved in Bone Degradation and Inflammation

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**ABSTRACT.** Proinflammatory cytokines, notably interleukin 1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), play an important role in initiating and perpetuating inflammatory and destructive processes in the rheumatoid joint. These cytokines regulate many nuclear factor  $\kappa$ B inducible genes that control expression of other cytokines, cell adhesion molecules, immunoregulatory molecules, and proinflammatory mediators. The expression of cyclooxygenase-2 and inducible nitric oxide synthase (iNOS) and thereby production of prostaglandins (PG) and NO are regulated by cytokines. PGE<sub>2</sub> and NO further promote inflammation and likely participate in destructive mechanisms in the rheumatoid joint. In some experimental systems, the effects of IL-1 and TNF- $\alpha$  appear synergistic, and correspondingly, concomitant inhibition of both cytokines provides greater than additive antiarthritic effects. Although the actions of IL-1 and TNF- $\alpha$  show a large degree of overlap, some differences have been observed in animal models. However, in patients with active rheumatoid arthritis, blockade of either cytokine results in clinical improvement and less radiographic progression. (J Rheumatol 2002;29 Suppl 65:3-9)

*Key Indexing Terms:*

CYCLOOXYGENASE-2

INTERLEUKIN 1

RHEUMATOID ARTHRITIS

INDUCIBLE NITRIC OXIDE SYNTHASE

TUMOR NECROSIS FACTOR- $\alpha$

In rheumatoid arthritis (RA), an immunological trigger begins an inflammatory process that ultimately manifests clinically by typical signs and symptoms of disease, such as joint swelling and tenderness<sup>1</sup>. Inflammation is also responsible for stimulating destructive mechanisms in the joint, which lead to structural damage and subsequently to functional declines and disability. The rheumatoid joint contains numerous cell types that are involved in these inflammatory and destructive processes<sup>2,3</sup>. The inflamed synovial membrane contains synovial macrophages and fibroblasts (synoviocytes), whereas plasma cells, dendritic cells, T lymphocytes, and mast cells are found in the subsynovial layer. The composition of the synovial fluid varies, but it is principally composed of neutrophils that have infiltrated from the circulation. As synovial proliferation continues, pannus invades from the joint margins, triggering cartilage thinning that is mediated in part by the release of matrix metalloproteinases from synovial fibroblasts in addition to chondrocyte mediated destruction and failure of repair mechanisms. Bone destruction is also initiated through the activation of osteoclasts.

The synovial macrophage plays an important role in orchestrating inflammation and joint destruction in the rheumatoid joint<sup>4</sup>. These macrophages are activated by Th1 cytokines, including interferon- $\gamma$  (IFN- $\gamma$ ), interleukin 12 (IL-12), and IL-18, which are released following T cell activation by antigen-presenting cells. Macrophage activation may also result from direct contact with T cells, as well as via stimulation by immune complexes or bacterial products found in the synovial fluid<sup>5</sup>. Once activated, macrophages release multiple cytokines and other inflammatory mediators that further amplify the inflammatory and destructive processes<sup>1</sup>.

## CYTOKINES

Cytokines are formed following cell activation (Figure 1), with increased transcription of cytokine genes leading to the synthesis and subsequent release of these mediators. The cytokines then interact with specific cell surface receptors, which initiate intracellular signal transduction in target cells and, ultimately, biological responses. Cytokines exert autocrine effects by interacting with receptors on cells that produce them, paracrine effects by binding to receptors on adjacent cells in the local microenvironment, and endocrine effects by acting on distant cells or organs<sup>6</sup>.

