Advances in antiarthritic therapeutics

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The number of drugs used in the treatment of rheumatoid arthritis has increased over the past 10–20 years, but there is still an urgent need for more specific modalities with reduced side effects. The currently available drugs treat the symptoms but do not affect the underlying disease. In spite of side effects, nonsteroidal anti-inflammatory drugs are still the first-line therapy, but more physicians are using disease-modifying antirheumatic drugs earlier in the disease and combination therapies are also being investigated. New therapies that focus on specific molecular or cellular targets are now in clinical trials and hold promise for the future treatment of this disease.

Rheumatoid arthritis (RA) is a chronic inflammatory immune-mediated disease that leads to varying degrees of damage of the articular cartilage and subchondral bone. It is the most common cause of physical disability in developed countries, and the prevalence ranges between 0.3% and 1.5% with a female to male ratio of 3:1. The initiating factors for the inflammation, local tissue destruction and systemic responses seen in RA are unknown, and although a variety of infectious agents have been implicated, the causal agent of this disease has not been identified and therefore it has not been possible to therapeutically target the disease in a rational manner. There is currently no single therapeutic agent, or combination of agents, fully capable of preventing disease progression or reversing joint destruction. First-line therapy has been directed towards the alleviation of pain and inflammation with nonsteroidal anti-inflammatory drugs (NSAIDs), but these compounds do little to halt disease progression. This treatment is followed by the use of slow-acting or so-called disease-modifying antirheumatic drugs (DMARDs) whose mechanisms of action are not well defined.

Over the past decade, the number of DMARDs in clinical use has increased considerably, and the current trend is to use more aggressive agents, such as methotrexate, earlier in the disease. Individual RA patients respond differently to these drugs both in terms of response and side effects and inevitably become unresponsive. This has led to the use of combinations of drugs in the hope that DMARDs with differing mechanisms of action might result in additive or synergistic effects on the disease process without an increase in toxicity (side effects). In efforts to produce more specific therapies with fewer side effects, newer approaches under investigation target the cells known to be involved in the inflammatory process as well as the mediators they produce.

Selective inhibition of the synthesis or the action of cytokines such as TNF-α and IL-1 may prove to be a viable option. Monoclonal antibodies have been evaluated as therapeutic agents for a variety of conditions since their discovery 20 years ago, but without significant success. More recently, the development of chimeric and subsequently primatized antibodies targeted to specific cell-surface antigens, cytokines and cytokine receptors has facilitated their evaluation in a number of disease entities. Examples of other approaches are the development of second generation NSAIDs with improved safety profiles, selective ablation or modulation of T-cell or macrophage function, inhibition of

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proteases involved in joint destruction as well as the development of safer immunomodulatory and/or immunosuppressive compounds. The clinical status of many of the compounds and protein agents that are being evaluated in RA and described in this review is shown in Tables 1 and 2.

Improved NSAIDs

NSAIDs inhibit the production of prostaglandins by inhibition of cyclooxygenase and thereby possess anti-inflammatory, analgesic and antipyretic activities. They are still used as first-line treatment for RA and osteoarthritis (OA). However, most of the agents of this class have side effects, the most serious being gastrointestinal ulceration and hemorrhage.

More recently, two isoforms of the cyclooxygenase have been identified; a constitutive form (COX-1) and an inducible form (COX-2), for which expression is associated with inflammation and pain[1]. Because most of the NSAIDs displayed little selectivity towards these isoforms, it became evident that isoform-specific inhibitors may have improved therapeutic value over the NSAIDs by reducing the gastrointestinal toxicities[2,3]. In Phase II trials, Searle's novel anti-inflammatory, celecoxib (SC58635; **1**; Figure 1) relieved RA and OA symptoms without the gastrointestinal side effects associated with traditional NSAIDs. Celecoxib, a highly selective COX-2 inhibitor, has no effect on platelet function. Other companies developing COX-2 inhibitors include Merck, which has MK966 in Phase IIb/III trials and Boehringer-Ingelheim, which is introducing meloxicam worldwide. Roche and Glaxo Wellcome are also active in this area. Tenidap (Pfizer; **2**; Figure 1) is a novel (non-selective) cyclooxygenase inhibitor of the oxindole class that has shown activity in RA patients in randomized multi-centered Phase II and III clinical trials[4]. The drug inhibits cyclooxygenases and the production of cytokines such as IL-1, IL-6 and

Table 1. Small molecular weight compounds in clinical development for rheumatoid arthritis and osteoarthritis

