The Mode of Action of Cytokine Inhibitors

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ABSTRACT. Tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1) are important mediators of inflammation and tissue damage in animal models of inflammatory arthritis and in patients with active rheumatoid arthritis (RA). Several inhibitors of these cytokines are now available for RA treatment, each having a different mode of action. Etanercept is a recombinant fusion protein of the soluble type II TNF receptor on a human IgG_1 backbone, whereas infliximab is a chimeric anti-TNF-α monoclonal antibody containing a murine TNF-α binding region and human IgG_1 backbone. Both agents potently and selectively bind TNF-α in the cellular microenvironment, thereby preventing TNF-α from interacting with membrane-bound TNF receptors on target cells. In comparison, anakinra is a recombinant human IL-1 receptor antagonist (IL-1Ra) that binds avidly to type 1 IL-1 receptors but does not stimulate any intracellular responses. Studies of these agents in animal models of inflammatory arthritis suggest that TNF-α plays a more important role in promoting inflammation, whereas IL-1 is more important in causing cartilage and bone destruction. However, these differential actions have not been borne out in clinical trials, where TNF-α blockers and anakinra similarly reduce clinical signs and symptoms of RA as well as slow radiographic evidence of disease progression. (J Rheumatol 2002;29 Suppl 65:16–21)

Key Indexing Terms:
ANAKINRA ETANERCEPT INTERLEUKIN 1
INFLIXIMAB RHEUMATOID ARTHRITIS TUMOR NECROSIS FACTOR-α

The etiology of rheumatoid arthritis (RA) is not completely understood. Many different approaches have been taken over the past decade to understand this disease, leading to numerous strategies for rational development of antiarthritic medications. In a leading rheumatology text published in 1966, RA was hypothesized to be an infectious disease, a hypersensitivity reaction, a nutritional or metabolic disease, an endocrine disorder, a psychosomatic condition, or a hereditary disease[1]. Many of these suggested mechanisms are still believed to be relevant in RA, but none of them alone satisfactorily explains the etiology of this disease. A detailed examination of the histological changes in the synovium provided new information that has served as a rationale for the development of therapeutic approaches using cytokine inhibition.

The initial synovial lesion in RA involves a variety of different cell types. How the disease process is initiated remains unclear, but it probably involves both the innate and adaptive immune systems[2]. Synovial tissue macrophages appear to be the major cell type responsible for the mechanisms that lead to tissue destruction (Figure 1). These macrophages release a variety of cytokines, notably interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α), which interact with other cells to cause cartilage and bone damage. IL-1 and TNF-α upregulate expression of cell adhesion molecules on endothelial cells, causing emigration of white blood cells from blood into the synovial tissue[3]. These cytokines also contribute to the process of angiogenesis, which is needed to sustain growth of the synovial pannus. They stimulate production of other cytokines, reactive oxygen intermediates, nitric oxide, and prostaglandins, which contribute to inflammation within the joint. IL-1 and TNF-α also interact with transformed synovial fibroblasts, known as synoviocytes, and chondrocytes in the superficial layers of cartilage, to stimulate the release of enzymes (matrix metalloproteinases) that degrade collagen and proteoglycans, resulting in cartilage destruction and joint space narrowing[4,5]. IL-1 and TNF-α, together with a number of other factors, promote osteoclast differentiation and stimulate activation of mature osteoclasts, which resorb bone, leading to joint erosions particularly at the marginal surfaces[6,7]. IL-1 in concert with IL-6 appears to be responsible for the periarticular osteopenia seen in RA.

Cytokines produced by synovial macrophages not only work locally in the joint but reach the circulation and contribute to the systemic manifestations of RA. IL-1 and IL-6 act on the liver to induce formation of acute phase proteins, and in the central nervous system they may contribute to the somnolence and low grade fever that accompany this disease. TNF-α is primarily responsible for the increase in muscle metabolism in RA. Each of these cytokines affects the bone marrow, contributing to the anemia of chronic disease seen frequently in patients with RA[8].

