Effects of Antirheumatic Agents on Cytokines

Pilar Barrera, Agnes M.Th. Boerbooms, Leo B.A. van de Putte,

and Jos W.M. van der Meer

A review of the literature concerning the effects of traditional antirheumatic drugs on cytokines and the cytokine and anticytokine approaches already used in the therapy of rheumatoid arthritis (RA) is presented. Many antirheumatic drugs are capable of cytokine modulation in vitro. Corticosteroids inhibit the transcription of a broad spectrum of genes including those encoding monocyte, T cell–derived cytokines and several hemopoietic growth factors, whereas drugs such as cyclosporin A and D-penicillamine interfere with T cell activation more specifically by suppressing interleukin 2 (IL-2) production. The in vivo effects of drug therapy on cytokines in RA patients are less well established. Gold compounds reduce circulating IL-6 levels and the expression of monocyte-derived cytokines, such as IL-1, tumor necrosis factor (TNF), and IL-6, in the rheumatoid synovium. Decreases in circulating IL-6, soluble IL-2 (sIL-2R), and TNF receptors and in synovial fluid IL-1 levels have been reported with methotrexate. Reductions in circulating IL-6 and sIL-2R concentrations have also been observed with cyclosporin and corticosteroids, whereas azathioprine reduces IL-6 but not sIL-2R. Studies on sulfasalazine are conflicting and the in vivo effects of D-penicillamine and antimalarials have not been studied yet. Interferon γ therapy is not effective in RA but may prove a useful antifibrotic for systemic sclerosis. Colony stimulating factors improve the granulocytopenia associated with Felty's syndrome or drug toxicities but can induce arthritis flares and should be reserved to treat infectious complications. Promising results are being obtained with selective antagonism of TNF and IL-1 in RA, and combinations of anticytokine strategies with traditional antirheumatic drugs have been already envisaged. These should preferably be based in a broader knowledge of the effects of antirheumatic agents on the cytokine network.
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THE PHARMACOLOGICAL agents used in the therapy of rheumatoid arthritis (RA) have been traditionally subdivided into nonsteroidal anti-inflammatory drugs (NSAIDs), disease modifying or slow-acting antirheumatic drugs (DMARDs or SAARDs), and corticosteroids. All of these drugs are clinically effective and, according to a recent classification, can be denominated symptom-modifying anti-rheumatic drugs (SMARDs). The term disease-controlling antirheumatic therapy (DC-ART) is reserved for those SAARDs capable of modifying not only clinical symptoms, but also radiological and functional outcome[1] (Fig 1). Despite broad experience with antirheumatic drugs, their mechanisms of action are only partially understood. This is especially true for the SAARDs, a heterogeneous group that includes antimalarials, thiol compounds, sulfonamides, and antimetabolites, as well as cytotoxic and immunosup-

From the Departments of Rheumatology and Internal Medicine, University Hospital Nijmegen, The Netherlands.

Pilar Barrera, MD: Rheumatology Fellow, Departments of Radiology and Internal Medicine; Agnes M.Th. Boerbooms, MD, PhD, Department of Rheumatology; Leo B.A. van de Putte, MD, PhD: Professor of Rheumatology, Department of Rheumatology; Jos W.M. van der Meer, MD, PhD: Professor of Internal Medicine, Department of Internal Medicine, University Hospital Nijmegen, The Netherlands.

Address reprint request to Pilar Barrera, MD, Department of Rheumatology, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

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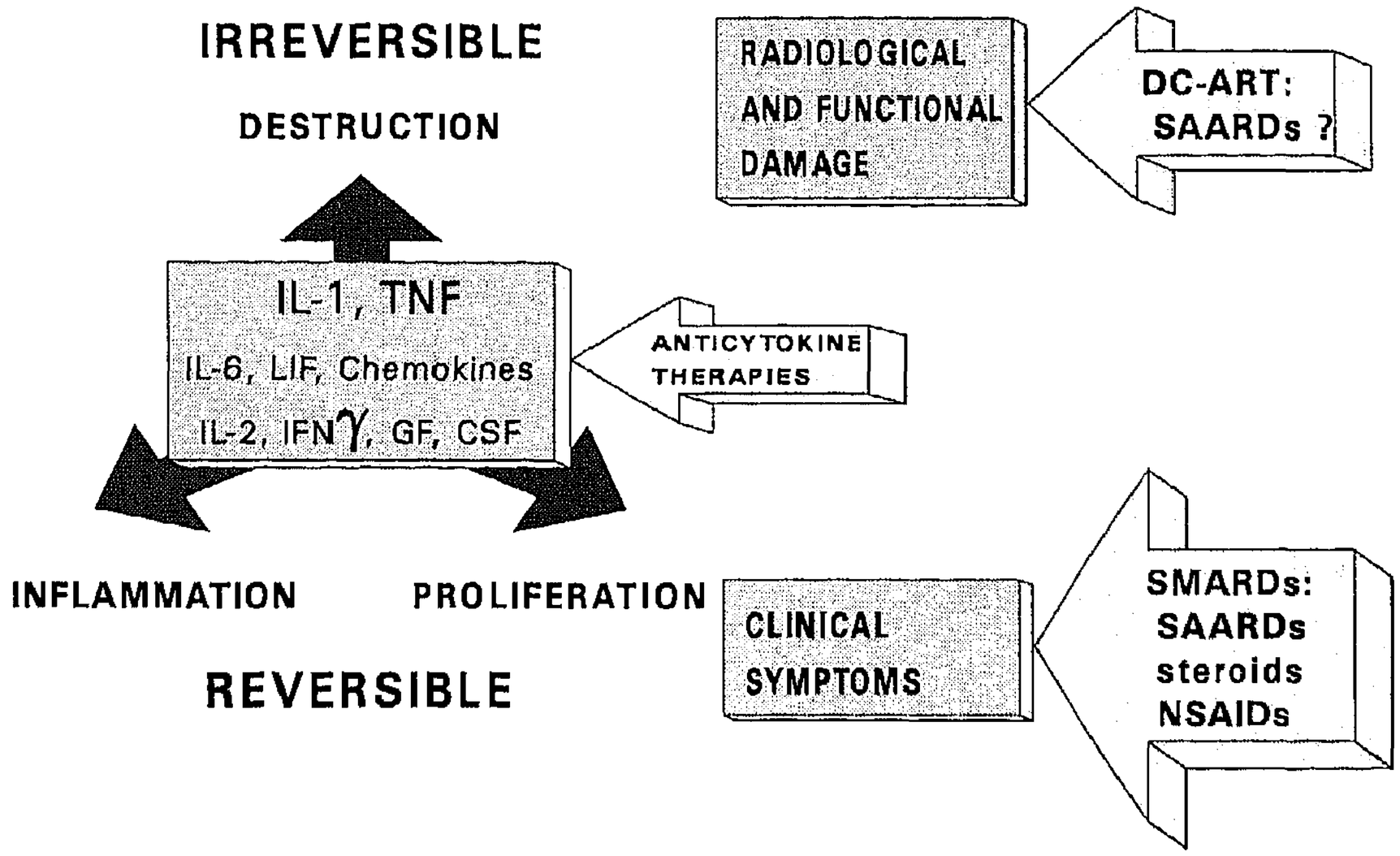