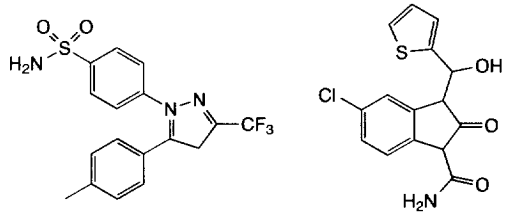
Company	Compound	Mechanism	Clinical phase
Searle	Celexocib	COX-2 inhibitor	Phase II
Merck	MK966	COX-2 inhibitor	Phase IIb/III
Pfizer	Tenidap	CO and cytokine inhibitor	Phase II/III for OA
Celgene	Thalidomide	Inhibits TNF	Phase II
Hoechst Marion Roussel	Leflunomide	Pyrimidine synthesis inhibitor	Phase III
Takeda	TAK603	Immunomodulator	Phase I/II
ISIS/Boehringer Ingelheim	ISIS2302	Inhibitor of ICAM-1 synthesis	Phase II
Novartis	Neoral	Cyclosporin A microemulsion	Phase IV
AnorMED	Atiprimod	Macrophage-targeting agent	Phase I/II
Roche	Ro31-9790	MMP inhibitor	Phase I
Chiroscience	D5410	MMP inhibitor	Phase I

Abbreviations: COX-2, cyclooxygenase-2; ICAM-1, intercellular adhesion molecule-1; MMP, matrix metalloprotease; OA, osteoarthritis; TNF, tumor necrosis factor.

Table 2. Protein agents in development for rheumatoid arthritis and osteoarthritis

Company	Compound	Mechanism	Clinical phase
Centocor	CenTNF (CA2)	Mab to TNF	Phase II/III
Bayer	CDP571	Mab to TNF	Phase II/III
Immunex/American Home Products	Enbrel	TNFR (p75)–IgG fusion protein	Phase III
Genentech/Hoffman-LaRoche	Lenercept (Ro45-2081)	TNFR (p55)–IgG fusion protein	Phase II
Amgen	Antril	IL-1 receptor antagonist	Phase II/III
SmithKline Beecham	Keliximab	Mab to CD4	Phase III
Johnson & Johnson	Orthoclone	OKT4A Mab	Phase II
Glaxo Wellcome	IgG4 anti-CD4	Mab to CD4	Phase II

Abbreviations: IL-1, interleukin-1; Mab, monoclonal antibody; TNFR, tumor necrosis factor receptor.



1, celecoxib (SC58635) 2, tenidap

Figure 1. *Nonsteroidal anti-inflammatory drugs.*

TNF-α (Ref. 4), although the effects on TNF-α are disputed. One side effect, proteinuria, was observed to be associated with doses that show efficacy in patients with RA. This adverse effect proved to be unacceptable and, together with a decrease in bone mineral density in treated patients, caused the withdrawal of tenidap for the treatment of this disease. However, lower doses may be of benefit in OA, and the compound is still in clinical development for this indication. Tenidap was shown to reduce markedly the severity of OA lesions in a canine experimental model[5].

Inhibition of nitric oxide synthase

Another new approach addressing inflammatory mediators of chronic inflammation is the inhibition of nitric oxide production. Nitric oxide is synthesized via an enzymatic oxidation of arginine by the nitric oxide synthases. It has long been thought that nitric oxide produced by inflammatory cells may contribute to tissue damage and the role of nitric oxide in a number of experimental inflammatory models has been described[6]. Among the isoforms of the enzyme, the inducible form of nitric oxide synthase (iNOS) is regulated primarily at the level of gene transcription. Arginine analogs represent the largest class of compounds used as NOS inhibitors, and they have been shown to be protective in inflammatory disease models[6]. Nitric oxide generation in various systems differs on the basis of the NOS isoform involved. Therefore, it is important to identify specific inhibitors, some of which have clear isoform selectivity[7,8]. No clear clinical candidate has emerged from the Phase I trials.

Cytokine inhibition

Several cytokines are thought to be involved in the inflammation and cartilage/bone damage that is characteristic of RA, and regulating their production and/or effects has been a major target for intervention in the disease. Of the many cytokines involved in RA, IL-1 and TNF-α have been the primary therapeutic targets, and a number of agents have been discovered that inhibit their synthesis, receptor interactions, processing or effects on cell signaling processes.

Antagonism of cytokine effects

A primary cytokine target for new drug development is TNF-α (Refs 9,10). The approaches taken to interfere with TNF-α activity include the use of antibodies directed at the cytokine itself as well as using the TNF receptor fused to IgG. The most promising agents currently available are Centocor's CA2 and ENBREL™ from Immunex/American Home Products. Both reduce inflammation, decrease swollen and tender joints and maintain efficacy for prolonged periods. CA2 (CenTNF) is a chimeric monoclonal antibody (Mab) against TNF-α which is under development for RA, inflammatory bowel disease (IBD) and Crohn's disease. Phase I studies are completed and Phase II trials are currrently under way[11]. Treatment is well tolerated with no significant adverse effects, although antibodies against the Mab were observed in some patients. Another antibody that neutralizes human TNF-α is CDP571, which is being developed by Celltech and Bayer. This is an engineered human anti-TNF-α Mab that has been shown to produce some improvements in disease activity in patients with active RA (Ref. 12).

