Emerging biologic therapies in rheumatoid arthritis: cell targets and cytokines
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Purpose of review
Biologic therapy for rheumatoid arthritis targets specific molecules, both cell-bound and soluble, that mediate and sustain the clinical manifestations of this complex disease. The aim of all the therapeutic strategies is to achieve complete and sustained suppression of inflammation, in the absence of unacceptable short-term and long-term toxicity. Despite the success of the currently available biologic inhibitors of tumor necrosis factor-α and interleukin-1, a substantial number of rheumatoid arthritis patients are refractory to these treatments. The purpose of this review is to highlight recent clinical trials of emerging biologic treatments for rheumatoid arthritis.

Recent findings
T cell co-stimulation has been targeted by the use of cytotoxic T lymphocyte–associated antigen 4-Ig, a genetically engineered fusion protein. In a large controlled clinical trial, this nondepleting approach was shown to achieve impressive clinical responses, without evidence of short-term toxicity. Likewise, rituximab, a B cell–deleting monoclonal antibody, was shown in a controlled clinical trial to have sustained benefit in patients with refractory rheumatoid arthritis. Despite profound B cell depletion with rituximab, there was an acceptable safety profile with this treatment. MRA, a monoclonal antibody that inhibits interleukin-6 by binding to its receptor interleukin-6R, demonstrated clinically significant improvement in rheumatoid arthritis and a particularly impressive reduction in the acute phase response.

Summary
The response of rheumatoid arthritis to a wide spectrum of therapeutic strategies attests to the complexity and heterogeneity of the disease and provides further impetus for studies that use these therapies to enhance our understanding of disease pathogenesis.

Keywords
B cells, biologic agents, cytokines, rheumatoid arthritis, T cells

Abbreviations
APC antigen-presenting cells
CTLA4 cytotoxic T lymphocyte–associated antigen 4
DMARD disease-modifying antirheumatic drug
IL interleukin
NSAID nonsteroidal anti-inflammatory drug
RA rheumatoid arthritis
RF rheumatoid factors
TNF-α tumor necrosis factor-α

Introduction
The pathogenesis of rheumatoid arthritis (RA) remains incompletely understood. It involves complex interactions between T and B lymphocytes, macrophages, and fibroblast-like synoviocytes, involving a network of cytokines acting in an autocrine and paracrine manner [1]. In recent years, the development of biologic agents that target specific soluble or membrane-bound molecules has revolutionized the treatment of RA. The success of tumor necrosis factor-α (TNF-α) inhibition, and to a lesser extent interleukin (IL)-1 inhibition, has firmly established these therapies in the clinical management of RA. Moreover, this approach has allowed unprecedented opportunities for developing a better understanding of the pathogenesis of this complex and heterogeneous disease. Despite the success particularly of the TNF-α inhibitors, data from both clinical trials and real-life clinical experience have clearly suggested that a substantial proportion of RA patients either do not respond, or lose their initial responses, to these agents [2*]. Thus, there continues to be a compelling need for the development of new therapeutic strategies. This review highlights recent research activity in the clinical development of novel biologic therapies for RA, focusing on therapies that target specific immune cells, and therapies that target cytokines.

Cell-targeted therapies
There has been a long-standing interest in manipulating cells of the immune system to achieve control of RA. Because of the prominence of T lymphocytes in rheumatoid synovitis, early attempts focused on the depletion of this cell population in the hope of ameliorating the disease. Clinical experience with T cell–depleting agents such as the CAMPATH-1H antibody was disappointing and was associated with long-lasting lymphopenia, although not an excess of morbidity or mortality after prolonged follow-up [3]. Interestingly, the T cells persisted in the synovial
membrane despite profound peripheral lymphopenia [4]. A host of other T cell–depleting strategies were associated with either unacceptable toxicity or modest efficacy or both. More recently, interest has focused on modulating T cell function rather than depleting large number of T cells or subsets of T cells. The important role of co-stimulation in the activation of T cells is now well understood, and this process has been targeted therapeutically with the cytotoxic T lymphocyte-associated antigen 4-Ig (CTLA4Ig) fusion protein, which interferes with co-stimulation without depletion of T cells.

In contrast to T cells, B cells had largely been ignored in RA pathogenesis until recently. After a period of prolonged indifference, the potential therapeutic utility of manipulating B cells in RA has been explored in recent years. These cells are well known to be responsible for producing rheumatoid factors (RF) and other RA-associated autoantibodies such as anti-cyclical citrullinated peptide. Importantly, B cells, which are abundant in the synovium of most patients with well-established RA, also act as highly efficient antigen-presenting cells (APC) to T cells and thus may play an important role in synovial T cell activation [5]. It has thus been postulated that depletion of B cells or modulation of their function may be associated with clinical benefit in RA.