Fig 1: Schematic representation of the pathogenesis, manifestations, and therapeutic interventions in RA. Joint inflammation, involving processes such as homing, chemotaxis and activation of cells together with the proliferation of synovial tissue, pannus formation, and angiogenesis eventually result in irreversible destruction of cartilage, bone, and periarticular structures. Recognition of the involvement of cytokines in the initiation and perpetuation of these events has prompted the introduction of anticytokine therapies for RA. NSAIDs, steroids, and SAARDs are SMARDs capable of alleviating the local and systemic manifestations of RA. Only those SAARDs that alter disease outcome should be considered DC-ART.

pressive agents. Many SAARDs interfere with cellular metabolic processes and have nonspecific anti-inflammatory, immunoregulatory, and anti-angiogenic effects, which may contribute to their efficacy or toxicity, but usually do not fully explain their therapeutic effects.

Cytokines are a complex family of peptides with specific receptors that mediate intercellular communication and play a crucial role in immunologic and inflammatory reactions. The evidence implicating cytokines in the pathogenesis of RA and other autoimmune diseases has had repercussions on the treatment of these disorders. It is known that several drugs, including SAARDs, modulate the cytokine network. This may be important because cytokines control the proliferation, activation, traffic, and homing of immunocompetent cells in the joint, as well as the growth and function of synovial, cartilage, bone, and endothelial cells. Furthermore, some circulating cytokines or cytokine receptors may act as markers of disease activity or immune activation and prove useful in monitoring the effects of treatment. Finally, and more importantly, selected cytokines and anticytokines are being used to treat RA, and the search for pharmacological agents with specific cytokine-suppressive properties has just begun. In this report, we review the modulatory properties of several SAARDs and other pharmacological agents on the cytokine network and summarize the clinical experience with cytokine and anticytokine strategies in the treatment of RA.

THE CYTOKINE NETWORK IN RA

A detailed review of the cytokine network is beyond the scope of this paper. Therefore only relevant aspects will be briefly mentioned. According to their biological effects, cytokines can be classified as proinflammatory or anti-inflammatory, fibrotic, growth and chemotactic factors (Table 1). Such a functional classification is an oversimplification because cytokines are characterized by pleiotropism (one cytokine possesses multiple functions), overlap (several cytokines share similar effects), and mutual regulation. Nevertheless, by considering their function, better insight into the links between cytokines and

Table 1: Cytokines Relevant for Rheumatoid Arthritis: Functional Classification

Cytokine	Main Cell Source	Function	Other Effects
Interleukins and tumor necrosis factor			
IL-1 and TNFα	MØ, many other cells	Proinflammatory	Fever, anorexia, acute phase reaction Cartilage and bone breakdown, fibroblast proliferation, adhesion molecule expression Stimulate synthesis of many other cytokines, such as IL-6, LIF, chemokines, growth- and colony-stimulating factors
IL-2	T cell (Th1)	Immunostimulation	T and B cell activation and proliferation, DTH reaction, ↑ CTL, NK, and LAK activity
IL-4	T cell (Th2)	Anti-inflammatory	B, T, and mast cell growth, synthesis of IgE, IgG1 and IgG4 ↑ IL-1Ra and ↓ IL-1, TNF, IL-6, IL-8, IFNγ production
IL-6	MØ, many other cells	Proinflammatory	B and T cell proliferation and activation, Ig synthesis, thrombopoiesis, mediates some IL-1 effects
		Anti-inflammatory	Acute phase reaction, synthesis of metalloproteinase inhibitors, feedback mechanism for IL-1 and TNF
IL-10	T cells	Anti-inflammatory	B cell differentiation and Ig synthesis, ↓ class II HLA expression ↑ IL-1Ra and ↓ IL-1, TNF, IL-6, IL-8, IFNγ and IL-2 production
IL-13	T cells	Anti-inflammatory	IL-4–like activities
Chemokines			
IL-8 family	MØ, other cells	Proinflammatory	Chemotactic for neutrophils and T cells, neutrophil activation and degranulation
MCP-1 family	MØ, other cells	Proinflammatory	Chemotactic for mononuclear cells, monocyte activation
Growth factors			
TGFβ	MØ, many other cells	Anti-inflammatory	Matrix and collagen synthesis, ↓ IL-1 and TNF and ↑ IL-1Ra production, ↓ IL-1R expression
		Proinflammatory	Chemotactic for neutrophils and fibroblasts, fibroblast proliferation, angiogenesis, osteophyte formation
		Immunosuppressive	↓ T and B cell proliferation, ↓ CTL, NK and LAK activity
FGF, PDGF, PD-ECGF, VEGF	MØ, endothelial cells, platelets, other	Proliferative	Fibroblast proliferation
		Profibrotic	Matrix synthesis, angiogenesis
Colony stimulating factors			
GM-CSF	MØ, many other cells	Proinflammatory	Growth and differentiation of granulocyte and MØ progenitors in bone marrow ↑ expression of class II HLA and adhesion molecules MØ activation, phagocytosis and cytokine production, chemotactic for monocytes
G-, M-CSF	MØ, other cells	Proinflammatory	Growth and differentiation of granulocyte and MØ progenitors in bone marrow Neutrophil and monocyte activation
Interferons			
IFNγ	T cells	Immunostimulation	T, NK cell, and MØ activation, ↑ expression of class II HLA and adhesion molecules Antiproliferative, antifibrotic
Other			
LIF	T cells	Proinflammatory	IL-6-like activities, bone and cartilage resorption

Abbreviations: CSF, colony stimulating factor; G, granulocytes; M, macrophages; CTL, cytotoxic T lymphocytes; DTH, delayed type hypersensitivity; GF, growth factor; FGF, fibroblast GF; HLA, human leucocyte antigen; Ig, immunoglobulin; IFN, interferon; IL, interleukin; IL-1R, IL-1 receptor; IL-1Ra, IL-1 receptor antagonist; LIF, leukemia inhibitory factor; LAK, lymphokine activated killer cell; MØ, macrophage; MCP-1, monocyte chemoattractant protein-1; MCAF, monocyte chemotactic and activating factor; NK, natural killer cell; PDGF, platelet-derived GF; PD-ECGF, platelet-derived endothelial cell GF; TGFβ, transforming GF-β; TNF, tumor necrosis factor; VEGF, vascular endothelial GF.

other processes involved in RA and their potential consequences for therapy can be gained.