ENBREL™ is a TNF receptor (p75)–IgG molecule for which Phase I and II clinical trials have been completed. Immunex has developed a hybrid protein consisting of two TNF-binding domains derived from the p75 TNF-α receptor fused with the Fc fragment of human immunoglobulin. This molecule binds both membrane-bound and soluble TNF-α as well as soluble TNF-β. A statistically significant reduction in RA symptoms has been observed in placebo-controlled clinical trials. Patient enrolment for Phase III is completed, and data should be available in late 1997. Genentech and Roche are jointly developing a TNF-α receptor (p55)–IgG1 fusion molecule called lenercept (Ro45-2081), which is currently undergoing clinical trials for sepsis, multiple sclerosis and rheumatoid arthritis. It is in Phase III trials for septic shock and in Phase II for multiple sclerosis and RA.

Another approach to the inhibition of cytokine effects is to interfere with receptor binding, and to this end Amgen is developing an IL-1 receptor antagonist (IL-1ra), Antril™. A Phase I study has shown the compound to be well tolerated and to have some efficacy in RA patients[13]. Amgen has recently completed a Phase II clinical trial with Antril in RA patients, the results of which were encouraging, with

modest improvement seen in the American College of Rheumatology (ACR) composite score. X-ray studies also showed significantly fewer eroded joints versus placebo. Of interest is a recent study in experimental OA where intra-articular injections of IL-1ra were shown to be chondroprotective[14]. The most common side effects were injection site reactions, and these may affect patient compliance considering the long-term dosing required for such a chronic disease; this compound is administered subcutaneously on a daily basis. As with other protein agents discussed above, long-term effects such as anti-idiotypic antibody responses to the protein agent and the potential increase in cancers (lymphomas) or autoimmune conditions (lupus-like syndrome) must also be addressed.

