Rheumatoid arthritis (RA), the commonest inflammatory joint disease seen in clinical practice, causes significant morbidity and mortality and shortens the lifespan by 10 years. Aspirin, an anti-inflammatory agent, was the mainstay of RA therapy till the 1950s. The management of RA was revolutionized with the advent of corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs) like methotrexate (MTX), sulphasalazine, and leflunomide as the disease course could now be modified favorably. Conventional DMARDs, however, have several limitations like slow onset of action, induction of partial remission and modest 5-year retention rates. The quest for an ideal DMARD thus continues. A better understanding of the pathophysiology of RA has enabled scientists to develop designer drugs termed ‘Biologicals’ that tackle the key inflammatory cytokines like TNF-α.

‘Biologicals’ or ‘biological response modifiers’ are therapeutic agents that have the potential to inhibit the behaviour of cytokine, cellular activation, and inflammatory gene transcription by various means. These include monoclonal antibodies, soluble cytokine receptors and natural antagonists. The first two biologicals developed for the treatment of RA were the TNF-α inhibiting agents, namely etanercept and infliximab. Thereafter newer agents were developed, including anakinra, a recombinant form of the naturally occurring IL-1 receptor antagonist, and adalimumab, a fully human monoclonal antibody against TNF-α. These biologicals represent a major advance in the treatment of RA. The Lasker Award for medical research, was awarded to Prof. Marc Feldmann and Sir Ravinder N Maini in 2003 for the discovery of anti-TNF therapy which has the potential to provide an effective treatment for RA and other autoimmune diseases.

This article deals with the molecular pathogenesis of the inflammatory cascade in RA, and reviews the current status of various approved and experimental biological agents in the treatment of RA.

**Pathogenesis of RA**

RA is a disorder characterized by persistent inflammatory synovitis, predominantly affecting the peripheral joints. This is associated with pannus formation, cartilage destruction, bone erosions and joint destruction. The synovial membrane in patients with RA is characterized by hyperplasia, increased vascularity and an infiltrate of inflammatory cells that are predominantly CD4+ T cells.

RA has been linked to the major-histocompatibility-complex (MHC) Class II antigens HLA-DRB1*0404, DRB1*0401, DRB1*0405, DRB1*0101 and DRB1*1402. The β chains of all the above HLA-DR molecules contain the same amino acids at positions 67 through 74, a concept known as ‘shared epitope’. The main function of MHC Class II molecules is to present antigenic peptides to CD4+ T cells, which suggests that RA is caused by an unidentified arthritogenic antigen. The antigen could be either an exogenous antigen, such as a viral or bacterial protein, or an endogenous protein such as citrullinated protein, human cartilage glycoprotein 39 or heavy-chain–binding protein.

Antigen-activated CD4+ T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce various cytokines. TNF-α, IL-1 and IL-6 and are the key cytokines that drive inflammation in RA and cause joint damage (Figure 1).
They are potent stimulators of synovial fibroblasts, osteoclasts, and chondrocytes that release tissue-destroying matrix metalloproteinases (MMP), which contribute to joint damage.\textsuperscript{12} The serum and synovial concentrations of IL-1 and TNF-\textalpha are high in patients with active RA.\textsuperscript{13,14} Activated CD4+ T cells also stimulate B cells to produce immunoglobulins, including rheumatoid factor (RF). RF may involve the activation of complement through the formation of immune complexes.

The products of activated macrophages, lymphocytes, and fibroblasts stimulate angiogenesis.\textsuperscript{15} Inflammatory cells are recruited into the joint by expression of adhesion molecules in endothelial cells in the synovium. This leads to the formation of hyperplastic, proliferating, inflamed synovium, also called ‘pannus’.\textsuperscript{15} Activated macrophages and synovial fibroblasts are present in the interface between the pannus and cartilage and cause damage to the joint. The release of enzymes like elastases and proteases leads to the degradation of proteoglycans in the superficial layer of cartilage. Activated CD4+ T cells along with the macrophages express the receptor activator of nuclear factor-kB ligand (RANK-L) that stimulates osteoclastogenesis.\textsuperscript{16}