Biologic therapies targeting T cells and B cells have been developed over the past 5 years and have been evaluated in well-designed clinical trials in patients with RA.

**Inhibition of T cell co-stimulation: abatacept**

T cells require two signals from APC for complete activation [6]. The first signal is antigen specific and occurs between a T cell receptor and the major histocompatibility complex—peptide complex on the APC. A second co-stimulatory signal occurs between CD28 molecules on T cells and CD80 or CD86 molecules on APCs. These two signals cause T cell proliferation and cytokine production, which in turn activate other inflammatory cells. If the second co-stimulatory signal is missing, the T cells may be poorly responsive to stimuli, and apoptosis may occur.

Cytotoxic T lymphocyte–associated antigen 4 (CTLA4) is an immunoregulatory protein expressed on the T cell surface after activation. It binds to CD80 or CD86, blocks their interaction with CD28, and thus acts as an off-switch for cell activation. CTLA4Ig is a genetically engineered fusion protein that consists of a human CTLA4 portion fused to a constant IgG1 region. This molecule binds to CD80 and CD86 and thereby inhibits T cell co-stimulation. On the basis of the central role of T cell activation in the pathogenesis of RA, it was hypothesized that inhibition of this process using CTLA4Ig would achieve clinically meaningful improvement in RA [7].

An initial 3-month, dose-finding pilot study of CTLA4Ig therapy in RA had demonstrated that at a 10 mg/kg dosage given every 2 weeks, 53% of patients had an ACR 20 response, whereas 16% had an ACR 50 response after 85 days of therapy [8]. The treatment was well tolerated, with no evidence of major toxicity. This study led to a larger clinical trial that was published in late 2003. In this publication, Kremer et al. [9] reported the results of a 6-month randomized, double-blinded, placebo-controlled trial studying the efficacy of CTLA4Ig in RA. All 339 patients in the study had active RA despite taking methotrexate (10–30 mg weekly). Two hundred fifty-nine patients completed 6 months of treatment. Methotrexate was continued in all patients, but all other disease-modifying antirheumatic drugs (DMARDs) were discontinued. Stable low-dose corticosteroids (prednisolone ≤10 mg/d) and nonsteroidal anti-inflammatory drugs (NSAIDs) were allowed. The patients were randomly assigned to three arms: placebo with methotrexate, CTLA4Ig (2 mg/kg) with methotrexate, and CTLA4Ig (10 mg/kg) with methotrexate. CTLA4Ig or placebo was infused at days 1, 15, and 30 and thereafter monthly for 6 months. ACR 20, 50, and 70 were measured at 6 months to assess clinical response. Responses to the Short Form Health Survey-36 were also assessed at baseline, 90 days, and 180 days. ACR 20 responses in the CTLA4Ig 10 mg/kg group were improved from months 2 to 6. There was no statistically significant difference in ACR 20 response between the placebo group and the CTLA4Ig 2 mg/kg group at 6 months ($P = 0.31$). ACR 50 and ACR 70 responses were higher at 6 months in both CTLA4Ig groups than in the placebo group. There was also significant improvement in all Short Form Health Survey-36 subscales in the CTLA4Ig 10 mg/kg group ($P < 0.05$), but no statistically significant difference between the CTLA4Ig 2 mg/kg and placebo groups. The drug was well tolerated. No deaths, malignancies, or opportunistic infections were reported. The most commonly reported adverse symptoms included headache, upper respiratory tract infection, musculoskeletal pain, nausea, and vomiting. The rate of serious side effects was actually lower in the CTLA4Ig 10 mg/kg group than in the other two groups. These data suggested that the 10 mg/kg dose has a favorable benefit-to-toxicity ratio and is the most suitable for clinical use.

As a follow-up to this study, Kremer et al. [10] and Dugados et al. [11] published data from a 1-year open-label extension of this study in abstract form. In this study, ACR 20, 50, and 70 values and DAS-28 suggested that efficacy was maintained at 2 years. Moreland et al. [12] presented data indicating similar rates of serious adverse effects when the CTLA4Ig 10 mg/kg (plus methotrexate) and the control (methotrexate alone) groups were compared. Together, these data suggest that abatacept (Bristol-Myers Squibb, Princeton, NJ, USA) combined
with methotrexate demonstrates sustained ACR response at 2-year follow-up and is well tolerated.

This promising strategy is now being evaluated in large phase III trials. It remains unclear whether CTLA4Ig therapy in RA should be considered for patients who are refractory to TNF-α inhibitors, or whether there is a subset of patients who are particularly well suited for treatment with this approach.