Each cytokine may have multiple, pleiotropic actions, and as a result, cytokine functions are frequently redundant. For example, IL-1 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) share many proinflammatory actions in RA, whereas IL-4 and IL-13 cause overlapping antiinflammatory effects in RA

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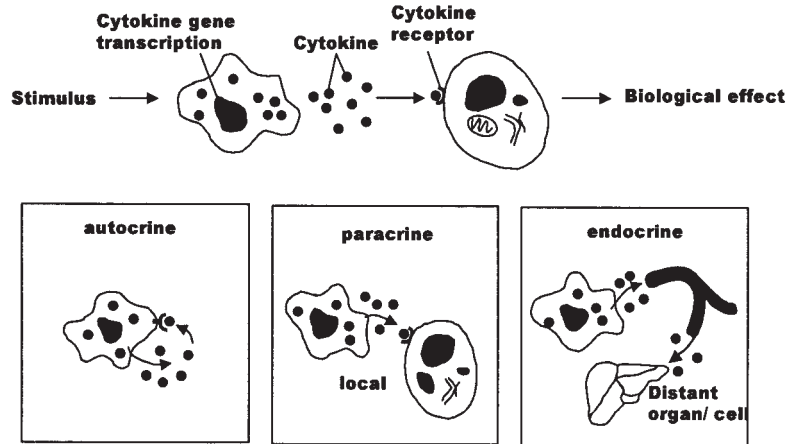


Figure 1. Steps leading to biological effects of cytokines. From Bingham, *et al*<sup>6</sup>, with permission.

while serving as integral mediators of asthmatic and allergic responses. When multiple cytokines are present, they may interact in a synergistic or antagonistic manner depending on the system under investigation<sup>7,8</sup>. Cytokines can influence the production of one another, as is the case with IL-1 and TNF- $\alpha$ , each of which can induce the expression of the other<sup>9</sup>. Finally, cytokines can affect the expression and/or function of cytokine receptors, thereby regulating cytokine action.

The rheumatoid joint contains a variety of proinflammatory cytokines besides IL-1 and TNF- $\alpha$ , which include IL-6, IL-15, IL-16, IL-17, IL-18, IFN- $\gamma$ , granulocyte macrophage-colony stimulating factor, and chemokines such as IL-8, macrophage inflammatory protein-1 $\alpha$  and monocyte chemoattractant protein-1. Under normal physiologic conditions, the actions of these proinflammatory cytokines are maintained in balance by antiinflammatory cytokines, such as IL-4, IL-10, IL-11, and IL-13, and by natural cytokine antagonists, including IL-1 receptor antagonist (IL-1ra), soluble type 2 IL-1 receptor, soluble TNF receptor (sTNF-RI), and IL-18 binding protein. In the rheumatoid joint, however, the balance swings in favor of the proinflammatory cytokines<sup>10</sup>.

IL-1 and TNF- $\alpha$  have numerous functions throughout the body, many of which are important in RA<sup>2,11,12</sup>. Both cytokines upregulate expression of cell adhesion molecules on endothelial cells, which is important in the recruitment of inflammatory cells to the inflammatory site<sup>13,14</sup>. Both IL-1 and TNF activate a variety of cell types found in the rheumatoid joint, including macrophages, fibroblast-like synoviocytes, chondrocytes, and osteoclasts, resulting in the release of other proinflammatory mediators and degradative enzymes<sup>15-18</sup>. Both stimulate proliferation of synovial cells leading to pannus formation. Both cytokines influence immunological activity by causing T cell and B cell activation, and both stimulate hepatocytes to release acute phase

reactants, notably IL-6. Both have actions in the central nervous system: TNF- $\alpha$  stimulates endogenous glucocorticoid production via an action in the hypothalamus, and IL-1 induces fever and slow wave sleep.

A significant body of evidence suggests that IL-1 and TNF- $\alpha$  are important in RA pathogenesis. First, both cytokines are arthritogenic when injected into the joints of experimental animals<sup>19,20</sup>. Second, spontaneous arthritis develops in transgenic animals overexpressing either cytokine<sup>21-23</sup>. Moreover, a spontaneous erosive arthritis is found in IL-1ra knockout mice<sup>24</sup>. Third, IL-1 or TNF- $\alpha$  blockade reduces inflammation and joint destruction in many animal models of RA<sup>25,26</sup>. Synergistic effects are evident, with simultaneous inhibition of both cytokines. Finally, the most important piece of evidence comes from controlled clinical trials, where IL-1 antagonism or TNF- $\alpha$  blockade reduces the signs and symptoms of active RA and slows radiographic evidence of joint destruction<sup>27-34</sup>.

