Abstract: The emergence and use of the biological agents is one of the major recent advances in the treatment of rheumatoid arthritis. Although the tumor necrosis factor alpha (TNFα) inhibitors are superior to conventional disease modifying anti-rheumatic drugs in terms of efficacy, certain patients may still be unresponsive or intolerant to them. Moreover, infection such as tuberculosis and the possibility of long term adverse effects such as malignancies are major concerns of the anti-TNFα agents. Thus, newer and, hopefully, more effective but less toxic biological agents have to be developed as alternative therapies. This article reviews the preliminary clinical data on several non-TNFα biological agents in the treatment of rheumatoid arthritis.

Keywords: Abatacept, anakinra, anti-IL-6 receptor, anti-IL-15, cytokines, rituximab

Introduction

The treatment approach to rheumatoid arthritis (RA) has undergone a major evolutionary change in recent years in part as a result of the growing awareness of the severity of this condition and in part due to the considerable progress in the understanding of the important roles of cytokines in the immunopathogenesis of the disease. Anti-tumor-necrosis-factor alpha (anti-TNFα) therapy represents an example of delivering new treatment by targeting a single molecule within the immunopathogenic pathway. Although anti-TNFα agents are highly effective in the treatment of RA, some patients do not benefit either because of contraindications or refractoriness to these agents. Others have to be withdrawn from treatment because of adverse effects. Since many of these patients would have failed multiple disease modifying anti-rheumatic drugs (DMARDs) prior to anti-TNFα therapy, alternative treatment modalities have to be sought. In this article, biologic therapies other than the anti-TNFα agents such as the interleukin(IL)-1 receptor antagonist, anti-IL-6 receptor antibody, anti-IL-15 antibody, cytotoxic T-lymphocyte antigen 4-human immunoglobulin Fc construct (CTLA4-Ig) and anti-CD20 antibody will be briefly reviewed.

IL-1 Receptor Antagonist (Anakinra)

Besides TNFα, IL-1 is another key mediator of immune and inflammatory processes in RA. IL-1 is involved in the recruitment of neutrophils, activation of macrophages, and promotion of growth and differentiation of T- and B-lymphocytes. It also leads to tissue destruction through the induction of proteolytic enzymes. Animal studies showed that intra-articular administration of IL-1 could lead to chronic arthritis resembling RA.1 IL-1 is detectable in the plasma and synovial fluid of RA patients and its concentration correlates with disease activity.2

IL-1 receptor antagonist (IL-1Ra) specifically inhibits IL-1 when it is present in excess (10 to 100 fold of IL-1Ra to IL-1). It occurs naturally to modulate IL-1-induced proinflammatory processes by preventing the binding of IL-1 to the IL-1 receptor.
Anakinra is a recombinant, non-glycosylated form of native human IL-1Ra. A large, 24-week, multicenter European trial showed that RA patients treated with anakinra (150 mg daily subcutaneous injection) was significantly more effective than placebo in reaching the ACR20 response (43% vs. 27%, p=0.014). The therapeutic benefit of the combination of anakinra with methotrexate (MTX) was also demonstrated in a dosage-ranging, multicenter, placebo-controlled trial. Patients on 1 mg/kg/day or 2 mg/kg/day of anakinra together with MTX 15-25 mg/week were significantly more likely to achieve an ACR20 response than MTX alone at 12 weeks (46% and 38% vs. 19%, p<0.01). ACR50 and ACR70 response rates were also higher in the anakinra 1 mg/kg and 2 mg/kg groups than the placebo group. The benefit of anakinra was sustained at 24 weeks. Functional improvement in terms of reduction of the Health Assessment Questionnaire-Disability Index (HAQ-DI) was also demonstrated in this study.

Another study using fixed dose of anakinra (100 mg/day) in addition to MTX also showed its efficacy when compared with MTX alone. Radiographic progression was also retarded by the use of anakinra.

The safety profile of anakinra as monotherapy or as combination with MTX has been satisfactory. Apart from injection site reaction, which was common (50-70%), other adverse events were infrequent and were comparable to placebo groups in previous clinical studies. The use of anakinra alone or with other may result in higher injection rates although the risk is low - 4.3 cases per 100-patient years. In contrast to anti-TNFα therapies, there was no report of increased incidence of tuberculosis or opportunistic infections.