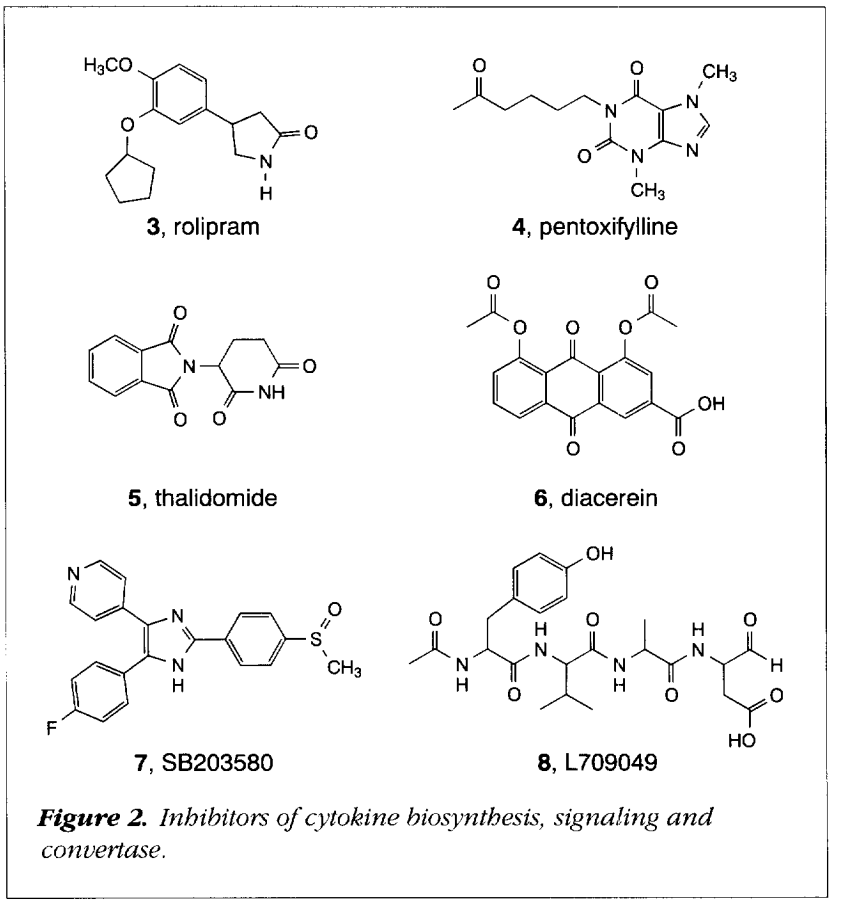


Figure 2. *Inhibitors of cytokine biosynthesis, signaling and convertase.*

Cytokine biosynthesis inhibitors

Inhibitors of cyclic nucleotide phosphodiesterases (PDEs) suppress lipopolysaccharide (LPS)-induced TNF-α production in monocytes/macrophages through the activation of adenylate cyclase[15–17]. This indicates that increased intracellular cyclic adenosine 3′,5′-monophosphate (cAMP) is a suppressive signal for TNF-α that could be exploited as a potential target. PDE 4 has been shown to be the predominant cAMP-metabolizing enzyme in immune and inflammatory cells[18]. Rolipram (**3**; Figure 2) is a PDE 4 inhibitor that suppresses TNF-α production in human monocytes[19] and is 500-fold more potent than pentoxifylline (**4**; Figure 2), a nonspecific PDE inhibitor. Rolipram and other PDE 4 inhibitors such as CP77059 (Pfizer) have been shown to have beneficial effects in the adjuvant arthritic (AA) rat[20], and Chiroscience is investigating orally active PDE 4 inhibitors as potential asthma and anti-inflammatory treatments including RA.

Another compound that inhibits TNF-α synthesis is thalidomide [Synovir (Celgene); **5**; Figure 2]. This compound, introduced in the 1950s as a sedative and withdrawn following discovery of its teratogenic effects, is being re-evaluated in a range of indications including inflammatory skin disorders and autoimmune disease[21,22]. The compound is thought to inhibit TNF-α production by enhancing degradation of TNF-α messenger RNA[23]. Celgene has synthesized a series of thalidomide analogs that may have potential in autoimmune diseases such as RA, diabetes and IBD, although the evidence for efficacy in animal models is controversial[22]. In an early open trial in RA, pain and joint inflammation disappeared after several weeks of treatment with thalidomide[24]. If the side effects of teratogenicity and peripheral neuropathy are lacking in a thalidomide analog, then the inhibitory effects on TNF-α synthesis may provide the rationale for investigating the potential efficacy of thalidomide or an analog in RA.

Diacerein (**6**; Figure 2) is already being used as an antiarthritic and has attracted attention recently because it may inhibit the production of IL-1 and TNF-α. Another proposed mechanism is that diacerein may halt the progression of arthritis by blocking the release of chondrocytic lysosomal enzymes that break down cartilage. In preclinical studies diacerein has shown some beneficial effects in a dog model of OA (Ref. 25).

Inhibitors of pro-inflammatory cytokine signaling

Pro-inflammatory cytokines, such as IL-1, act through specific high-affinity receptors to induce gene expression. However, the signaling mechanisms involved have not been totally elucidated. Recently, many of the molecular

events leading to gene activation have been attributed to protein phosphorylation. At least three different types of mitogen-activated protein (MAP) kinases and β-casein kinase have been implicated in IL-1 signaling. Bacterial endotoxins – lipopolysaccharides (LPS) – employ a similar biochemical signaling cascade leading to activation of protein kinases. The first definitive report suggesting that LPS signaling involves protein phosphorylation, by Han and coworkers, identified a novel member of the MAP kinase family, p38 kinase[26]. Another member of the same protein kinase family, c-Jun N-terminus protein kinase (JNK), has also been implicated in stress signaling in mammalian cells[27–30]. Subsequently, it has been observed that IL-1 and TNF are particularly potent activators of both protein kinases[31–33]. Thus, both LPS and IL-1/TNF can trigger signaling responses involving p38 and JNK MAP kinases (Figure 3).

An inhibitor that interferes with such signaling pathways will have the benefit of inhibiting both pro-inflammatory cytokine production and cytokine action. SmithKline Beecham's SB203580 (**7**; Figure 2), a pyrimidyl imidazole compound originally described as an inhibitor of cytokine biosynthesis[34], binds to and selectively inhibits p38 MAP kinase (IC_{50} = 0.08 μM). The compound has no effect on JNK (the other member of the MAP kinase family activated by cellular stress) or Erk (the classical mitogen-activated kinase) and their respective activating kinases. It inhibits p38 MAP kinase activity, but not its activation, and prevents activation of a known physiological substrate, MAPKAP kinase 2. In mammalian cells this results in inhibition of the subsequent phosphorylation of heat-shock protein (HSP) 27, which can be activated by chemicals, osmotic stress or IL-1 (Refs 35,36). SB203580 possesses therapeutic activity in collagen-induced arthritis in DBA/LACJ mice, with a dose of 50 mg/kg resulting in significant inhibition of paw inflammation and serum amyloid protein (SAP) levels. Antiarthritic activity is observed in adjuvant-induced arthritis in the Lewis rat, and evidence for disease-modifying activity in this model was indicated by an improvement in bone mineral density (BMD) and by histological evaluation. Additional evidence for beneficial effects on bone resorption was provided in the fetal rat long bone assay. SB203580 was found to reduce mortality in a murine model of endotoxin-induced shock. This novel profile of activity strongly suggests that cytokine inhibitors could provide significant benefit in the therapy of chronic inflammatory disease[37].