The similar biological actions of IL-1 and TNF- $\alpha$  may be explained, in part, by the similar intracellular signaling pathways that their receptors activate. The type 1 IL-1 receptor and TNF-receptors (TNF-R) activate a family of adapter proteins called TNF-R associated factors (TRAF) — TRAF2 in the case of TNF- $\alpha$  and TRAF6 in the case of IL-1<sup>35-37</sup>. In turn, these TRAF activate downstream signaling pathways including nuclear factor  $\kappa$ B (NF- $\kappa$ B)-mediated transduction mechanisms. NF- $\kappa$ B is normally found in the cytoplasm in an inactive complex with I $\kappa$ B. The TRAF activate an enzyme known as I $\kappa$ B kinase, which phosphorylates I $\kappa$ B, leading to its dissociation from the complex and resulting in the liberation of active NF- $\kappa$ B. The NF- $\kappa$ B then enters the nucleus and binds to promoter regions of multiple genes, resulting in their transcription and subsequently in generation of multiple products.

Many of the products of NF- $\kappa$ B inducible genes are involved in inflammation and immune responses (Table 1).

These genes encode adhesion molecules, cytokines, growth factors, cytokine receptors, immunoregulatory molecules, and acute phase proteins. In addition, NF- $\kappa$ B-inducible genes encode enzymes that are important in inflammatory mediator biosynthesis: cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). These enzymes are responsible for production of prostanoids and nitric oxide, respectively.

### INFLAMMATORY LIPID MEDIATORS

The eicosanoids are a family of lipid mediators that are derived from arachidonic acid found in membrane phospholipids. Arachidonic acid is released by the action of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzymes and is then converted into prostanoids, prostaglandins, and thromboxanes, through the intermediate COX-1 and 2, and into leukotrienes through the intermediate 5-lipoxygenase enzyme acting in concert with 5-lipoxygenase activating protein. The intermediate prostaglandin product PGH<sub>2</sub> formed by COX-1 and 2 is subsequently converted into prostaglandin or thromboxane end products by terminal synthase enzymes. For example, PGE<sub>2</sub> synthase (PGES) is responsible for producing PGE<sub>2</sub>. Many of the enzymes involved in prostaglandin synthesis exist in several forms. One form may be expressed constitutively, including cytosolic group IV- $\alpha$  PLA<sub>2</sub> (cPLA<sub>2</sub>), COX-1, and cytosolic PGES (cPGES), whereas other forms of these enzymes are induced preferentially in inflammation and following cytokine stimulation, including COX-2, microsomal PGES (mPGES), and multiple types of secretory PLA<sub>2</sub> (sPLA<sub>2</sub>)<sup>38,39</sup>.

COX-2 is highly expressed in the rheumatoid synovium. Strong COX-2 immunostaining was found in the synovial lining layer, synovial vessel endothelium, and in cells of the subsynovium<sup>40</sup>. COX-2 expression was significantly higher in specimens from patients with RA as compared with osteoarthritis (OA). In comparison, COX-1 expression was

almost exclusively localized to the synovial lining layer, with no significant difference between RA and OA samples. Both IL-1 and TNF- $\alpha$  upregulated expression of COX-2 and mPGES in rheumatoid synoviocytes without affecting COX-1 or cPGES<sup>41,42</sup>. The stimulation of COX-2 and mPGES was inhibited in a concentration-dependent manner by dexamethasone.