Since animal models of inflammatory arthritis had predicted the excellent clinical responses of combining anti-IL-1 and anti-TNFα therapy, the potential of synergistic effects of these two agents was investigated in human RA. A pilot safety study on the combination of etanercept and anakinra unexpectedly showed a high rate of adverse events, although better efficacy was suggested. A larger double-blind controlled trial involving 244 patients on this combination did not show any additional clinical benefit to etanercept alone. In that study, ACR50 response was reached by 31% of patients in the group with etanercept plus anakinra, compared with 41% of patients treated with etanercept alone (p=0.914). The incidence of serious infections (0% for etanercept alone, 3.7-7.4% for combination therapy), injection-site reactions, and neutropenia was increased with combination therapy.

Because of the relatively lower treatment efficacy and cost-effectiveness of anakinra in comparison to anti-TNFα agents, the National Institute of Clinical Excellence of the United Kingdom no longer recommends the use of anakinra in the management of RA. However, in patients who failed anti-TNFα therapy, anakinra may still be a useful alternative. Patients with juvenile idiopathic arthritis may also represent another group who may benefit from anti-IL-1 therapy.

Anti-IL-6 receptor antibody (MRA)

IL-6 is one of the most abundant cytokines that is present in the joint and serum of patients with active RA. Serum IL-6 concentration correlates with disease activity and radiological joint damage. IL-6 is a 26 kDa pleiotropic cytokine. It was known initially as B cell stimulatory factor 2 or hepatocyte stimulating factor. It is produced by a wide range of cell types including lymphocytes, monocytes, fibroblasts, synoviocytes and endothelial cells. It binds to either soluble or cell surface IL-6 receptor (IL-6R) and then the complex binds to a cell surface molecule, gp130, which then leads to cellular activation (Figure 1). Physiologically, it stimulates the

![Figure 1. IL-6 causes activation through the action of IL-6-IL-6R complex on gp130.](image-url)
differentiation of B cells into plasma cells, proliferation and differentiation of T-lymphocyte into cytotoxic T cells. It also induces acute phase response, stimulates haematopoiesis, and the formation of osteoclasts. Interestingly, in vitro, IL-6 can also inhibit inflammation by antagonizing IL-1 and TNF-α. Hence it has been argued that IL-6 may regulate rather than mediate inflammation in RA.

In animal models, IL-6 knockout mice were resistant to antigen-induced arthritis. Monoclonal antibody to IL-6 reduced the disease severity in mice if given early in the disease process of collagen-induced arthritis. Similarly, blocking of IL-6 could improve disease in a primate model of RA. Taken together, these studies suggested that inhibition of IL-6 in patients with RA may be of clinical benefit.

In an open label study, Wendling et al treated 5 patients with a murine anti-IL-6 monoclonal antibody (B-E8, IgG1) given at 10 mg/day intravenously for 10 consecutive days. Clinical improvement appeared rapidly and no side effects were noted. However the production of anti-globulin and increased risk of anaphylaxis precluded its use in clinical practice.

A recombinant humanized anti-IL-6R monoclonal antibody of the IgG1 subtype (MRA) inhibits the formation of IL-6-IL-6R complex and subsequent cellular activation via gp130. A phase I/II double blind, randomized, placebo-controlled, single dose trial demonstrated its efficacy in the treatment of RA. The ACR20 response was met by 50% of patients in the 5 mg/kg group at week 2 but none in the placebo group. Improvement was maintained until week 8.

A phase II multicenter European study on 359 RA patients also showed good response rates at doses of 4 mg/kg or 8 mg/kg, especially in combination with MTX. Eighty-two percent of patients in the 8 mg/kg plus MTX group achieved EULAR good or moderate response but only 47% of patients in the MTX group achieved this response.

In a multicenter, double-blind, placebo-controlled trial, MRA at doses of 4 mg/kg or 8 mg/kg or placebo was given to 164 RA patients resistant to at least 1 DMARD. At 3 months, 78% of patients in the 8 mg group, 57% in the 4 mg group, and 11% in the placebo group achieved the ACR20 response (p<0.001). ACR50 response was also significantly higher in the 8 mg group than in the placebo group (40% vs. 1.9%, p<0.001). Similar incidence of adverse events was noted in all groups and was not dose dependent. Raised serum cholesterol levels (triglyceride, total cholesterol and HDL cholesterol) were noted although there were no increased cardiovascular events in the study period.

Overall, the promising results of these studies suggested that antibody to IL-6 receptor is a potential new treatment for RA.