**B cell depletion: rituximab**

The CD20 antigen is present on the cell surface of all preplasma cell stages of B cell differentiation, although the role of this molecule remains unclear. The mature plasma cell loses the CD20 antigen, and thus it serves as a relatively specific marker for B cells [13]. Rituximab (Roche Pharmaceuticals, Basel, Switzerland; Genentech, South San Francisco, USA; IDEC Pharmaceuticals, San Diego, USA), a genetically engineered human-mouse chimeric monoclonal antibody against the CD20 antigen, has been used successfully in the treatment of B cell malignancies like non-Hodgkin lymphoma, chronic lymphocytic leukemia, and others. Rituximab binds to the CD20 antigen on the B cell surface and efficiently depletes B cells by antibody-dependent and complement-dependent cell lysis [13,14].

In an initial report, Edwards and Cambridge [15] described an open-label study of rituximab in combination with cyclophosphamide and prednisolone in five patients with refractory RA. These patients all demonstrated dramatic and sustained clinical improvement, with two of the patients continuing to show ACR 70 responses at 1 year. In these patients, RF had become undetectable. Despite profound B cell depletion, no significant toxicity was seen. This group published an expanded series of 22 patients that suggested the need for a dosage of at least 600 mg/m² to achieve clinical benefit [16]. A subsequent analysis of the serologic effects of this treatment suggested that IgA-RF, IgG-RF, and anticyclic citrullinated peptide antibodies decreased out of proportion to a decrease in total serum immunoglobulins, and in antibodies to specific pathogens [17]. Moreover, the decrease in autoantibodies paralleled a decrease in C-reactive protein, and the disease relapsed when autoantibody levels increased again, although the return of B cells was unpredictable.

These data led to a controlled phase II trial, the results of which were published in 2004 [18**]. In this multicenter, double-blind, controlled study by Edwards et al. [18*], 161 patients with methotrexate-refractory RA were randomized into four treatment groups: continuing oral methotrexate (≥10 mg/wk), rituximab (1000 mg on days 1 and 15), rituximab and cyclophosphamide (750 mg on days 3 and 17); and rituximab and methotrexate. No other DMARD was allowed during the trial. Stable doses of NSAIDs and corticosteroids (prednisolone ≥12.5 mg/d or equivalent) were allowed. The primary endpoint of the study was the ACR 50 response at week 24. The ACR 50 response for rituximab combination therapy with either methotrexate or cyclophosphamide was significantly higher than the control methotrexate group. ACR 50 responses for methotrexate, rituximab, rituximab plus methotrexate, and rituximab plus cyclophosphamide were 13%, 33%, 41%, and 43%, respectively. ACR 50 response differences between methotrexate and rituximab monotherapy did not reach statistical significance (P = 0.059) but did trend towards increased values. Interestingly, the ACR 20, ACR 50, and ACR 70 responses even at week 48 for the rituximab plus methotrexate group were 65%, 35%, and 15% respectively (P ≤ 0.001, P = 0.002, P = 0.03, respectively). This demonstrates a sustained clinical response after just two doses of rituximab.

Rituximab was associated with almost complete depletion of peripheral B cells. The greatest decline in peripheral B cells was noted in the rituximab-cyclophosphamide group. The control group showed initial decline followed by rebound in cell numbers. Rituximab treatment groups also showed a rapid decline in RF levels. The methotrexate (control) group experienced an initial decrease, but the RF levels returned to baseline by week 24. Immunoglobulin levels did not change significantly. Despite the profound peripheral B cell depletion in the rituximab groups, the overall incidence of infection was similar in all groups at weeks 24 and 48. All the treatment groups had a similar overall incidence of adverse effects; however, the highest incidence of serious adverse events was noted in the rituximab plus cyclophosphamide group. Serious infections occurred in one patient in the control group, two patients in the rituximab monotherapy group, and two patients in the rituximab plus cyclophosphamide group. Fatal bronchopneumonia occurred in a patient in the rituximab monotherapy group.

An extension to the trial was published in abstract form by Emery et al. [19]. The patients were evaluated at week 104. ACR 50 values for the rituximab plus methotrexate and methotrexate (placebo) groups were 21% and 11%, respectively. Also, 13% of the rituximab plus methotrexate group reached a major clinical response (defined as ACR 70 maintained for ≥6 months). No significant differences in infections were noted between the different treatment groups.