**Key inflammatory cytokines in RA**

TNF-\textalpha is a soluble 17-kd trimeric protein that is produced mainly by monocytes and macrophages. Newly synthesized TNF-\textalpha is inserted into the cell membrane and subsequently released through the action of TNF-\textalpha converting enzyme, to become biologically active.\textsuperscript{17} It binds to TNF receptors on a variety of target cells, setting up a signalling cascade in that cell. There are two distinct TNF receptors, the p75 and the p55, that are trans-membrane proteins and activate different intra-cellular signal-transduction pathways.\textsuperscript{18,19,20} TNF receptor signalling occurs through two arms. One arm has death-domain proteins which lead to apoptosis or programmed cell death, giving it the name, tumour necrosis factor. The second and dominant signalling pathway goes through a series of kinases, leading to the activation of nuclear-factor kappa B (NF-kB) that is a key transcription factor for activating genes involved in inflammation.\textsuperscript{18} TNF-\textalpha triggers production of other cytokines, induces endothelial adhesion molecules, stimulates

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**Figure 1: Key cytokines in RA**

- TNF-\textalpha, IL-1, IL-6
- IL-4, IL-10
- soluble TNF receptor
- IL-Ra, IL-1 decoy receptor

**Figure 2: A simplified schematic diagram of pathogenesis of RA**

- T cell activates B cell
- Macrophage activates TNF-\textalpha
- Endothelial cell increased expression of adhesion molecules T cells and macrophages recruited
- Fibroblast IL-1 predominant action on fibroblast
- Pannus T cells activated MMP, IL-6, PGE2 secreted
- Cartilage destruction
- Osteoclast joint erosions periarticular osteopenia
- Immunoglobulin produced (Rheumatoid factor)
- RANK-L differentiates to Osteoclast
- Macrophage IL-3
- Macrophage differentiates to Osteoclast
Table 1: Biologic agents used to treat RA

<table>
<thead>
<tr>
<th>Agent</th>
<th>Usual Dose/Route</th>
<th>Efficacy (with MTX)</th>
<th>Side-effects</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infliximab</td>
<td>3 mg/kg IV infusion at weeks 0, 2, and 6 followed by maintenance dosing every 8 weeks. Has to be combined with MTX.</td>
<td>ACR-20 = 59%</td>
<td>Infusion reactions (fever, chills, urticaria, chest pain, dyspnoea, hypotension), Antibody formation, Predisposition to infections, URI, reactivation of TB, exacerbation of demyelinating disease</td>
<td>Active infections, uncontrolled DM, Surgery (withhold for 2 weeks postoperatively)</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>40 mg sub-cutaneously every 2 weeks (fortnightly). May be given with MTX or as monotherapy.</td>
<td>ACR-20 = 70%</td>
<td>Injection site reactions, URI, rash, headache, sinusitis, exacerbation of demyelinating disease</td>
<td>Active infections, uncontrolled DM, Surgery (withhold for 2 weeks postoperatively)</td>
</tr>
<tr>
<td>Etanercept</td>
<td>25 mg sub-cutaneously twice a week or 50 mg once a week. May be given with MTX or as monotherapy.</td>
<td>ACR-20 = 70%</td>
<td>URI, injection site pain, rash, headache, sinusitis, reactivation of TB, exacerbation of demyelinating diseases</td>
<td>Active infections, uncontrolled DM, Surgery (withhold for 2 weeks postoperatively)</td>
</tr>
<tr>
<td>Anakinra</td>
<td>100 mg sub-cutaneously once daily. May be given with MTX or as monotherapy.</td>
<td>ACR-20 = 70%</td>
<td>Injection site reactions, headache, sinusitis, infections, neutropenia</td>
<td>Active infections, uncontrolled DM, Surgery (withhold for 2 weeks postoperatively)</td>
</tr>
</tbody>
</table>

ACR—American College of Rheumatology; ANA—Antinuclear antibody; DM—diabetes mellitus; MTX—Methotrexate; TB—Tuberculosis; URI—Upper respiratory infection (Modified with permission from Handa R. Management of Rheumatoid arthritis. Natl Med J India 2004, 17:143-51)

collagenase and stromelysin, and stimulates osteoclast differentiation. Hence, the blockade of TNF-α has a more global effect on inflammation than the blockade of other cytokines (Figure 2).

IL-1 is a 17-kd protein that is mostly produced by monocytes and macrophages. IL-1 is a 17-kd protein that is mostly produced by monocytes and macrophages. IL-1β is the main protein in the joints of RA (Figure 3). IL-1 binds to two types of cell-surface receptors, Types I and II. Only Type I receptors are capable of intracellular signalling while Type II receptors are decoy receptors that bind to circulating IL-1. Soluble forms of IL-1 receptors compete with cell-surface receptors, thus decreasing IL-1-mediated activation of cells. Additionally, IL-1Ra is produced and secreted by all cells that express IL-1 and binds to the Type I receptor with high affinity without triggering a signal, thus providing another mechanism for the inhibition of IL-1 activity.