In normal physiology, the actions of IL-1, TNF-α, and other proinflammatory cytokines are maintained in balance

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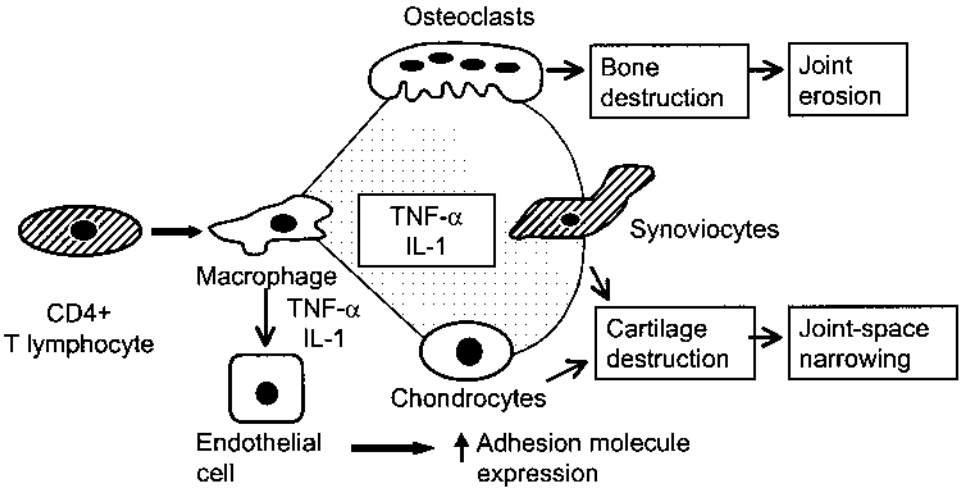

Figure 1. Mechanisms of structural damage in rheumatoid arthritis.

by a variety of antiinflammatory factors, which include IL-1 receptor antagonist (IL-1Ra), soluble IL-1 receptor type II (s-IL-1RII), soluble TNF receptors (sTNF-R), and a number of antiinflammatory and immunoregulatory cytokines (IL-4, IL-10, IL-11, and IL-13)[9-11]. In rheumatoid synovitis, however, the balance is shifted in favor of the proinflammatory cytokines. One approach for restoring balance to the cytokine network in the synovium is through use of agents that block the effects of proinflammatory cytokines.

STRATEGIES FOR CYTOKINE INHIBITION

There are numerous approaches to inhibition of cytokine effects in the synovium. Several widely used drugs, including corticosteroids, methotrexate, and cyclosporin A, are known to block cytokine production from macrophages and T cells. Other approaches provide a more selective method for blocking cytokine effects. The release of active cytokines can be prevented by inhibition of TNF-α converting enzyme, which is required for TNF-α release[12], and of IL-1 converting enzyme (ICE; caspase 1), which is needed for IL-1ß release[13]. Inhibitors of these enzymes are effective in animal models of inflammatory arthritis and some have recently entered clinical trials. A second approach is to block the effects of cytokines once they are released. Several such drugs have been developed and shown to be effective in randomized clinical trials of patients with active RA[14-16]. The mode of action of these agents will be discussed in greater detail in the following sections. Finally, a newer approach for blocking cytokine action targets downstream intracellular signaling molecules, such as p38 mitogen activated protein kinase[17]. Such kinase inhibitors are under active investigation.

BLOCKING TNF-α EFFECTS

TNF-α is produced primarily by macrophages in response to lipopolysaccharide and other cell-activating stimuli. Once released, TNF-α interacts with specific receptors that are found on the surface of most cells in the body. Two types of TNF receptors have been identified: the 55 kDa type I receptor (TNF-RI; p55) and the 75 kDa type II receptor (TNF-RII; p75)[18]. It is important to recognize that the effects of TNF-α differ depending on which receptor it activates. Activation of TNF-RI is important in host defence and leads to inflammation, tissue destruction, and cytotoxicity. In contrast, activation of TNF-RII may be somewhat protective in RA, particularly at later stages of the disease process. Activation of this receptor leads to T cell proliferation, T cell apoptosis, and immunosuppression, which may be important in how the host attempts to combat the complex disease process. Accordingly, some investigators believe that it would be preferable to block TNF-RI while leaving TNF-RII unaffected[19]. Further, TNF-deficient mice exhibit reduced but not absent inflammatory arthritis, indicating that TNF is important but not essential for this disease process[20].