Because of their systemic and local effects, interleukin 1 (IL-1) and tumor necrosis factor α (TNFα) are the prototypes of pro-inflammatory cytokines.[2] Compelling evidence from in vitro studies and experimental arthritis models shows that tissue destruction and joint inflammation are independently regulated by IL-1 and TNF and that these mediators orchestrate the arthritic process.[2,3] The IL-1 "family" consists of three related peptides: IL-1α, which is primarily cell bound, IL-1β, which is primarily secreted, and the IL-1 receptor antagonist (IL-1Ra). These three molecules are each capable of binding to the membrane receptors, but only IL-1α and β are biologically active. The IL-1Ra occupies the receptor but does not activate signal transduction, and therefore it acts as a competitive IL-1 inhibitor.[4] There are two types of IL-1 receptors. Type I (IL-1RI) mediates signalling activity and has a higher affinity for IL-1α and IL-1Ra, whereas type II (IL-1RII) is a decoy receptor, which lacks signalling capacity and has a higher affinity for IL-1β.[5] Membrane-bound and soluble forms of the IL-1RII decoy receptor and the IL-1Ra together represent natural mechanisms for counteracting the potentially toxic role of IL-1 in vivo.

TNFα and the related lymphotoxin (TNFβ) bind to two forms of membrane receptors, type 1 or p55 and type 2 or p75, which are active in signal transduction, and can be cleaved from the cell membrane yielding soluble forms (sTNFR). The sTNFRs behave as endogenous antagonists by binding TNFα, preventing it from attaching to the membrane receptors, but may also function as carrier molecules that preclude TNF degradation.[6]

IL-1, IL-1Ra, and TNFα are present in the rheumatoid synovial membrane and can be detected in plasma and in synovial exudates of RA patients together with soluble forms of both TNFR and type II IL-1R. Compared with IL-1Ra and sTNFR, the concentrations of IL-1 and TNF in biological fluids are low but this excess of antagonists may not be sufficient to block the activity of IL-1 and TNF completely.[2]

IL-1 and TNFα are arthritogenic in several animal models, and antagonizing their effects reduces the severity, or even prevents arthritis.[2,3] Moreover, these cytokines occupy a high position in the cytokine hierarchy because they stimulate the production of each other and of many proinflammatory cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, leukemia-inhibitory factor, IL-8 and other chemotactic factors.[7] It is therefore not surprising that ongoing anticytokine strategies for RA are primarily focused on suppressing the actions of IL-1 and TNF.

IL-6 stimulates B and T cell differentiation and activation, as well as immunoglobulin production, and shares proinflammatory activities with IL-1, TNF, and colony stimulating factors. However, IL-6 is not arthritogenic, does not induce local prostaglandin-E_2 or collagenase production, and is anti-inflammatory by promoting the acute phase reaction, upregulating metalloprotease inhibitors,[7] suppressing IL-1 and TNF synthesis in vitro, and inducing IL-1 and TNF endogenous antagonists in vivo.[8] Moreover, IL-6 is readily detected in biological fluids and may be a useful marker of disease activity.

Several other cytokines, including colony-stimulating and growth factors, chemotaxins, and the leukemia-inhibitor factor (LIF), are also present in the rheumatoid synovial membrane and are thought to perpetuate the ongoing inflammation by stimulating cell recruitment and activation.[7]

In contrast to the abundance of monocyte-derived cytokines, T cell products are scant in the inflamed joint,[9,10] which has led to speculation about the role of T cells in RA. T helper cells can be divided into Th1 (secreting IL-2 and IFNγ) and Th2 subsets (secreting IL-4 and IL-5). Other cytokines such as transforming growth factor β (TGFβ), IL-10, and IL-13 are not confined to these subsets and are also produced by non–T cells.[11,12] IL-2 is implicated in the activation and proliferation of T, B, and natural killer (NK) cells, whereas IFNγ induces the expression of class II HLA and adhesion molecules, activates macrophages, lymphocytes, and NK cell activity and has antiproliferative and antifibrotic effects. Lymphocytes from RA patients defectively produce IL-2 and IFNγ and are hyporresponsive to IL-2, which could account for the low proliferative responses to mitogens and delayed type hypersensitivity in RA. Because of its immunostimulatory proper-

ties, IL-2 has been used in the therapy of malignancies, whereas several studies have examined the effects of IL-2 inhibition[13] and IFNγ therapy in RA.[14]

In contrast with the low levels of IL-2, high concentrations of its soluble receptor (sIL-2R) have been detected in several autoimmune diseases including RA. These are considered to reflect lymphocyte activation and may be useful in monitoring disease activity.[15]

Finally, cytokines such as IL-4, IL-10, IL-13, and TGFβ are considered anti-inflammatory because they inhibit the production of proinflammatory cytokines such as IL-1 and TNF and upregulate their inhibitors.[12,16] These anti-inflammatory cytokines are being tested in animal models and may find therapeutic application in human autoimmune diseases.

EFFECTS OF SAARDS AND OTHER ANTIRHEUMATIC DRUGS IN CYTOKINE MODULATION

Our knowledge of the cellular and soluble mediators of inflammation has advanced our understanding of the mode of action of SAARDs in RA. Knowing that cytokines play a key role in the cascade of inflammatory responses has stimulated research on the effects of antirheumatic drugs on these mediators. Several in vitro studies and observations in animal models and in patients treated with SAARDs suggest that some of these agents have cytokine-modulating properties. The present section summarizes the literature on several SAARDs, corticosteroids, and other drugs with "cytokine suppressive" properties.

Antimalarials

The antimalarial agents hydroychloroquine (HCQ) and chloroquine (CQ) are quinoline derivatives that exert a variety of effects on immunocompetent cells. These agents accumulate in lysosomes, inhibit several enzymes, and interfere with the function of monocyte-macrophages, which may represent their main mode of action. Early studies showed that HCQ and CQ inhibit the cartilage degradation induced by IL-1.[17] More recently, it was shown that therapeutic concentrations of HCQ suppressed the production of IL-1 and IL-6 by monocytes, probably acting at a posttranscriptional level.[18,19] The effects of these drugs on TNFα production is more controversial, although combinations of HCQ with sulfasalazine inhibit IL-1 and TNF production synergistically.[18,19] HCQ does not alter either IL-8 gene expression or synthesis by endothelial cells and monocytes[20] or the production of IL-2, IL-4, or IFNγ by T cells.[18] However, the drug inhibits IL-6 synthesis by T cells, suggesting a selective effect of HCQ on some proinflammatory cytokines (IL-1 and IL-6).[18] The effects of HCQ therapy on cytokines in RA have not been investigated, but Wallace et al[21] observed lower circulating concentrations of IL-6 and sIL-2R in systemic lupus erythematsus (SLE) patients treated with HCQ than in those receiving only corticosteroids or no therapy. In a prospective study by the same authors, significant decreases in serum IL-6 levels occurred after 6 and 12 weeks of treatment with HCQ, though sIL-2R concentrations remained unchanged.[22] This seems to confirm the relative lack of effect of HCQ on T cell activation.