**Anti-IL-15 antibody**

IL-15, a cytokine with structural similarities to IL-2, is produced by macrophages when T cells are activated or when synovial fibroblasts are exposed to TNFα or IL-1β. There are two isoforms of IL-15 - secreted and intracellular forms. IL-15 binds a widely distributed heterotrimeric receptor (IL-15R). IL-15 sustains T cell/macrophage interactions to promote activation and cytokine release by the latter via a cell contact-dependent mechanism. It can also promote T cell migration and survival, NK cell activation, synovial neutrophil activation and survival (Figure 2).

In a murine model of arthritis, the administration of a soluble fragment of IL-15Rα into DBA/1 mice profoundly suppressed the development of collagen-induced arthritis. Treatment of DBA/1 mice with a brief course of CRB-15, a lytic and antagonistic IL-15 mutant/Fcγ2a fusion protein, at the time of collagen challenge markedly inhibited the incidence and severity of arthritis.
A phase I/II, double-blind, placebo-controlled trial tested a humanized monoclonal antibody against IL-15 (HuMax-IL15) in RA patients failing at least one DMARD. HuMax-IL15 was well tolerated, and ACR20/50/70 responses were reached in 63%/38%/25% of patients, respectively, in all dosage groups after 4 weekly injections. Although no placebo group was included in the efficacy analysis, this study suggested that targeting IL-15 may prove beneficial in the treatment of RA.

Another multicenter, randomized, double-blind, placebo-controlled phase II trial was conducted on the human monoclonal antibody against IL-15 (AMG 714). One hundred and ten patients with active RA despite DMARD therapy were randomized to 5 arms with different dosages of AMG 714 (40, 80, 160 and 280 mg) or placebo administered by subcutaneous infusion every 2 weeks for 12 weeks along with a stable dose of MTX 25 mg/week. A significantly higher proportion of patients in the highest dosage group reached ACR20 compared with the placebo group (62% vs. 26%, p=0.017). The incidence of adverse events was similar in the treatment and placebo groups (60.9% vs. 56.5%). This study demonstrated anti-IL-15 monoclonal antibody is safe and effective in treating RA.

Cytotoxic T-lymphocyte antigen 4-immunoglobulin (CTLA4-Ig/Abatacept)

Resting T cells require at least two separate signals to achieve full activation. The first occurs through the interaction of processed peptide presented in the setting of the major histocompatibility complex (MHC) and the T-cell receptor. The second occurs when a costimulatory signal is delivered because of engagement of CD28 on T cells with CD80/86 on the surface of the antigen-presenting cell.

CTLA4 is a second, high affinity receptor for both CD80 and CD86, with an avidity of 500-2500 times that of CD. Thus CTLA4 effectively blocks the second signal for T-cell activation (Figure 3).

CTLA4-Ig is a fusion protein consisting of the extracellular domain of human CTLA4 and a fragment of the Fc domain of human IgG1. The ability of CTLA4-Ig to block T-cell dependent antibody responses was demonstrated in murine and primate models. CTLA4-Ig was also shown to prevent and ameliorate collagen-induced arthritis.

The first study of CTLA4-Ig in RA patients was a pilot, dose-finding study on the agent and a similar molecule, LEA29Y. ACR20 response was observed in a dose-dependent manner. Similar incidence of adverse events was observed across all treatment groups, with one case of septic arthritis in CTLA4-Ig 2 mg/kg group.

Subsequently, a phase IIb multicenter, international study was conducted in RA patients with an inadequate response to MTX. Three hundred and ninety-nine patients were given CTLA4-Ig (2 mg/kg or 10 mg/kg) or placebo infusion biweekly for 3 doses then monthly for a total of six months. All patients also received MTX. ACR20 response was achieved in 60% of patients on CTLA4-Ig 10 mg/kg, 41.9% of patients on 2 mg/kg, and 35.3% on placebo, respectively (p<0.001, 10 mg/kg vs. placebo). The ACR50 and ACR70 responses also favored CTLA4-Ig in a dose response fashion. Clinically meaningful and statistically significant improvement in all subscales of SF-36 was also seen in 10 mg/kg group. The overall safety profile of CTLA4-Ig was similar to that of placebo.

Currently, two phase III studies of CTLA4-Ig are underway in patients with RA refractory to MTX and in subjects who have failed treatment with the TNF inhibitors.