TNF-RI and TNF-RII exist on the cell surface as trimers. Under conditions of cell stimulation, these receptors are cleaved by proteolysis and released locally into the cell microenvironment as soluble molecules, termed sTNF-RI and sTNF-RII, respectively. These soluble receptors may act naturally as antiinflammatory factors or they may have another role that has not yet been elucidated. TNF-α also exists as a trimer, and it binds to the soluble receptors. It is important to recognize that the effects of TNF-α depend on a dynamic equilibrium among the concentrations of TNF-α itself, its soluble receptors, and its cell-bound receptors, as well as the affinities of TNF-α for each type of receptor. Moreover, the biological effects of exogenously administered soluble TNF receptors may differ from those of endogenous soluble receptors.

Two TNF-α blockers are currently available for treatment of RA. Etanercept is a dimer of sTNF-RII attached to the Fc region of IgG_1. This molecule contains 2 arms, with sTNF-RII attached to the larger molecular weight Fc portion that provides it with better retention in the circulation. The sTNF-RII arms of etanercept bind TNF-α in solution, preventing its interaction with both types of TNF receptors on the cell surface. Although this desired effect is achieved with exogenously administered soluble receptors, other mechanisms of action are also possible. Studies from animal models and *in vitro* systems indicate that low concentrations of soluble TNF receptors stabilize the structure of TNF-α, but higher concentrations inhibit TNF-α binding to TNF-RI and TNF-RII on target cells[21]. Depending on the affinity of the ligand for the soluble receptor relative to the membrane-bound receptor, in some situations soluble receptors may actually deliver ligands to their target tissues[22].

The other TNF-α blocker, infliximab, is a chimeric IgG_1 monoclonal antibody that contains a murine binding site for TNF-α. A totally human anti-TNF-α monoclonal antibody (D2E7) has been developed and is currently in clinical development[23]. Both monoclonal antibodies bind to TNF-α with high affinity and specificity, and thereby prevent TNF-α from binding to its membrane-bound receptors on target cells. However, macrophages and certain T cell subsets express TNF-α on their cell surfaces, and the monoclonal antibodies bind as tightly to this membrane-bound TNF-α as to soluble TNF-α. This interaction may alter cellular function, possibly promote removal of the cell from the circulation, or lead to cell death. Soluble TNF-α receptors also bind to membrane-bound TNF-α, but it is not known if this interaction has any consequences. The presence of a murine segment in infliximab may lead to production of human anti-mouse antibodies (HAMA) during treatment. However, the concomitant use of methotrexate appears to inhibit the production of HAMA.

BLOCKING IL-1 EFFECTS

The IL-1 system, like the TNF-α system, is also complex. The IL-1 family comprises 3 members: the proinflammatory ligands IL-1α and IL-1ß and the naturally occurring antagonist IL-1Ra[24]. There are 2 types of IL-1 receptors, but these do not correspond to the receptors in the TNF-α system. The type 1 IL-1 receptor (IL-1RI) contains a long intracellular domain; IL-1 binding to this receptor triggers intracellular signaling and cellular activation leading to biological actions[25]. In contrast, the type 2 IL-1 receptor (IL-1RII) contains a very short intracellular domain, and as a result, it is unable to transduce any signals in response to IL-1 binding[26]. IL-1RII is essentially inert and does not influence cell function. However, when IL-1RII is present, it may compete with the biologically active type 1 IL-1RI for IL-1 binding. Accordingly, IL-1RII appears to function as a dummy or decoy receptor[27]. The extracellular domain of both receptors are readily cleaved enzymatically, and the resulting soluble receptor (sIL-1R) may be found in significant concentrations in the cell microenvironment, synovial fluid, and systemic circulation[28]. Of the two IL-1 receptors, IL-1RII appears to be more important in generating high concentrations of sIL-1R.

The production of IL-1α and IL-1ß differs. Both are generated as precursor proteins without a leader sequence, indicating that they are not purposely intended for immediate secretion from the cell. The IL-1ß precursor is not biologically active within the cell, but it is cleaved by ICE (caspase 1) into the mature IL-1ß protein and subsequently released by the cell[13]. In contrast, the IL-1α precursor is biologically active, can be expressed on the cell surface and exert proinflammatory effects, or can move into the nucleus and influence transcriptional events[29]. The IL-1α precursor can also be cleaved and released by the cell as mature IL-1α. However, in terms of treating inflammatory disorders, such as RA, the secreted mature IL-1ß is the more important form of IL-1.