CQ and HCQ have similar effects on monocytes, although the former may have additional effects on T cells. CQ inhibits monocyte production of IL-1,[23] TNFα, and IL-6[24] and acts synergistically with cyclosporin in suppressing IFNγ production by T cells.[25] CQ therapy does not affect IL-1 production in animal models of adjuvant arthritis[26] or haemorrhagic shock but inhibits TNF, IL-6 and prostaglandin release in the latter.[27]

Gold Compounds

Gold compounds modulate the functional activities of virtually every immunocompetent cell, but inhibition of mononuclear phagocyte function may be of paramount importance. This is supported by the suppressive action of these agents on the production or activity of monocyte-derived proinflammatory cytokines.

Most in vitro studies have shown that gold sodium thiomalate (GST) and auranofin (AF) inhibit the synthesis of IL-1 by monocytes[28-32] and act synergistically when combined.[19] Gold compounds also antagonize some of the biological effects of IL-1,[28,33,34] and therapy with these agents decreases IL-1 production in adjuvant arthritis.[26,35] Danis et al studied the production of IL-1 by monocytes from RA patients treated with GST. Patients considered "high IL-1 pro-

ducers" at baseline had transient clinical improvement associated with decreases in IL-1 secretion, whereas "low producers" had a better clinical response and an increase in IL-1 secretion during therapy.[36] The authors suggested that IL-1 production before treatment might be predictive of the clinical response to GST, but several confounding factors, including the use concomitant medications, render this study inconclusive.

Gold does not alter IL-1Ra synthesis by normal monocytes[32] although increases have been reported in patients with RA.[37] Further studies should elucidate whether gold therapy induces a beneficial shift in the IL-1/IL-1Ra balance.

Gold compounds may modulate other proinflammatory cytokines besides IL-1. GST inhibits the production of IL-6, but not of TNF, by monocytes,[32] suppresses the synthesis of IL-8 and monocyte chemoattractant protein (MCP-1) by rheumatoid synoviocytes,[38] and interferes with the production of IL-8 by monocytes and endothelial cells.[20,39] AF, however, inhibits the production of TNFα by monocytes,[40] and several effects of this cytokine on neutrophils.[41,42] Serial studies in patients with RA receiving chrysotherapy have reported reductions in circulating IL-6 levels,[43,44] monocyte production of IL-6,[45] and, interestingly, cytokine expression in the synovial membrane as recently demonstrated by Yanni et al.[46] Using sequential synovial biopsies, the authors observed a striking decrease in the expression of monocyte-derived cytokines, including IL-1β, IL-1α, IL-6, and TNFα, and in the number of infiltrating monocytes and macrophages after 12 weeks of treatment with GST, whereas the number of T and B cells remained unchanged.[46] Whether such reduction in monocyte infiltration results from systemic effects, like inhibition of myelopoiesis,[47] or from a local action of gold is unknown, but these results indicate that the monocyte-macrophage is the principal target cell for gold compounds. Nevertheless, T cell proliferation and activation and cytokine production are also affected by these drugs at least in vitro. Gold compounds inhibit T cell proliferation induced by mitogens, antigens,[48,49] and IL-2[50,51] and suppress the synthesis of IL-2 and the expression and release of IL-2R by activated T cells at a transcriptional level.[49] Some of these effects may require low intracellular glutathione concentrations.[52] The effect of gold on IFNγ production is controversial,[53,54] but GST inhibits several IFNγ activities such as the induction of class II HLA expression in monocytes[55] and endothelial cells.[51] This, together with the inhibition of endothelial cell proliferation[56] and expression of adhesion molecules by gold compounds,[57] may result in decreased angiogenesis and cell recruitment in the joint.

Chrysotherapy normalizes depressed IL-2 production in adjuvant arthritis[35] and patients with pemphigus vulgaris.[58] In contrast, circulating sIL-2R levels in RA remain unchanged after 24 weeks of gold therapy, despite clinical improvement,[59-61] and only one study reported lower sIL-2R levels in patients with clinical remission than in those with active disease after prolonged treatment.[60] It seems, therefore, that despite the inhibitory effects of gold on T cells in vitro, chrysotherapy for RA does not affect T cell infiltration in the joint[46] or lymphocyte activation evidenced by sIL-2R, at least in the short term.

D-Penicillamine

D-penicillamine and the related drug bucillamine, are structural analogues of cysteine, which, in contrast to gold and antimalarials, may predominantly affect lymphocytes (especially T helper cells) without altering macrophage function. Whether therapy with these drugs results in cytokine modulation in RA has not been assessed, but available in vitro studies corroborate this hypothesis.

The drugs' effect on T cell function is mediated by their capacity to generate hydrogen peroxide in the presence of copper salts. This results in a direct inhibition of T cell activation and production of IFNγ and IL-2, and indirectly in decreased immunoglobulin synthesis by B lymphocytes.[62,63]

Initial in vitro studies suggested that D-penicillamine might decrease the production of monocyte products with IL-1-like activity.[64] Later it was shown that IL-1 synthesis and bioactivity are suppressed only with high concentrations of the drug[33]; however, therapeutic concentrations do not alter the production of either IL-1[23,30,65] or TNF[19] by human monocytes. Moreover,

therapy with D-penicillamine does not inhibit IL-1 production in the adjuvant arthritis model.[26,66] D-penicillamine blocks the binding of IL-1β to α-2-macroglobulin, an acute phase protein that may function as carrier for IL-1 and other cytokines.[67] This dissociating effect is not specific for IL-1,[68] and its relevance for the therapeutic effect of the drug is speculative. In the presence of copper, D-penicillamine and other thiol compounds inhibit spontaneous and IL-1–induced fibroblast proliferation. This is due to the production of hydrogen peroxide rather than suppression of IL-1 synthesis.[69] D-penicillamine inhibits the proliferation and IL-8 gene expression and synthesis by endothelial cells,[20] and decreases neovascularization in vivo.[70] These antiangiogenic and antifibrotic properties may be important for therapeutic efficacy in RA and systemic sclerosis. It has been suggested that bucillamine is superior to D-penicillamine in treating RA,[71] possibly because of its additional immunosuppressive effects. In vitro, both drugs inhibit T cell function, including IL-2 and IFNγ production, in the presence of copper,[72] but only bucillamine forms an intramolecular disulphide and inhibits B cell function whether or not copper salts are present.[62,72] This effect is not due to decreased IL-6 production by B cells.[62] Furthermore bucillamine-, like D-penicillamine, does not affect IL-1 production by human monocytes.[30,65]

Sulfasalazine

Sulfasalazine is an azo compound of sulfapyridine and 5-aminosalicylic acid. Sulfapyridine seems to be the active component in RA, though sulfasalazine itself might also have antirheumatic properties. The exact mechanism of action of this drug remains also unclear despite its multiple anti-inflammatory and immunoregulatory effects.