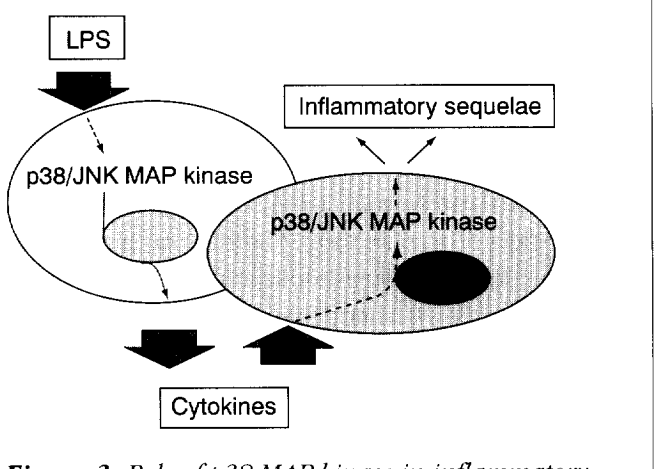


Figure 3. *Role of p38 MAP kinase in inflammatory cytokine signaling.*

Cytokine convertase inhibitors

IL-1 is synthesized as an inactive, secreted precursor molecule and requires proteolytic cleavage to yield the mature active form[38]. The enzyme responsible for proteolytic cleavage of IL-1β has been identified as a unique cysteine proteinase, IL-1 converting enzyme (ICE)[39,40]. Potent inhibitors of ICE have been identified, such as Merck's L709049 (**8**; Figure 2; K_i = 0.76 nM), which inhibits mature IL-1β release in human whole blood (IC_{50} = 4 μM)[40]. Sterling Winthrop's WIN67094 and a compound being developed by Vertex (VE13045) have been shown to decrease effectively IL-1β production *in vivo*. Such prototypic inhibitors provide impetus to the design of more potent and selective agents useful in inhibiting IL-1 production.

TNF-α is synthesized as an integral membrane protein and, as is the case with IL-1, requires proteolytic cleavage to yield an active protein. It has now been shown that the TNF-α converting enzyme (TACE) is a member of the adamalysin family of matrix metalloproteinases (MMPs) with a unique Zn^{2+} binding domain[41]. In addition, the hydroxamic acid-based pseudopeptide MMP inhibitors have been reported to inhibit endotoxin-induced production of TNF-α by inhibition of a TNF-α processing enzyme[42–44]. Several companies, including British Biotech, Glaxo Wellcome and Immunex, have reported on MMP inhibitors with an ability to decrease TNF both *in vitro* and *in vivo*, but these compounds are not selective TACE inhibitors, and many other 'shed' proteins, including the TNF receptors, can be affected.

Metalloproteinase inhibitors

The zinc-dependent MMPs described above participate in the progressive breakdown of the connective tissues of the articular joint, as well as TNF-α production. Inhibition of MMPs as therapeutic intervention in different diseases is being investigated by many pharmaceutical companies[45]. British Biotech is developing MMP inhibitors for a range of indications, including RA and cancer. BB2516 (marimastat) is in Phase III clinical trials for cancer, but the development of MMP inhibitors with good oral bioavailability for RA is proving to be more difficult.

These compounds, primarily of the hydroxamate class, are also nonspecific inhibitors of TNF-α release and inhibit shedding of TNF-α receptor proteins (p55 and p75), which adds weight to the rationale for their potential use in RA. British Biotech is developing one such compound with Glaxo Wellcome (BB2983) and Phase I studies have been initiated. Roche is examining a series of hydroxamic acids for therapeutic intervention in RA, and one of these, Ro31-9790 (**9**; Figure 4), has been evaluated in a Phase I clinical trial. Compounds in clinical development are Ro32-3555 (**10**; Figure 4), a cartilage protective agent with selectivity for collagenase over gelatinase and stromelysin, and RS113456, an MMP inhibitor with selectivity for collagenases -2 and -3 over stromelysin. RS113456 is in preclinical development and has oral activity in mouse and rabbit models of arthritis.

The anti-inflammatory MMP inhibitor D5410 (Chiroscience) is expected to enter Phase II studies for RA in late 1997. This compound has been shown to inhibit TNF convertase as well as MMPs. It is orally available and active in animal models of arthritis. Novartis has a lead MMP inhibitor, CGS27023A (**11**; Figure 4), which is a potent collagenase inhibitor but does not inhibit TNF convertase. It has been shown to have beneficial effects in guinea pig and rabbit models of OA (Ref. 46), but has yet to enter the clinic for either RA or OA.

Tetracycline and its derivatives minocycline and doxycycline (**12**; Figure 4) are weak MMP inhibitors independent of their antimicrobial activity. They have been evaluated in periodontal disease and RA. Minocycline was shown to have some benefit in an open study in RA (Ref. 47), whereas, in an earlier study, tetracycline was inactive[48].