Anakinra is a recombinant human IL-1Ra that is identical to the naturally occurring nonglycosylated form with the exception of one N-terminal methionine. Like the natural protein, anakinra is a pure IL-1R antagonist, which accounts for its ability to inhibit the effects of IL-1. On a molecular level, IL-1α or IL-1ß binding to IL-1RI allows a complex to form with another membrane molecule termed IL-1 receptor accessory protein (IL-1R-AcP) (Figure 2)[30]. This complex then generates intracellular signals leading to cell activation and biological responses. IL-1Ra exhibits equal avidity as IL-1 in binding to IL-1RI. However, if IL-1Ra (or anakinra) first binds to IL-1RI, IL-1R-AcP cannot be complexed with IL-1RI and, consequently, a signal is not generated. However, it is important to recognize that cells contain a small number of IL-1RI, perhaps 500 to 1000 per cell, and only a few must be activated to produce a full biological response. Because of this exquisite sensitivity to IL-1 and the presence of spare IL-1RI receptors, large amounts of IL-1Ra must be present in order to effectively reduce the biological effects of IL-1[31]. Unfortunately, in RA, the production of IL-1Ra by synovial tissues is not adequate to balance the effects of IL-1[32]. Although in some patients greater amounts of IL-1Ra than of IL-1 may be produced, the 10- to 100-fold excess needed to block IL-1 is not achieved.

BLOCKING IL-1 AND TNF-α IN ANIMAL MODELS

The effect of blocking IL-1 or TNF-α on early inflammatory events and subsequent erosive arthritis has been evaluated in numerous animal models of inflammatory arthritis (Table 1)[33-37]. In these studies, the cytokines were blocked with different types of therapeutic agents, including IL-1Ra, monoclonal antibodies, and soluble receptors. Although the effects were dependent on the study design and the model used, it appears that TNF-α generally plays a greater role in

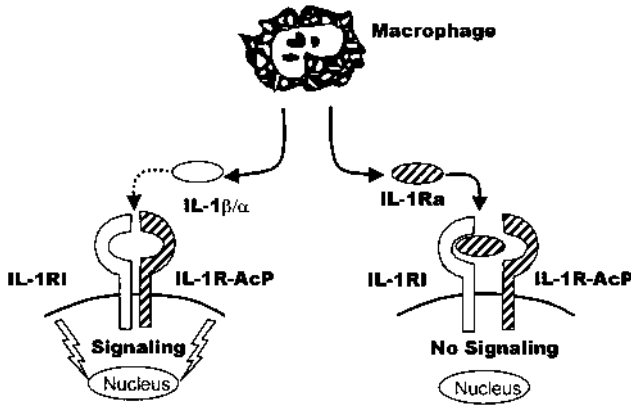

Figure 2. Mechanism of action of IL-1Ra. IL-1ß and IL-1Ra are produced by macrophages, and both bind with high avidity to IL-1RI. IL-1ß binding to IL-1RI leads to engagement of the IL-1 receptor accessory protein (IL-1R-AcP), resulting in intracellular signaling and cell activation. Conversely, IL-1Ra binding blocks the binding site for IL-1ß and prevents engagement of IL-1R-AcP. As a result, intracellular signaling and cell activation are blocked. From Bresnihan[30], with permission.

the early events of inflammation, whereas IL-1 is more involved in the later events involving tissue destruction. In the chronic relapsing streptococcal cell wall arthritis model, for example, sTNF-R reduced joint swelling but did not significantly affect chronic cellular infiltration or cartilage erosion, a profile that was also observed when the model was induced in TNF-α knockout mice[38,39]. In comparison, anti-IL-1 significantly inhibited chronic cellular infiltration and cartilage erosion while partially reducing joint swelling[38]. Similar results were obtained when streptococcal cell wall arthritis was induced in IL-1 knockout mice[39].

The differential effects of blocking IL-1 and TNF-α suggest that additive or synergistic actions may be achieved by concomitantly using inhibitors of both cytokines. This hypothesis was evaluated in the adjuvant arthritis and collagen induced arthritis models in rats[40]. When used individually at relatively low doses, PEGylated sTNF-RI and IL-1Ra modestly inhibited joint swelling and bone resorption in adjuvant arthritis, and they inhibited joint swelling and paw weight in collagen induced arthritis (Figure 3).

Table 1. Role of TNF-α and IL-1 in animal models of arthritis. Adapted with permission from van den Berg, *et al,* 2001[38].