In vitro, sulfasalazine inhibits the production of IL-1β and TNFα by monocytes[19,30,73-75] and may suppress the binding of TNFα to its receptors.[76] Neither the production[45,74,77] nor the activity of IL-6 are markedly altered by sulfasalazine.[78] However, the drug down-regulates the synthesis of IL-2 and IFNγ by T cells,[79,80] which may explain its inhibitory effect on lymphocyte proliferation and antibody production. Moreover, sulfasalazine inhibits the proliferation as well as IL-8 gene expression and synthesis[20] in endothelial cells, which may account for its antiangiogenic effects. Interestingly, most of the effects of sulfasalazine on cytokines and immunoregulation are not shared by its metabolites, which suggests that the parent compound possesses therapeutic effects.

Studies on the effect of sulfasalazine therapy on circulating cytokines in RA have yielded conflicting results. In a 6-month, placebo-controlled study, Danis et al[77] observed decreases in IL-1α, IL-1β, and TNFα but unchanged IL-6 concentrations during sulfasalazine therapy,[77] whereas other authors reported no changes in IL-1β and TNFα levels[81] and decreases in serum concentrations and monocyte production of IL-6.[45,81,82] Circulating sIL-2R concentrations did not change after 12 weeks of therapy with sulfasalazine in several studies.[61,81,82] The number of patients in these studies generally were too small to draw definitive conclusions and the effects of sulfasalazine therapy need further investigation.

Combinations of SAARDs are increasingly used to treat RA and there are in vitro data suggesting that some of these combinations suppress cytokine production in a synergistic manner.[19] In an open study conducted in our center, the combination of sulfasalazine with methotrexate proved clinically superior to methotrexate alone.[83] Serial cytokine measurements showed decreases in IL-6 and sIL-2R levels with both therapies, but the concentrations of sTNFR and the production of IL-1β and IL-1Ra by monocytes fell only in patients treated additionally with sulfasalazine. These results suggest an additional or specific sulfasalazine on these cytokines[84] and encourage further studies on the effects of other SAARD combinations.

Methotrexate

Intensive efforts have been made to elucidate the mechanism of action of methotrexate. Because of its antifolate effect, methotrexate may impair DNA, RNA, and protein synthesis, inhibit transmethylation reactions and polyamine synthesis, and stimulate adenosine release. Moreover, the drug can inhibit 5-lipoxygenase activity and has modulatory effects on cytokines.[85]

Methotrexate inhibits some biological activities of IL-1 including the proliferation of thymocytes[86,87] and synovial fibroblasts but does not affect the production of collagenase, prostaglandin E, hyaluronic acid, or metalloprotease inhibitors induced by IL-1.[88,89] This suggests that the drug antagonizes the proliferative but not the secretory activities of IL-1.[88] The suppression of IL-1 bioactivity by methotrexate is due to its antifolate effect because it can be reversed by folinic acid,[87] although binding of methotrexate to IL-1, which has structural homology to dihydrofolate reductase (DHFR), or receptor down-regulation have been also proposed to explain this effect.[90] Methotrexate therapy decreases the production of IL-1 in adjuvant arthritis,[91-93] although this has not been consistently observed in RA. Two studies have assessed the effect of a single dose of methotrexate on monocyte IL-1β production: Segal et al[86] reported no changes in 9 patients treated with methotrexate for variable periods of time, whereas we observed significant decreases after the first methotrexate dose, but not in patients treated for longer than 1 year who already had lower IL-1β production and less active disease.[94] In another study, Chang et al[95] reported decreases in IL-1 production in 4 of 8 patients after 6 weeks of methotrexate therapy.[95] These studies suggest that if MTX down-regulates IL-1 production the effect occurs in the early phase of treatment. To test this hypothesis, we monitored 26 patients treated with methotrexate for 24 weeks. Some decrease in IL-1 production occurred after 4 weeks, but the effect was not progressive and measurements after 24 weeks were not significantly lower than those at baseline.[84] Therapy with methotrexate did not alter circulating IL-1β concentrations in this and another study,[84,95] but decreases in serum[96] and in synovial fluid concentrations of IL-1β have been described.[97] Taken together, methotrexate may inhibit the proliferative effects of IL-1, and decrease monocyte IL-1 production in the short term in certain patients, although this effect is not sustained and unlikely explains the long-term efficacy of methotrexate. Further investigation is needed to elucidate whether therapy with methotrexate decreases the production of IL-1 in the joint.

In-vitro, methotrexate inhibits IL-6 but not TNF bioactivity[86] and decreases the production of IL-8 by monocytes.[39] The synthesis of IL-8 by endothelial cells[20] or RA synoviocytes[38] is not affected by the drug. Treatment with methotrexate reduces circulating IL-6,[84,96,98,99] IL-8,[96] and p55 soluble TNF receptor (sTNFR) concentrations[98] in patients with RA but does not affect circulating levels[84,98] or monocyte production of TNFα.[84,94] Decreases in IL-6, IL-8, and p55 sTNFR levels probably reflect clinical improvement and are not a specific effect of methotrexate. Several studies have investigated the effects of methotrexate on T cell–derived cytokines. In vitro this drug enhances the cytotoxic[100] and proliferative[86] effects of IL-2 and the production of this cytokine by lymphocytes.[101] Methotrexate therapy normalizes the defective production of IL-2 in the adjuvant and streptococcal cell wall arthritis models.[91,93] Lymphocyte IL-2 synthesis increases after a single dose of methotrexate in patients with RA[102] and in patients receiving the drug for longer periods.[96,103] Kremer et al[96] reported increases in serum IL-2 concentrations during treatment with methotrexate,[96] and decreases associated with disease flares 4 weeks after withholding the drug.[104] The defective IL-2 production in RA might be due to excessive polyamine production,[105] which is inhibited by methotrexate.[106] This effect could explain the enhanced IL-2 production observed during methotrexate therapy.[107] In contrast with this up-regulation of IL-2, treatment with methotrexate reduces the circulating sIL-2R levels in patients with RA[84,98,99,108] and juvenile chronic arthritis.[109] This effect does not predict response to treatment[108] but rather reflects clinical improvement.[98]