Elastase inhibitors

Cortech is developing a series of orally active elastase inhibitors for the treatment of chronic inflammatory diseases including RA, obstructive pulmonary disease and IBD. Preclinical studies in RA are ongoing in the USA and Japan.

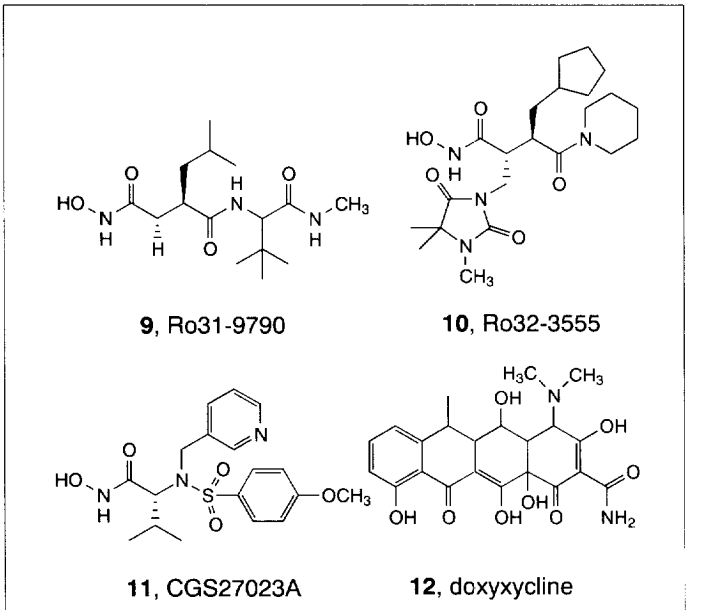


Figure 4. *Metalloproteinase inhibitors.*

Immunosuppressive and immunomodulatory approaches

Historically, most of the immunosuppressive/immunomodulatory agents used in the treatment of RA have displayed unacceptable side effects. Recent efforts have addressed the use of combinations of effective compounds at non-toxic doses, in the hopes of achieving efficacy with fewer side effects. In addition, there are now more selective approaches under investigation, including the induction of tolerance and the ablation (or modulation) of specific cell subsets known to participate in the disease process.

Induction of tolerance to specific antigen

Numerous investigators have shown that systemic hyporesponsiveness can be induced to a specific antigen by oral administration of that antigen. The mechanisms by which orally administered antigens induce tolerance involve active suppression or clonal anergy; this concept has been tested extensively in experimental models of autoimmune disease[49]. Extending the findings in experimental animals to human disease has proven more difficult than hoped. In a placebo-controlled trial using bovine type II collagen, a minority of patients responded to treatment[50]. Orally administered type II chicken collagen has been evaluated in

patients with juvenile RA (Ref. 51) and Colloral™, a liquid form of type II collagen is being evaluated by AutoImmune with modest success. However, some recent studies in animal models have shown that this strategy may not always have beneficial effects and that unexpected exacerbation of disease can occur following oral administration of the disease-causing antigen[52,53].

Ablation of T-cell subsets/inhibition of function

Anti-CD4. The concept of selective T-cell immunosuppression in transplantation therapy was initiated shortly after the identification of the CD4 and CD8 subsets of lymphocytes in the late 1970s[54]. More recently, the concept has been applied to the treatment of RA. Studies with depleting anti-CD4 monoclonal antibody therapy in RA yielded disappointing efficacy results and concerns regarding universal and sustained $CD4^+$ T-cell lymphocytopenia[55,56]. Recently, SmithKline Beecham's SB210396 (IDEC CE9.1), a PRIMATIZED® anti-CD4 monoclonal antibody has, for the first time, proven the concept of this approach in RA in a double-blind, placebo-controlled trial. In a Phase II trial, significant improvements in signs and symptoms of RA (measured using the ACR 20 criterion) have been observed following intravenous administration of the antibody. Administration has not been associated with serious infusion-related adverse events[57]. More than half of the patients who responded to an induction regimen sustained the clinical benefit for more than three months. Johnson & Johnson/Ortho and Glaxo Wellcome also have anti-CD4 monoclonal antibodies in clinical trials.

Antibodies to the aβ T-cell receptor. Antibodies directed against T cells have been used extensively to analyze the mechanism of T-cell activation in humans and mice. Mabs with specificity for the αβ T-cell receptor have been proposed as potential therapeutic agents for several diseases, and there are reports of activity in animal models of arthritis[58] and autoimmune diabetes[59]. BMA031, a murine/human chimeric antibody[60], has been evaluated in organ transplantation and graft-versus-host disease but not in RA.