Two recent case reports provide further evidence on the potential role of rituximab in DMARD-refractory RA. Kramm et al. [20] reported five patients with RF-positive erosive arthritis. All five patients experienced lack of efficacy with multiple DMARDs, including anti-TNF therapy. The DMARDs being taken at the time of the trial were continued. All five patients were given four weekly doses
of 375 mg/m² of rituximab. Swollen and tender joint counts were evaluated before and after the rituximab therapy. Four of the five patients achieved remission lasting 5 to 12 months. All patients experienced relapse after a mean duration of 8 months after rituximab therapy.

Similarly, Kneitz et al. [21] performed an open study of five patients with refractory RA. The patients had been unsuccessfully treated with at least three other DMARDs. All DMARDs except methotrexate were stopped at least 2 weeks before the patients entered the study. Methotrexate was continued at the same dosage if a partial response had been previously observed. Four of the five patients had not responded to anti–TNF-α therapy. All five patients reached the primary efficacy point (improvement in Disease Activity Score 28 ≥ 1.2).

These studies suggest that B cell depletion may be effective in treating refractory RA and in producing prolonged and sustained improvement in disease activity parameters. It has been speculated that RF-positive patients would potentially be the most responsive to this approach, although this contention remains unanswered. A theoretic risk of immune-system reaction against chimeric antibodies exists, but no significant reactions of this nature have been reported to date in RA patients [22]. Fully human monoclonal antibodies are, however, currently being developed.

**Cytokine-targeted therapy**

Cytokines are molecules that play both pro-inflammatory and anti-inflammatory roles. Indeed, our knowledge of their central role in inflammation has been used for therapeutic benefit with the advent of TNF-α and IL-1 blocking agents in RA. Other cytokines have been evaluated as potential therapeutic targets.

**Inhibition of interleukin-6 using MRA, an anti-interleukin-6 receptor antibody**

Interleukin-6 is a glycoprotein composed of 184 amino acids. Numerous cells can produce this inducible cytokine, including macrophages, B cells, T cells, fibroblasts, endothelial cells, mesangial cells, and many types of tumor cells [23]. IL-6 gene expression is regulated primarily through the nuclear factor-κB pathway, which is activated by both TNF-α and IL-1, along with several other pro-inflammatory stimuli [1]. IL-6 signals through a receptor complex formed by IL-6R and gp130, which activates several members of the JAK-STAT pathway. This ultimately leads to the transcription of genes with IL-6 response elements, the best known of which are the acute-phase proteins. IL-6 signaling is inhibited by suppressor of cytokine signaling and the protein inhibitors of activated STATS [23]. The effects of IL-6 are pleiotropic, occurring at both a systemic and a local tissue level, and involving a wide variety of cells (Fig. 1). Of particular relevance to RA are the effects on the differentiation of B and
T lymphocytes, as well as the differentiation of macrophages, megakaryocytes, and osteoclasts. IL-6 is now known to be the primary regulator of the hepatic acute-phase response, stimulating hepatocytes to produce C-reactive protein, fibrinogen, serum amyloid A protein, and a spectrum of other acute-phase proteins, while suppressing albumin production.

Interleukin-6 is elevated in the serum and synovial fluid in RA patients [24,25]. The excessive production of IL-6 is postulated to play a role in the pathogenesis of several inflammatory diseases such as RA, Crohn disease, and juvenile idiopathic arthritis [26]. In RA, IL-6 participates in immune cell activation and autoantibody production, osteoclastogenesis, and bone loss, and the often debilitating systemic and constitutional symptoms associated with the acute-phase response. It also plays a role in activating the hypothalamic-pituitary axis, leading to the release of anti-inflammatory hormones [27].

MRA (Chugai Pharmaceutical Co. Ltd., Tokyo, Japan) is a humanized anti-IL-6 receptor antibody that inhibits the binding of IL-6 to its receptor IL-6R and prevents IL-6 signal transduction. The ultimate development of this approach as an effective treatment for RA by Kishimoto and his colleagues in Japan has been a spectacular example of the path from bench to bedside. In 2004, Nishimoto et al. [28**] published the results of a multicenter, double-blind, placebo-controlled trial with 162 patients with active RA who had been unsuccessfully treated with at least one DMARD or immunosuppressant. The study population represented a group of patients with refractory RA for whom four to five DMARDs had been tried in the past without success. No DMARDs, immunosuppressants, or parenteral or intraarticular corticosteroids were allowed, but stable doses of oral corticosteroids (prednisolone ≤10 mg/d) and NSAIDs were. Patients were divided into three groups: placebo, MRA 4 mg/kg, and MRA 8 mg/kg; they were administered the allotted study drug three times every 4 weeks for 3 months. The primary end point was ACR 20 measured at week 12. Twenty-five of the 53 patients in the placebo group withdrew. The reasons included exacerbation of disease requiring DMARD (12 patients), patients’ request (3 patients), lack of efficacy and patients’ request (6 patients), and adverse events (4 patients).