Recent studies suggest that methotrexate may not only upregulate Th1-cytokines, like IL-2, but also may downregulate Th2-derived cytokines. Taylor et al[110] observed an increased mRNA expression of IL-4 and IL-10 in mononuclear cells from RA patients compared with healthy controls. Treatment with methotrexate reduced IL-4 and IL-10 but enhanced IFNγ expression in patients with RA.[110] Consistent with these effects, Kremer et al[96] reported decreases in serum IL-4 during methotrexate therapy and increases in circulating IL-4 levels associated with disease flares after stopping treatment.[102] The effects of methotrexate on IL-2, IL-4, and IL-10 are puzzling because they

suggest a shift in cytokine expression towards a "pro-inflammatory" pattern.

Azathioprine

The purine analogue azathioprine is converted in 6-mercaptopurine (6-MP) which follows pathways similar to endogenous purines and is incorporated into DNA and RNA. The cytotoxicity of azathioprine is unlikely to account for its therapeutic efficacy in RA, but the levels of endogenous purine enzymes responsible for its metabolism may influence its toxicity.[111]

In vitro studies suggest that azathioprine does not alter IL-2 production,[112,113] whereas both azathioprine and 6-MP inhibit IL-6 bioactivity.[78] Studies concerning the effect of therapy with azathioprine on cytokines in patients with RA are scant. In a 48-week trial of azathioprine versus methotrexate, we observed no significant changes in the circulating concentrations of either TNFα or the soluble TNF and IL-2 receptors during treatment with azathioprine. Decreases in IL-6 concentrations reflected clinical improvement and were not specific to the drug.[98] In a smaller study, Crilly et al[114] reported no changes in either circulating sIL-2R levels or in the production of IL-6 by monocytes after 24 weeks of azathioprine therapy.

Cyclosporin A

Cyclosporin A is an immunosuppressive agent that specifically interferes with T cell activation. Complexes of cyclosporin A with its specific receptor, cyclophilin, bind to and block the activity of calcineurin, preventing the activation of transcription factors involved in cytokine gene expression.[115] The major effect of cyclosporin A is the suppression of T cell activation, which is due to inhibition of the transcription and synthesis of IL-2 and the expression of receptors for IL-2 in lymphocytes.[115,116] This results in secondary inhibition of B lymphocyte, natural killer (NK) cell and macrophage activities. Besides its effect on IL-2, cyclosporin A inhibits the transcription of IL-3, IL-4, IL-5, IFNγ,[116] and TNFα[117] in T cells. The synthesis of IL-1 by monocytes is not affected by cyclosporin A,[23] although the drug may antagonize some of this cytokine's effects in bone and cartilage.[116] Under certain conditions, cyclosporin A decreases monocyte TNFα production.[116,118]

Decreases in the number of circulating cells bearing the IL-2R[119] and in circulating sIL-2R and IL-6 concentrations[120,121] have been reported in RA patients treated with cyclosporin A. Decreases in sIL-2R levels associated with clinical improvement also have been observed during cyclosporin A therapy for Crohn's disease[122] but not in systemic sclerosis.[123]

Corticosteroids

Glucocorticoids (GCs) affect virtually every cellular and humoral mechanism involved in inflammation and immune response, including cytokines. The mechanism of action of GCs is complex but involves regulation of gene transcription. GCs form complexes with specific cytoplasmic receptors, which are translocated to the cell nucleus. Interestingly, mononuclear cells from patients with RA shown a low GC receptor density, although this does not result in GC resistance.[124] The binding of GC-receptor complexes to regulatory elements of DNA results in either positive or negative regulation of gene transcription. GCs interfere with cytokine production not only by suppressing their transcription, but also by decreasing messenger RNA stability and affecting other posttranscriptional events.[125-128] This inhibits the synthesis of proinflammatory and chemotactic cytokines such as IL-1, TNFα, IL-6, IL-8, and monocyte chemotactic and activating factor (MCAF), and hemopoietic growth factors such as IL-3 and GM–CSF.[20,38,128-132] In addition, GCs interfere with T cell activation by down-regulating the gene expression of IL-2 and IL-2R, inhibit the production of IL-4 and IFNγ by lymphocytes,[133,134] and induce IL-1 and IL-6 receptor expression in some cells.[135-137] In vitro experiments have shown that the combination of GCs with IL-4 suppresses the synthesis of IL-1 and TNFα by monocytes[138] and synovial tissue[139] synergistically, which may find applications in future therapies.

Several studies have provided evidence for a modulatory role of GC therapy on cytokine production or release in vivo. In humans, therapy with GCs attenuates the toxicity induced by IL-2 and lymphokine activated killer cells, OKT3, and experimental endotoxinemia by re-

ducing the release of TNFα, IFNγ, and IL-2 in the circulation.[140-142] These drugs inhibit IL-4 and IL-5 expression by bronchoalveolar cells[143] and monocyte TNF production[144] in asthmatic patients and reduce circulating IL-6 levels in patients with polymyalgia rheumatica and giant cell arteritis.[145] In RA, circulating levels of IL-6, IL-8, and sIL-2R[146] and the production of IL-1 by monocytes[147] decrease significantly after pulse therapy with high-dose corticosteroids, and reductions in sIL-2R[148] and TNFα[149] concentrations are associated with improvement during treatment with low-dose GCs.