Anti-adhesion molecule therapy

ISIS2302 is a novel anti-inflammatory agent that acts by inhibiting the synthesis of intercellular adhesion molecule-1 (ICAM-1) through its ability to block specifically its mRNA expression. ISIS2302 is a first-generation antisense oligonucleotide under joint development by ISIS Pharmaceuticals and Boehringer Ingelheim for use in rheumatoid arthritis, psoriasis, kidney transplant rejection, ulcerative colitis and Crohn's disease. ISIS2302 has so far demonstrated none of the toxicities associated with traditional DMARDs or immunosuppressive drugs such as cyclosporin A, and in a Phase I study was well tolerated. Phase II trials with the compound have begun for rheumatoid arthritis as well as other indications.

Celltech/Boehringer Ingelheim are developing humanized chimeric anti-ICAM-1 antibodies for use in autoimmune diseases and it has been demonstrated that interference with adhesion using such antibodies is effective in animal models of arthritis[61,62]. Clinical studies with anti-ICAM-1 have shown the molecule to have beneficial effects in approximately 60% of the patients, with some responding for up to 11 months[63].

T-cell receptor peptides as vaccines

Immune Response Corporation is developing an immune-based therapy consisting of a combination of three T-cell receptor peptides in adjuvant. The premise is that treatment will selectively inhibit clonal expansion of cells that may be responsible for triggering inflammatory reactions in the rheumatoid joint. Positive results with this peptide combination (IR501) have been described following intramuscular injection in RA patients and were described as statistically significant. AnervaX™ (Anergen) is an immunosuppressant major-histocompatibility-complex peptide vaccine that includes the DR4/1 motif associated with increased risk for RA and is designed to elicit an immune response that will block presentation of self-antigens to T cells. AnervaX is currently in Phase IIa trials in RA patients in the USA.

Antiproliferative compounds

Leflunomide. Leflunomide (HWA486; **13**; Figure 5) is an isoxazole derivative being developed by Hoechst Marion Roussel (HMR). It is a heterocyclic oral prodrug of the active compound A771726 (**14**; Figure 5). The mechanism of action of leflunomide is to interrupt *de novo* pyrimidine nucleotide biosynthesis by inhibition of dihydro-orotate dehydrogenase, thereby disrupting DNA synthesis in immune cells[64,65]. It is active in several animal models of autoimmune disease including adjuvant arthritis, lupus erythematosus and autoimmune uveitis[66] and also prolongs experimental graft survival. Leflunomide is undergoing Phase III trials in Germany and the USA and Phase II trials in Japan for rheumatic disease.

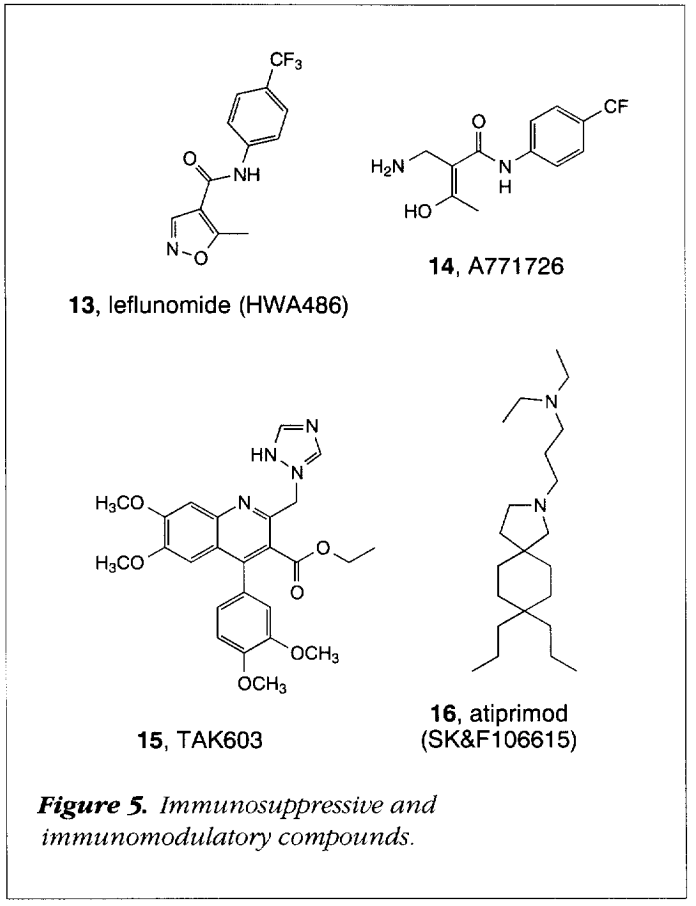


Figure 5. *Immunosuppressive and immunomodulatory compounds.*

Clinical responses to leflunomide include significant improvements, compared with placebo, in swollen-joint counts and the physician's, patient's and global assessment of disease activity. At doses of 10 mg and 25 mg per day, the drug also improved erythrocyte sedimentation rate, C-reactive peptide levels, the number of rheumatoid factor-positive patients, the pain assessment and results of the Health Assessment Questionnaire[67]. Adverse events associated with leflunomide were diarrhea, abdominal pain, maculopapular rash, weight loss, transient alopecia and reduced white blood cell count. HMR expects to apply for marketing approval in Europe for the treatment of RA in 1997.