B-cell depletion therapy – anti-CD20 antibody (Rituximab)

Evidence for involvement of B lymphocytes in RA dated back to the nineteenth century, when germinal centres were noted in RA synovium and when rheumatoid factor was
identified in mid-twentieth century. However since then the significance of autoantibodies in RA remained obscure. Then the interest shifted to T cells because of the findings of T lymphocytes in RA tissue, and the linkage of RA to HLA-DR4.

In recent years there has been growing interest in, and enhanced understanding of, the contribution of B cells to the immunopathogenesis of RA. There are several possible mechanisms by which B cells may play a part in the disease process: (1) B cells may function as antigen presenting cells and provide important co-stimulatory signals required for CD4+ T cell clonal expansion and cellular functions; (2) B cells in RA synovial membrane may secrete proinflammatory cytokines; (3) Rheumatoid synovial membrane contains an abundance of B cells that produce the rheumatoid factor (RF). Seropositive RA is associated with more aggressive articular disease, more extra-articular manifestations. RF may also be a self-perpetuating stimulus for B cells, and RF immune complex mediates complement activation and propagation of the inflammatory cascade; and (4) B cells may directly activate T lymphocytes.

Rituximab is a genetically engineered chimeric anti-CD20 monoclonal antibody. It comprises human IgG1Fc constant regions and small variable light and heavy chain regions from the anti-CD20 murine antibody fragment. CD20 is a pan-B cell surface antigenic phosphoprotein that is restricted in its expression to pre-B and mature B cells. CD20 is not present on stem cells and is lost before differentiation into plasma cells. Treatment with rituximab results in a transient depletion of selective B cells that lasts for up to six months. This is the result of antibody dependent cell-mediated cytotoxicity, complement dependent cytotoxicity, and/or promotion of CD20+ B cell apoptosis.

In an open label study, five patients with active RA despite at least five previous DMARDs were given four infusions of rituximab (300-600 mg) over 22 days along with cyclophosphamide, together with medium high dose prednisolone.32 All five patients showed a rapid improvement in synovitis. They all satisfied the ACR50 response by six months while three of them achieved ACR70 response. B cells dropped to an undetectable level but immunoglobulins remained within normal limits. Flare up of arthritis was associated with repletion of B cells in two patients. Good clinical response was also noted in another open label study in which rituximab at 375 mg/m2 given weekly for four infusions to five patients with low dose steroid in the absence of cyclophosphamide.33

A double-blind, randomized controlled study recruited 161 seropositive patients with active RA failing DMARDs including MTX.34 They were randomized into four groups: MTX alone, rituximab alone (two 1 g infusions), rituximab plus cyclophosphamide (two 750 mg infusions), and rituximab plus MTX. All patients also received prednisolone for 17 days (total 910 mg). At week 24, ACR50 responses achieved by patients in the rituximab-MTX (43%) and rituximab-cyclophosphamide groups (41%) were significantly higher than in the MTX monotherapy group (13%) (p=0.005 for both groups vs. MTX alone). The proportion of patients achieving ACR20 and EULAR responses were also higher in all groups treated with rituximab. ACR responses were maintained for up to 48 weeks for the rituximab-MTX group. The majority of adverse events (transient hypotension or hypertension, cough, pruritus, and rash) occurred within the first rituximab infusion and tended to be less frequent during subsequent infusions. Serious infections occurred in one patient (corneal abscess) in the control group and in four patients (two septic arthritis, two pneumonia - one fatal) in the rituximab groups. Anti-chimeric antibody developed in 4.3% of patients without specific clinical manifestations.

Taken together, these studies showed that B cell depletion by rituximab is effective in refractory RA and sustained improvement can be achieved with a cycle of treatment. Interestingly, evidence is accumulating that patients with RF negative disease do not respond to this form of therapy. At the University College of London, four seronegative RA patients have been treated with rituximab but none of them showed an ACR20 response.35 In the study by De Vita,33 the patient with negative RF also did not respond to rituximab treatment. It is possible that seronegative RA has immunopathology different from its seropositive counterpart.

Conclusions

In recent years we see an explosive development of biologic therapies for RA. The success of anti-TNF therapy has led to development of other biologic agents targeting specific pathways in the immunopathogenesis of RA. Although no long term cure can be achieved as yet, these biologic agents have brought us closer to the ultimate aim of treatment of RA.
NON-TNF BIOLOGICAL TREATMENT IN RA

References