Other Drugs

The number of pharmacological agents with potential antirheumatic and cytokine-modulating activities is too long to be fully enumerated. Several drugs used for other indications were discovered, by chance, to have antiarthritic effects. Among these agents, thalidomide, a sedative, and pentoxifylline, a phosphodiesterase inhibitor used for peripheral vascular disorders, are worthy of mention because of their TNFα-suppressive activities. Thalidomide inhibits its TNFα production, neutrophil chemotaxis, and superoxide generation, but its use in RA has been limited because of the drug's toxicity.[150] Pentoxifylline decreases TNFα production in vitro and in vivo[151] and has anti-inflammatory, immunomodulatory, and anti-fibrotic properties. In an open, 3-month trial, performed in patients with severe RA refractory other therapies, additional treatment with pentoxifylline resulted in clinical improvement. TNF production remained unchanged after 3 days of treatment but was, unfortunately, not measured thereafter.[152]

More selective cytokine inhibitors already used in clinical trials for RA include IX 207-887 and the dual cyclooxygenase/lipoxygenase inhibitor tenidap. IX 207-887 inhibits IL-1, IL-6, and superoxide production and is effective in animal arthritis. A placebo-controlled study in RA reported improvement in clinical and laboratory variables in patients treated with IX 207-887, although adverse reactions were frequent.[153] Tenidap inhibits the synthesis of IL-1, IL-6, and TNFα by human monocytes and rheumatoid synovium[154-155] and decreases T cell proliferation and IFNγ synthesis.[156] Furthermore, the drug inhibits metalloprotease production by chondrocytes exposed to IL-1,[157] bone resorption induced by IL-1 and TNF,[158] and the expression of HLA class II antigens in synoviocytes exposed to IFNγ.[159] Patients with RA receiving tenidap show reductions in acute phase reaction and circulating IL-6 and sIL-2R concentrations.[160-161]

Several immunoregulatory agents under current investigation for treating autoimmune diseases may affect cytokine production. These encompass drugs with immune-enhancing effects (levamisole, tilomisole and imuthiol) inhibitors of nucleotide synthesis (mycophenolate, brequinar, and mizorbine) and immunosuppressive agents (15-deoxyspergualin, leflunomide, FK506, and rapamycin).[162,163] Leflunomide, an isoxazol-derivative currently undergoing clinical trials for RA, inhibits lymphokine-dependent T and B cell proliferation and decreases IL-1 and TNFα induced proliferation of RA synovial fibroblasts.[164] FK506 and rapamycin are cyclosporin-related drugs that share structural homology and the same immunophillin receptor but interfere with T cell activation at a different level. Like cyclosporin, FK506 decreases the activity of calcineurin, is a potent suppressor of IL-2 production and receptor expression, and inhibits the transcription of various cytokines (IL-3, IL-4, IL-5, IFNγ, TNFα,[118] and IL-8[165]). Rapamycin interferes with lymphokine signal transduction without affecting calcineurin activity. Both agents are effective in animal arthritis and are being evaluated in patients with autoimmune diseases and organ allografts.

THERAPY WITH CYTOKINES AND ANTICYTOKINES IN RA

Advances in molecular biology have provided new therapeutic approaches for autoimmune diseases. Among the immunomodulators, selected cytokines such as IFNγ, GM-CSF, and G-CSF have been used to treat RA or its complications and promising results are being obtained with specific antagonists of proinflammatory cytokines.

The rationale for the use of IFNγ therapy in RA was tenuous, being based on the relative deficiency of this cytokine in the joint and its inhibitory effects on B cells, neutrophils, fibroblasts, and cytokine release. The lack of signifi-

Table 2: Anticytokine Therapies for Rheumatoid Arthritis (1 and 2)

Reference	Compound, Study Design	No. of Patients	Dose Schedule	Comments
173	Murine anti-IL-6 MoAb (BE8) Open	8	10 mg/day for 10 days	Improvement transient, mean duration 2 months; increased serum IL-6 in 4 patients; antimouse immunization in 2 patients; fever, chills and hypertension in 1 patient
174	Murine anti-IL-2R MoAb (Campath 6) Pilot	3	25 mg/day for 10 days	Improvement for 3 months in 2 patients, no significant side effects reported
175	IL-2-diphteria fusion toxin (DAB_{486}) Open, phase I/II Dose ranging	19	75, 130, 260 kU/kg/day for 5 to 7 days, repeated courses in 13 patients	Improvement in 9 patients (medium or high dose), duration 4-30 weeks, maintained improvement with repeated courses, anti-diphteria toxin immunization in all patients, very common adverse events (transaminase evaluations)
176	Double-blind, phase II Placebo-controlled (first course), followed by open study IL-2-diphteria fusion toxin (DAB_{389})	45	0.7 mg/kg/day for 5 days repeated 3 monthly for up to 3 courses	Improvement in 4/22, 11/36, and 11/33 patients after first, second, and third course respectively; anti-DT immunization in all patients, very common adverse events
177	Open, phase I/II	20	75, 150 kU/kg/day for 5 days	Patients were refractory to- and continued methotrexate therapy; improvement in 5/16 patients receiving high dose course; common adverse events, often transaminase elevations
178	Extension study Open, phase I/II Recombinant IL-1Ra (Anakinra)	40	150 kU/kg/day for 5 days; repeated courses by flare, up to 4 courses in 2 years	Additional methotrexate in 10 patients; lack of effect in 17 patients, less common adverse events; maintained improvement with repeated courses
179	Double-blind, phase I Placebo-controlled Dose ranging	25	0.5, 1, 2, 4, 6 mg/kg, single dose	Improvement in tender joint count in the high-dose group
180	Open, dose ranging	15	1, 2, 4 mg/kg/day for 28 days	Improvement after 7 days, frequent skin reactions at the injection site, 3 patients withdrawn (side effects)
180	Double-blind, phase II Dose and frequency ranging	175	20, 70, 200 mg per dose; first 3 weeks: 1, 3, or 7 dose weekly; last 4 weeks: 1 dose weekly	Improvement most marked with daily dose; frequent skin reactions at the injection site (62%); 25 patients withdrawn (lack of effect [n = 12], adverse events [n = 13]) multicenter, placebo controlled trial ongoing

Table 2: Anticytokine Therapies for Rheumatoid Arthritis (1 and 2) (Cont'd)

Reference	Compound, Study Design	No. of Patients	Dose Schedule	Comments
181	Recombinant sIL-1R type I Double-blind, phase I Placebo-controlled Dose ranging	23	125, 250, 500, 1000 $\mu g/m^2$/day for 28 days	Tendency to improve observed in treatment versus placebo group, but no patient achieved significant improvement few side effects
	Chimeric anti-TNFα MoAb (cA2)			
182	Open, phase I/II	20	20 mg/kg divided in 2-4 doses during 2 weeks	Maximal improvement at 3 weeks, median 14 weeks few side effects: minor infections (n = 2), development of anti-dsDNA (n = 2), and anticardiolipine antibodies (n = 1)
183	Extension study, repeated doses	8	Up to 3 additional courses of 10 mg/kg	Shorter improvement duration after repeated doses? 3 patients withdrawn (side effects), development of antinuclear antibodies (n = 2), increase in anti-dsDNA titers (n = 1), antimouse immunization (n = 4)
184	Double-blind, placebo-controlled, dose ranging	73	1, 10 mg/kg, one dose	Rapid- and dose-dependent improvement, few side effects; increased infections?
185	Pilot in JCA	1	Two doses of 10 mg/kg	Unchanged joint symptoms, decreased fever and IL6 levels
	Humanized anti-TNFα MoAb (CDP571)			
186	Double-blind, placebo-controlled, dose ranging	36	0.1, 1, 10 mg/kg, one dose	Improvement at 1-2 weeks in the high dose group, dose-dependent response, no significant decrease in *swollen joints*
	Extension study, open	30	1, 10 mg/kg, second dose	Best response in the high dose group; 8 patients withdrawn (disease progression); study with repeated doses ongoing, some patients become ANF+
	Dimeric p55 sTNFR fusion protein (R045-2081) Double-blind, placebo-controlled, dose ranging	?	0.1, 0.2, 0.4 mg/kg every 4 weeks, up to 3 doses	Results unpublished, extension study ongoing
187	Dimeric p75 sTNFR fusion protein Phase I, placebo-controlled, dose ranging	16	Loading dose: 4-32 mg/m^2, then maintenance dose: 2-16 mg/m^2 twice weekly for 4 weeks	Some tendency to improvement but no dose response observed