		Cytokine Involvement			
		Early Inflammation		Erosive Arthritis	
Arthritis Model	Species	TNF-α	IL-1	TNF-α	IL-1
Streptococcal cell wall — acute	Mouse	++	–	–	++
Streptococcal cell wall — flare	Mouse	+	+	–	++
Streptococcal cell wall — flare	Rat	+	+	NR	++
Antigen induced arthritis	Mouse	±	±	NR	++
Antigen induced arthritis	Rabbit	+	+	±	++
Antigen induced arthritis—flare	Mouse	±	+	–	++
Collagen induced arthritis	Mouse	+	++	+	++
Immune complex arthritis	Mouse	–	++	–	++
Adjuvant arthritis	Rat	+	+	+	+

NR: not reported.

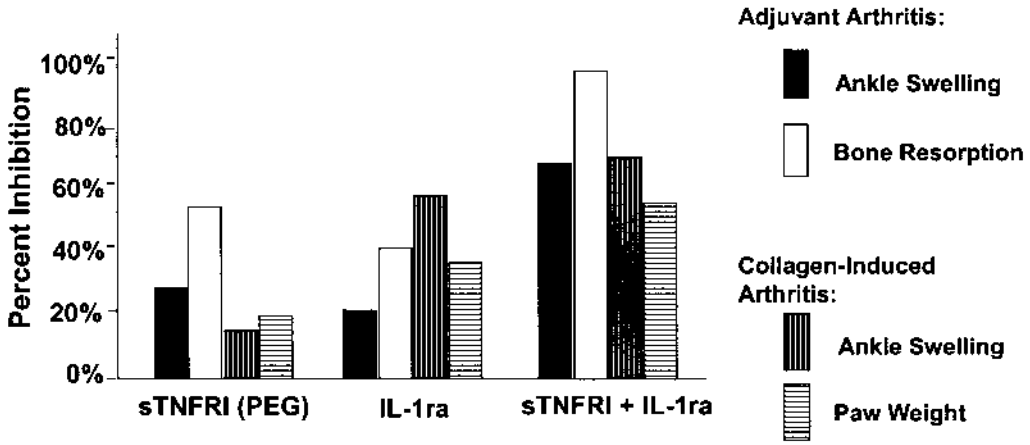

Figure 3. Effect of PEGylated sTNF-RI and IL-1Ra alone and in combination in adjuvant arthritis and collagen-induced arthritis. From Bendele, *et al*[40], with permission.

However, when used in combination, the effects of sTNF-RI and IL-1Ra were additive, resulting in marked inhibition of inflammatory and erosive variables. Moreover, when an inactive dose of IL-1Ra was administered in combination with a minimally active dose of PEGylated sTNF-RI, greater than additive effects were observed. These animal models show that TNF-α and IL-1 play key roles in causing inflammatory arthritis. Inasmuch as TNF-α was more effective in decreasing inflammatory variables, this observation suggests that TNF-α may be more important in causing various signs and symptoms of RA, such as joint swelling, tenderness, and pain. Conversely, inhibition of IL-1 leads to greater reduction of tissue damage, suggesting that IL-1 plays a more important role in stimulating mechanisms leading to cartilage and bone destruction. Nevertheless, it is important to remember that no animal model exactly resembles RA. The results of clinical trials show that active RA responds similarly to IL-1 and TNF-α inhibition. Etanercept, infliximab, and anakinra each significantly improved the clinical signs and symptoms of RA[14-16], and each significantly reduced radiologic evidence of joint damage in RA[41-43].

CONCLUSION

The use of cytokine inhibitors in RA and other inflammatory diseases is still an emerging field. Randomized clinical trials have shown that TNF-α and IL-1 inhibitors are effective in the treatment of active RA. Other approaches to preventing production of these cytokines or blocking their effects are still in early stages of development. Moreover, agents targeting other inflammatory cytokines and mediators as well as use of recombinant antiinflammatory cytokines are being evaluated for RA treatment. It is tempting to speculate that in perhaps 5 or 10 years, RA will be treated with a cocktail of agents, each of which has a different mechanism of action. In this cocktail, drugs would be administered in relatively low doses in order to minimize any safety and tolerability concerns, while maximizing efficacy through the different mechanisms of the individual drugs.

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