Like TNF, IL-1 is a key pro-inflammatory cytokine in RA with activities that are very similar to TNF. The effects of the two cytokines are often additive or synergistic.

IL-6 is an inflammatory cytokine produced by T cells, monocytes, macrophages, and synovial fibroblasts. IL-6 is involved in diverse biological processes such as final maturation of B cells into plasma cells, T cell activation, induction of acute-phase response, stimulation of growth and differentiation of haematopoietic precursor cells and proliferation of synovial fibroblasts.

Interferon-γ (IFN-γ) is a cytokine that is made by T cells and acts on a variety of other cells in RA synovium. It induces expression of antigen-presenting molecules, such as Class II MHC molecules and also upgrades regulation of Class I MHC on the antigen-presenting cells.

Anti-inflammatory cytokines in RA

There is a counter-regulatory mechanism in the form of anti-inflammatory cytokines, mainly in the form of IL-10 and IL-4. Some anti-inflammatory effect is also provided naturally by the presence of IL-1Ra, IL-1 decoy receptor and soluble TNF receptor. In vitro, these cytokines cooperate to inhibit the production of inflammatory cytokines. In vivo, however, they are inherently weak and prove inadequate. Monocytes, macrophages, B cells and T cells produce IL-10. However, within the RA joint, it is predominantly produced by macrophages. In vitro, it inhibits T cell proliferation and production of several cytokines including IL-1 and TNF-α. IL-10 has been shown to reverse the cartilage degradation seen in RA. IL-4 is produced by CD4+ Th2 cells and participates in the growth and differentiation of B cells. In vitro studies have shown it to inhibit the production of IL-1, IL-6 and IL-8 and increase the expression of IL-1Ra. To summarise, CD4+ T cells, stimulated by an as yet unidentified antigen, initiate RA. The immune response is amplified by the stimulation of other...
macrophages, synovial fibroblasts, chondrocytes, and osteoclasts. The release of cytokines, especially TNF-α, IL-1 and IL-6, causes synovial inflammation. Joint damage results from the degradation of connective tissue by MMP and the stimulation of osteoclastogenesis.

Cytokine antagonists in RA

A variety of cytokine-based strategies are being explored in RA. The FDA has currently approved TNF-α blocking agents and IL-1 antagonists for clinical use. The TNF blockers include etanercept, infliximab and adalimumab (Table 1). Anakinra is the recombinant form of IL-1 receptor antagonist (IL-Ra).

The commonly used indices to depict clinical response to therapy in RA include the American College of Rheumatology (ACR) response, Health Assessment Questionnaire (HAQ) score and Disease Activity Score (DAS). The ACR response is mentioned as ACR-20, 50 or 70. An ACR-20 response would mean a 20% improvement in tender and swollen joint counts from baseline and 20% improvement in 3 of the 5 remaining domains, namely, patient and physician global assessments, pain, disability, and an acute phase reactant. ACR-50 and 70 responses mean a 50% and 70% improvement respectively from baseline. HAQ is a scale used for assessment of global functional status in RA. DAS represents a composite score to denote disease activity that is assessed by evaluating tender joints, swollen joints, ESR and the patient’s own assessment on a visual analogue scale.

a) TNF-α Blocking Agents

Etanercept

Etanercept (Enbrel) is a dimeric fusion protein consisting of soluble p75-TNF receptor Type II and the Fc portion of human IgG1, which confers it a longer half-life of 4.8 days. The primary action of etanercept is to bind and inactivate soluble and cell-bound TNF-α and lymphotoxin-α. The efficacy of etanercept was first demonstrated in a placebo-controlled trial in refractory RA. In 234 DMARD refractory patients with a mean disease duration of 12 years, ACR-20, 50 and 70 response rates was observed in 59%, 40% and 15% patients respectively at the end of 6 months (p<0.001), which was sustained over 5 years of open label follow-up. Nearly one-third of those receiving steroids were able to discontinue the drug and there was a significant improvement in HAQ scores (p<0.0001). The TEMPO (Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes) trial compared combination therapy of etanercept and methotrexate (MTX) with etanercept monotherapy (25 mg twice weekly) and MTX monotherapy (mean 17 mg) in 686 patients with mean disease duration of 6.6 years. The efficacy of the combination was significantly greater than that of either monotherapy (P < 0.01) and demonstrated greater reduction in radiographic progression. The efficacy of etanercept monotherapy was significantly greater than that of MTX alone. The ACR-20, 50 and 70 responses at 52 weeks were 85%, 69% and 43% respectively for the combination group.