The sPLA<sub>2</sub> family consists of a large group of related molecules. The low molecular weight group IIA sPLA<sub>2</sub> was originally isolated from RA synovial fluid; it is induced by both IL-1 and TNF- $\alpha$ . Moreover, this sPLA<sub>2</sub> has proinflammatory activity of its own. At concentrations found in RA synovial fluid, type IIA sPLA<sub>2</sub> augmented TNF- $\alpha$  induced PGE<sub>2</sub> production by cultured synovial fibroblasts<sup>42</sup>. The expression and regulation of other members of the sPLA<sub>2</sub> family are largely unknown, but it is the subject of active investigation. It is apparent that these enzymes do not function solely by cleaving arachidonic acid from membrane phospholipids, but they may have unique receptor-mediated functions, such as inducing expression of COX-2 and other enzymes. It is notable that the expression of group V sPLA<sub>2</sub> in rheumatoid synovium closely parallels that of COX-2, with intense staining found in the synovial lining layer, blood vessel endothelial cells, and subsynovial lymphoid aggregates (Figure 2).

Upregulation of sPLA<sub>2</sub>, COX-2, and mPGES leads to enhanced production of PGE<sub>2</sub>. This prostaglandin causes several effects that may be relevant to bone and cartilage erosion in RA<sup>43,44</sup>. *In vitro* systems and animal models have demonstrated that PGE<sub>2</sub> induces bone resorption by osteoclasts<sup>18</sup>. On the basis of knockouts of the 4 PGE receptor subtypes, PGE<sub>2</sub> causes bone resorption by a cAMP dependent mechanism via the EP4 receptor<sup>45</sup>. This effect of PGE<sub>2</sub> is consistent with observations that nonsteroidal antiinflammatory drugs, such as indomethacin, and COX-2 selective inhibitors retard osteoclast formation and activation<sup>18</sup>. Moreover, COX-2 knockouts show impaired bone resorption in response to parathyroid hormone (PTH) or vitamin D. PGE<sub>2</sub> also stimulates new bone formation by osteoblasts by inducing expression of RANKL (receptor activator of NF- $\kappa$ B ligand), and depending on the experimental system, PGE<sub>2</sub> affects the synthesis and degradation of type II collagen and proteoglycans. Finally, at relatively high concentrations, PGE<sub>2</sub> stimulates release of matrix metalloproteinases that degrade cartilage.