Abbreviations: MoAb, monoclonal antibody; IL, interleukin; TNFα, tumor necrosis factor-α; sTNFR, soluble TNF receptor; ANF, antinuclear factor.

cant improvement observed in most studies for RA[14] is therefore not surprising. Considering the powerful immunostimulatory effects of IFNγ, disease flares rather than improvement could be expected. Induction of antinuclear antibodies, and SLE in patients with RA,[166-167] and disease exacerbation in patients with SLE may occur during IFNγ therapy,[168] and there is no indication for using IFNγ in these disorders. In contrast, the improvement of skin fibrosis and pulmonary function observed in open trials with IFNγ for systemic sclerosis[169,170] suggests that IFNγ may prove useful as antifibrotic therapy. The colony-stimulating factors GM-CSF and G-CSF improve the granulocytopenia associated with Felty's syndrome as well as that induced by antirheumatic and cytostatic therapies, but because these agents may induce arthritis flares, their use should be restricted to treating or preventing infections associated with long-lasting neutropenia.[171,172]

To date, IL-1, TNF, IL-6, and IL-2 have been the target of specific anticytokine approaches in RA (Table 2). The experience with anti-IL-2 and anti-IL-6 treatment is limited,[171-178] and most attention has been drawn by therapies counteracting the two pivotal mediators TNFα and IL-1.[179-187] Initial results suggest that these anticytokine therapies are well tolerated and result in rapid improvement of clinical symptoms and laboratory parameters. Because these effects are transient, repeated administration is necessary. Besides the therapies listed in Table 2, other approaches, including soluble IL-1 receptors, inhibitors of the specific IL-1 convertase,[16] antiinflammatory cytokines such as IL-4, IL-10, and IL-13, and the use of antisense oligonucleotides and gene therapy, are under investigation.

SUMMARY, CONCLUSIONS, AND FUTURE PROSPECTS

The search for evidence that antirheumatic drugs interfere with cytokines is hampered by several problems, not least among those being the difficulty in extrapolating experimental data obtained in vitro to the situation in vivo (or in humans): Some SAARDs alter cytokine production or activity only at drug concentrations that are not attainable during therapy, and most in vitro studies do not take into account the effect of drug metabolites or the complexity of the immune response in vivo. This is exemplified by the bimodal effect of gold compounds on IL-1, IL-2, and IL-8. Although low concentrations of these agents seem to stimulate IL-1 and IL-2 and inhibit IL-8 production, the converse occurs at higher drug concentrations.[19,20,31,52] Moreover, in the case of IL-2, either stimulation or strong inhibition can be attained with low concentrations of GST depending on the glutathione content of the cells studied.[52] In vitro results, therefore, need to be confirmed by observations in vivo. Experience with animal models has taught us about the arthritogenic potential of cytokines and the effects of therapy with drugs and other anticytokine strategies, but the differences between human and animal arthritis emphasizes the need for human studies. Reports on the effects of SAARD therapy on cytokines in RA are relatively scarce and pitfalls include methodological problems, interindividual variations in cytokine concentrations, and most importantly, the need to quantify an increasing number of cytokines, preferably in the joint. Moreover, because SAARDs also modify disease activity in RA, changes in cytokine concentrations or expression during therapy with these agents may reflect clinical improvement rather than a specific drug effect.

Despite these problems, some conclusions on the cytokine-modulating effects of antirheumatic drugs can be drawn. First, it seems likely that many effects of antirheumatic agents on inflammation and immune response are mediated by, or result in, cytokine modulation. Second, the effects of antirheumatic drugs are usually not confined to a single cytokine. Even cyclosporin, considered to downregulate IL-2 gene transcription most specifically, also inhibits the synthesis of other T cell–derived cytokines,[116] whereas corticosteroids, have the broadest spectrum of activity and inhibit gene transcription of virtually every cytokine gene. Third, in contrast to the usually low levels of IL-1 and TNFα, high concentrations of IL-6, sIL-2R, and sTNFR are present in the circulation and synovial fluid of RA patients, and correlate with several disease activity measures. Therapy with a number of SAARDs and corticosteroids decreases circulat-

ing IL-6 and sIL-2R concentrations and sTNFR levels apparently decrease with methotrexate given alone or in combination with SASP.[84,94] These effects probably reflect clinical improvement and suggest that IL-6, sIL-2R, and sTNFR may be useful markers of disease activity in RA. Fourth, preliminary evidence suggests that therapy with certain SAARDs may regulate cytokine production by circulating cells and, most importantly, cytokine expression in the joint, as recently shown during gold therapy.[46]

The most important conclusion from previous studies is that our knowledge of the effects of SAARD therapy on the cytokine network in RA is patchy. Properly controlled, longitudinal studies assessing both cytokine concentrations in biological fluids and their expression in the joint during SAARD therapy are needed.

The rheumatologists are familiar with the use of combinations of NSAIDs, SAARDs, and corticosteroids as well as with patients refractory to these treatments. This has resulted in a search for novel therapies aimed at interfering with specific sites of immunoregulation, inflammation, and tissue destruction. The targets envisaged include T cells, antigen presentation, adhesion molecules, metalloproteases, nitric oxide, and cytokines. Trials with anticytokine therapies have reported rapid but transient clinical benefits in RA and ongoing studies will show whether these effects can be maintained with repeated administration. There is wide clinical experience with SAARD therapy for RA and it is unlikely that novel therapies will supplant these agents in the near future, although combinations of cytokine-suppressive and traditional therapies offer interesting perspectives. More knowledge on the effect of SAARDs on cytokines is needed before we arrive at selected combinations that complement each other's actions and translate this into better treatment for RA.

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