There was significant improvement in ACR 20, 50, and 70 with MRA treatment compared with placebo. The efficacy was initially noted at week 4 and continued to increase up to week 12. The ACR 20 responses were 11.3%, 57.4%, and 78.2% for placebo, MRA 4 mg/kg, and MRA 8 mg/kg, respectively. The ACR 50 and ACR 70 responses for the MRA 8 mg/kg group were 40% and 16.4%, respectively. Similarly, improvements in DAS-28 were also noted. Furthermore, normalization of the C-reactive protein level occurred in 76% and 26% of patients in the MRA 8 mg/kg and 4 mg/kg groups, respectively. Only 1.9% of the placebo group patients showed C-reactive protein normalization. A decrease in RF titers was seen in the MRA 8 mg/kg group but no correlation was seen between decrease in RF titer and ACR response rate in this study.

The incidences of adverse events in the placebo, MRA 4 mg/kg, and MRA 8 mg/kg groups were 56%, 59%, and 51% respectively. Three serious adverse events were noted in the MRA group. One of the patients died of reactivation of chronic Epstein-Barr virus infection and hemophagocytosis syndrome after receiving a single dose of MRA 8 mg/kg. Other serious side effects noted in the MRA groups were allergic pneumonitis and infection of a leg burn. Increases in lipid levels (total cholesterol, triglycerides, and high-density lipoprotein cholesterol) were common in the MRA groups. There was no increase in cardiovascular complications associated with the increase in lipid levels, although the trial was too short and too small to enable adequate assessment of this. An elevation of transaminases was observed in 12.8% of the MRA group. Leukopenia was also noted. Both these abnormalities were transient. Anti-MRA antibodies were detected in 2 patients who received MRA. These patients were withdrawn from the study.

In a published abstract, Nishimoto et al. [29] reported a study in five patients who had achieved ACR 50 or 70 responses with MRA treatment. The MRA treatment was suspended until the patients no longer fulfilled ACR 50 criteria. ACR 50 criteria lasted for 3, 6, 9, and 22 months in four of the five patients. The authors also showed a correlation between reductions in IL-6 levels after MRA treatment and ACR 70 response. They concluded that once the IL-6 levels are normalized, the efficacy could be sustained even after MRA cessation.

Other cytokine targets

Preliminary trials targeting other cytokines including IL-12, IL-15, and IL-18 are under way. AMG 714 (Genmab, Copenhagen, Denmark) is a human monoclonal antibody that binds to IL-15 and inhibits its signaling. In a published abstract, McInnes et al. [30] demonstrated that patients receiving AMG 714 had clinically meaningful improvement compared with placebo, providing a first proof of the concept that IL-15 may be a rational target in the treatment of RA. In preclinical studies, an anti–IL-17 antibody significantly reduced the severity of collagen-induced arthritis [31].

Conclusion

Despite the success of TNF-α and IL-1 inhibitors, a substantial proportion of RA patients remain refractory to the available therapeutic modalities. There continues to be considerable investigative activity to develop new strategies
for treating RA patients. In the past year it has been shown that depletion of B cells with the monoclonal antibody rituximab results in sustained improvement in the signs and symptoms of RA, even after only two doses of this agent. Moreover, there is little evidence of excessive toxicity despite profound depletion of B cells. Likewise, the inhibition of T cell co-stimulation by the use of CTLA4Ig demonstrates impressive clinical efficacy and acceptable toxicity. This is in sharp contrasts to the previously observed unfavorable risk-to-benefit ratio associated with T cell-depleting agents such as Campath. Effective inhibition of IL-6, a central pro-inflammatory cytokine in RA, produces clinically meaningful improvement in the disease state, particularly in the acute-phase response associated with RA.

The response of RA patients to this wide spectrum of therapeutic strategies attests to the complexity and heterogeneity of the disease and provides further impetus for studies that use these therapies to enhance our understanding of disease pathogenesis.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest ** of outstanding interest


This is a state-of-the-art review of drug therapy in RA


This randomized, double-blind trial compared oral methotrexate, rituximab, rituximab plus methotrexate, or rituximab plus cyclophosphamide in 161 randomly assigned RA patients. The trial showed that rituximab, alone or in combination, provided significant and prolonged improvement in symptoms.


This multicenter placebo-controlled trial demonstrated a reduction in disease activity with MRA, an anti-human IL-6R monoclonal antibody