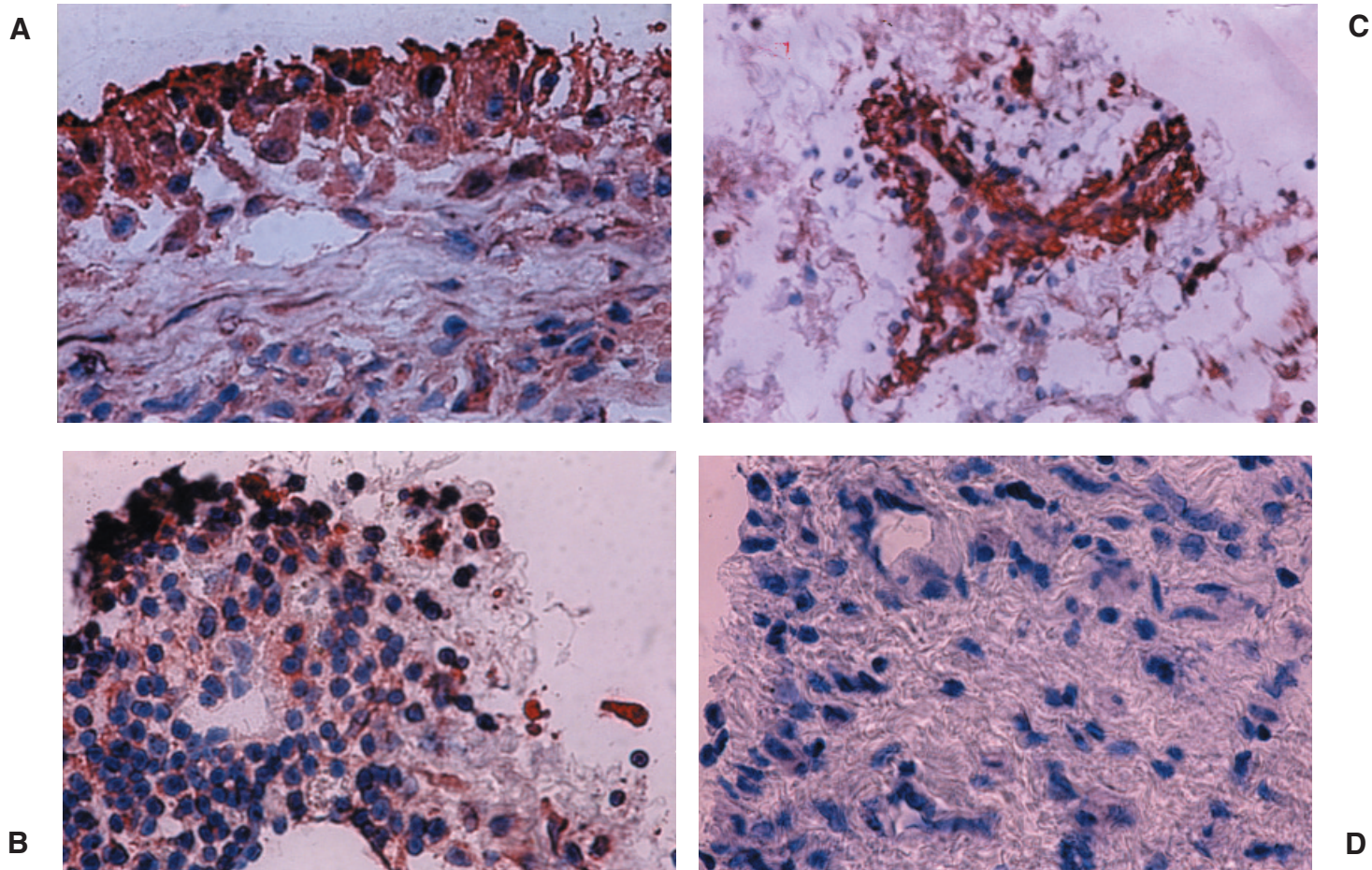
### NITRIC OXIDE

Nitric oxide (NO) is another mediator that appears to be important in cartilage and bone destruction<sup>46</sup>. Like the prostaglandins, NO is produced through constitutive and inducible pathways, which are responsible for its “house-keeping” and pathogenic roles, respectively. Like COX-2, the transcriptional control of iNOS is regulated by cytokines, such as IL-1 and TNF- $\alpha$ , as well as other cellular

Table 1. A partial list of NF- $\kappa$ B inducible genes involved in immune responses and inflammation.

Class	Genes
Inflammatory mediators	COX-2, iNOS
Cell adhesion molecules	ICAM-1, VCAM-1, E-selectin
Cytokines	IL-1, IL-2, IL-3, IL-6, IL-8, IL-12, TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\beta$
Growth factors	G-CSF, M-CSF, GM-CSF
Cytokine receptors	IL-2R
Immunoregulatory molecules	Igk light chain, MHC class I and II, T cell receptor $\alpha$ and $\beta$
Acute phase proteins	SAA, complement factors B, C3 and C4

ICAM: intercellular adhesion molecule; VCAM: vascular cellular adhesion molecule; G-CSF: granulocyte colony stimulating factor; M-CSF: macrophage colony stimulating factor; GM-CSF: granulocyte macrophage-colony stimulating factor; SAA: serum amyloid A.