Inhibition of NF-κB. The discovery that the mechanism of the immunosuppressive action of the glucocorticoids was inhibition of NF-κB (Refs 68,69) stimulated the search for inhibitors that would not have the undesirable features of classical steroids. Inhibition of the translocation of active NF-κB to the nucleus or inhibition of its binding to DNA constitute more selective approaches than the overall suppression of the immune response that results from steroid treatment. Therapies directed at NF-κB are being considered by Inflazyme and Signal Pharmaceuticals.

Cyclosporin A. Several studies have evaluated the use of cyclosporin A in patients with RA with some efficacy noted. Novartis has applied to have Neoral® (cyclosporin A for microemulsion) approved for the treatment of severe RA in patients in whom the slow-acting, second-line drugs have either failed or were not tolerated. Neoral® is currently indicated as an immunosuppressive agent to prevent organ rejection in kidney, liver and heart transplants.

TAK603. TAK603 (**15**; Figure 5) is an immunomodulatory agent under development by Takeda for the treatment of RA and OA. The compound suppressed the development of synovial lesions and joint destruction (bone and cartilage) in the AA rat. *In vitro*, TAK603 was shown to suppress mitogen-induced proliferation of mouse lymphocytes and Con A-induced interferon-γ and IL-2 production. In addition, the compound was shown to inhibit IL-1 induced matrix reduction in rabbit chondrocytes[70]. It is in Phase I trials in the USA and Phase II in Japan for RA.

Atiprimod (SK&F106615). Atiprimod (**16**; Figure 5) is a macrophage-targeting agent belonging to the azaspirane class of compounds; efficacy has been shown in animal models of autoimmune disease and transplantation[71]. The compound is being developed by AnorMED. It is particularly effective in the AA rat model where inhibition of inflammation as well as protection of cartilage and bone integrity in the joint is observed[72]. Phase I clinical studies have been completed in RA patients with no adverse side effects observed. The beneficial effects of atiprimod appear to be mediated by its effect(s) on monocyte/macrophages, where it intercalates into lipid membranes, accumulates in lysosomes, alters lysosomal pH and, as a consequence, modifies the activity of these cells. The compound inhibits phorbol myristate acetate (PMA)-stimulated superoxide release and protein kinase C activity in U937 cells, as well as inhibiting the activities of phospholipases A_2 and C (but not D) in a noncompetitive manner *in vitro* (unpublished observations).

Unlike some of the compounds already being used as DMARDs, atiprimod is minimally suppressive and has no effect on antibody synthesis in rats and mice and only mild effects on cellular responses to mitogen and antigen[73].

In addition, host defenses against infection are not compromized by the azaspiranes[74].

Future strategies

The next generation of antiarthritic compounds will be orally bioavailable, more potent, more selective and less toxic than current agents. It is conceivable that combinations of the different therapeutic approaches will be used to combat this disease. The current explosion of genomic technology will facilitate the discovery of new, rational targets for drug design. In addition, there is the potential for gene therapy and this approach is already being investigated at the University of Pittsburgh[75]. The purpose of this gene therapy

30 Sanchez, I. *et al.* (1994) *Nature* 372, 794–798
31 Derijard, B. *et al.* (1994) *Cell* 76, 1025–1037
32 Kyriakis, J.M. *et al.* (1994) *Nature* 369, 156–160
33 Sluss, H.K. *et al.* (1994) *Mol. Cell. Biol.* 14, 8376–8384
34 Lee, J.C. *et al.* (1994) *Nature* 372, 739–746
35 Cuenda, A. *et al.* (1995) *FEBS Lett.* 364, 229–233
36 Rouse, J. *et al.* (1994) *Cell* 78, 1027–1037
37 Badger, A.M. *et al.* (1996) *J. Pharmacol. Exp. Ther.* 279 (3), 1453–1461
38 Dower, S.K. *et al.* (1992) *Chem. Immunol.* 51, 33–64
39 Cerretti, D.P. *et al.* (1992) *Science* 256, 97–100
40 Thornberry, N.A. *et al.* (1992) *Nature* 356, 768–774
41 Solozano, C.C. *et al.* (1997) *J. Immunol.* 158, 414–419
42 Mohler, K.M. *et al.* (1994) *Nature* 370, 218–220
43 Gearing, A.J. *et al.* (1994) *Nature* 370, 555–557
44 McGeehan, G.M. *et al.* (1994) *Nature* 370, 558–561