**Figure 2.** Immunohistologic localization of group V phospholipase A<sub>2</sub> (PLA<sub>2</sub>) in rheumatoid synovium. Synovial biopsies were obtained from 3 patients with RA, and evaluated using standard immunohistochemistry. An affinity purified rabbit anti-peptide group V PLA<sub>2</sub> antibody<sup>54</sup> or affinity purified rabbit IgG, both at 10 µg/ml, were applied to acetone fixed sections. Biotinylated secondary antibody was applied; slides were developed with 3-amino-9-ethylcarbazole with H<sub>2</sub>O<sub>2</sub>, counterstained with Gill's hematoxylin, and examined under light microscopy. Representative samples are shown with group V PLA<sub>2</sub> antibody (A, B, C) or with control antibody (D): A. Synovial lining layer; B. synovial lining, subsynovial lymphoid aggregate; C. blood vessel, endothelial cells; and D. control antibody.

stimuli (Figure 3)<sup>47</sup>. The iNOS enzyme catalyzes the conversion of L-arginine in the presence of molecular oxygen and the cofactor NADPH into NO and L-citrulline. Among its many biological actions, NO activates matrix metalloproteinases, inhibits collagen and proteoglycan synthesis by chondrocytes, and promotes vasodilation, which leads to fluid and cellular influx into an inflammatory site. NO also combines with reactive oxygen species to produce peroxynitrite, which promotes chondrocyte apoptosis<sup>48</sup>.

In murine models, NO derived from iNOS may be essential for IL-1 induced bone resorption<sup>49</sup>. In iNOS deficient mice, IL-1 induced osteoclastic bone resorption was disrupted, but resorption caused by parathyroid hormone or vitamin D was unaffected. The defect in IL-1 induced bone resorption was attributed to a defect in NF-κB nuclear translocation and binding to promoter sites in the iNOS deficient animals.

#### SYNERGISM OF IL-1 AND TNF-α

Intraarticular injection of IL-1 or TNF-α into a rabbit knee joint markedly increases the number of infiltrating leukocytes<sup>7</sup>. The nature of the inflammatory infiltrate was predominantly monocytic with TNF-α, whereas it was both neutrophilic and monocytic with IL-1. When administered in combination at submaximal doses, IL-1 and TNF-α were synergistic with respect to both neutrophil and monocyte infiltration. The number of neutrophils and monocytes were 2.8 and 1.8 times higher, respectively, than would be expected if the effects were simply additive.

Similarly, greater than additive effects have been observed in animal models of RA when IL-1 and TNF-α blockers were combined<sup>50</sup>. In established collagen induced arthritis in rats, treatment with either recombinant human IL-1ra or PEGylated sTNF-RI partially reduced several disease variables evaluated histologically, including inflammation, pannus, cartilage damage, and bone resorption

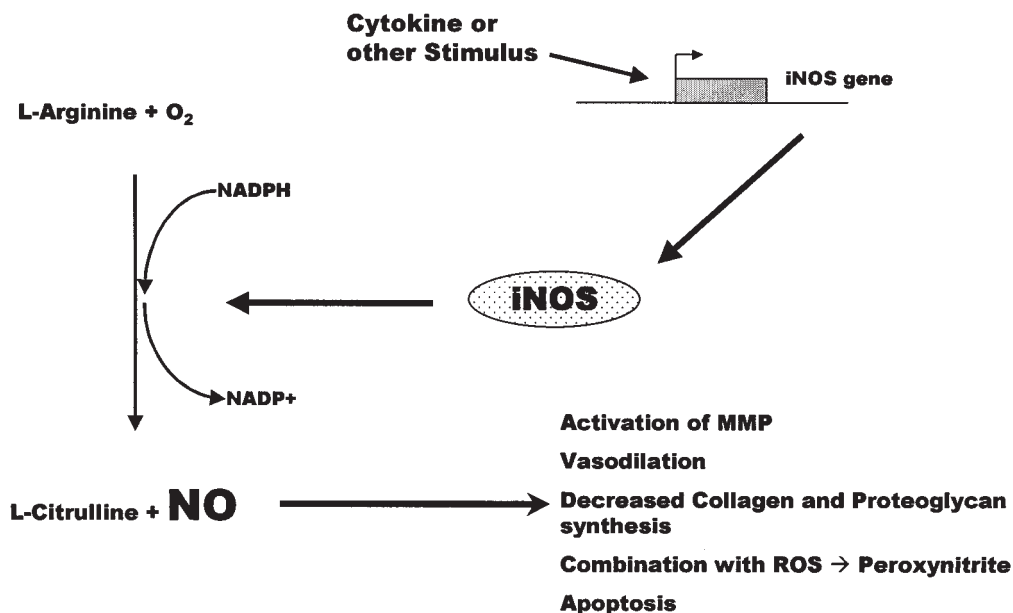


Figure 3. Nitric oxide induction by cytokines and other stimuli.

(Figure 4). When both agents were administered in combination, inflammation was reduced in an additive manner, whereas the other 3 variables were almost completely suppressed.

Some differences between IL-1 and TNF- $\alpha$  have been seen in animal models, and differences may be apparent in individual patients treated with inhibitors of these cytokines. In the rat adjuvant arthritis model, for example, IL-1ra inhibited angiogenesis in the joint, whereas blocking TNF- $\alpha$  did not reduce the number of capillaries<sup>51</sup>. In murine

collagen induced arthritis, IL-1 blockade inhibited cartilage and bone destruction, whereas TNF- $\alpha$  inhibition only reduced joint inflammation<sup>52</sup>. Nevertheless, it is important to recognize that animal models of RA do not necessarily reflect clinical disease. Importantly, treatment with either IL-1 or TNF- $\alpha$  blockers reduces inflammation and slows joint destruction in patients with active RA<sup>27-34</sup>. It remains to be determined, however, whether patients with poor treatment responses to either an IL-1 or TNF- $\alpha$  blocker will have better responses when an inhibitor of the other cytokine is

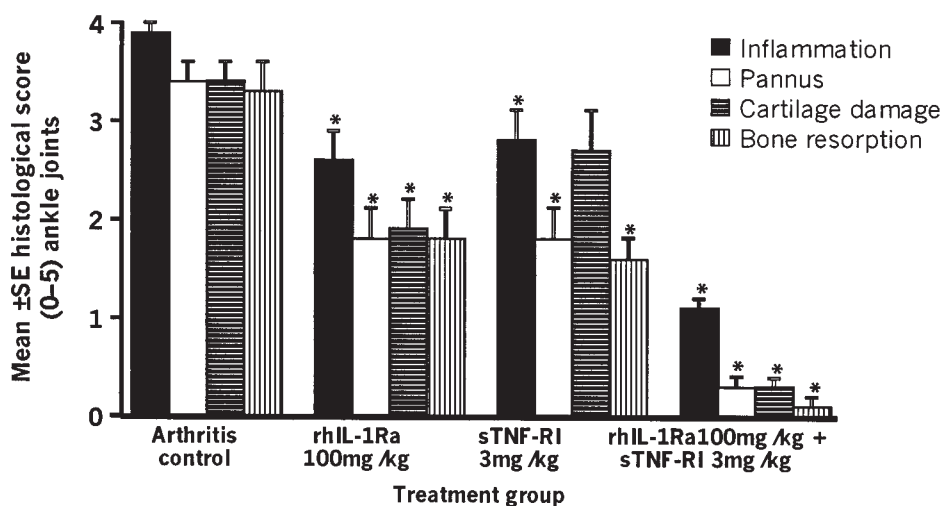


Figure 4. Synergism of combined IL-1 and TNF- $\alpha$  blockade in established collagen induced arthritis in rats. Shown are the effects of recombinant human IL-1ra 100 mg/kg and PEGylated sTNF-RI 3 mg/kg alone and in combination on histological scores of inflammation, pannus, cartilage damage, and bone resorption. \*P < 0.05, 2 tailed t test to control. From Bendele, *et al*<sup>50</sup> with permission.

administered. The question of synergy of effect using IL-1 and TNF inhibitors is being addressed in ongoing clinical trials of patients with rheumatoid arthritis; however, one report has suggested an increased incidence of severe infections when combinations of biologics are administered<sup>53</sup>.

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