Etanercept has been approved for use in DMARD-naive patients too. Unlike the above-mentioned trials that were done in patients with longstanding RA, the ERA (Etanercept in Rheumatoid Arthritis) trial was done in 632 patients with early arthritis (duration less than one year). Patients were given etanercept or MTX. The etanercept group showed faster improvement. Although clinical response rates were similar by one year, at two years the etanercept group had better ACR-20 response rate (72% versus 59%) with better HAQ scores. Etanercept was also more effective in reducing the rate of erosions. Combined data from 5-year follow-up of responders from both the ERA trial and the etanercept monotherapy trials have demonstrated sustained improvement.

Infliximab

Infliximab (Remicade) is a chimeric (75% human + 25% mouse) monoclonal antibody against TNF-α. In the initial trials antibody response directed against the murine component was detected in 17% of patients when given alone. Combination with MTX was able to suppress this immune response considerably. The Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis study compared the combination of infliximab and MTX with MTX alone in 428 patients with active RA. Infliximab was given in 4 different doses (3 mg/kg and 10 mg/kg every 4 or 8 weekly). The ACR-20, 50 and 70 responses in the infliximab groups ranged between 50-60%, 26-31% and 8-18% respectively at 30 weeks for different doses. At 52 weeks, ACR-50 responses were significantly better for both the 4-weekly infliximab regimens (34% and 39% vs. 8% for 8 weeks). A dose-dependent improvement in all parameters was observed including slowing of radiological progression. The efficacy of infliximab has been shown up to a follow-up of 4 years.

Like etanercept, infliximab too has been tried in patients with early arthritis. The ASPIRE trial compared the efficacy of the combination of infliximab (3 mg/kg or 6 mg/kg) and MTX (mean dose, 17 mg) with MTX alone in 1049 patients of early arthritis with mean disease duration of 7 months. At 52 weeks, greater improvement was reported with combination therapy for ACR-20 response and DAS 28 scores, with greater retardation of radiographic progression (P<0.05). Patients in the highest quartile of baseline radiographic scores demonstrated greater treatment benefit with combination therapy. Pilot studies in India, especially from the armed forces, have demonstrated safety and efficacy of infliximab.

Table 1: Expanding indications for TNF blockers

1. Rheumatoid arthritis
   a. Refractory RA
   b. Moderate to severe RA
   c. Early RA for induction of remission
2. Ankylosing spondylitis
3. Still’s disease and AOSD
4. Fistulising Crohn’s disease
5. Psoriasis
6. Vasculitis
   a. Refractory Wegener’s granulomatosis
   b. Sight-threatening Behcet’s disease
**Adalimumab**

Adalimumab is the first fully human monoclonal antibody, indistinguishable from naturally occurring human IgG1, and possesses a high specificity and affinity for TNF-α. It has low immunogenicity compared to infliximab and thus avoids the need for concomitant administration with immunosuppressants such as MTX. Adalimumab was found to be significantly more effective than placebo when given alone as well as in combination with MTX in several clinical trials. In the ARMADA trial, adalimumab (20, 40, or 80 mg S.C. every other week) and MTX combination was compared with MTX alone in 271 patients with refractory RA. At 24 weeks, significantly more subjects in the former group met ACR-20 response (47.8%, 65.7%, 65.8% vs. 14.5%; P < 0.001 for each). Significant differences were also observed in ACR-50 and ACR-70 responses, especially in the 40 mg and 80 mg dose groups.

**b) IL-1 Antagonism**

Anakinra (Kinera) is a recombinant form of IL-1Ra that competes with IL-1 for binding to cell surface-bound IL-1 receptor, but does not induce intracellular signalling. In a randomised controlled trial (RCT) comparing anakinra with placebo in 472 patients with severe RA, patients receiving 150 mg of anakinra achieved a significantly better ACR-20 response (43% v 27%; p=0.014) at 24 weeks. Radiographic progression was significantly slowed (p=0.0004). The combination of anakinra with methotrexate was shown to be significantly better than methotrexate alone over 24 weeks in another RCT on 419 patients with respect to ACR-20 response (42% vs. 23%, p=0.021) and HAQ score (p<0.01). An important drawback is its short half-life (6 hours) in plasma, which necessitates frequent daily treatment with high doses required to maintain a therapeutic effect.

**Clinical considerations**

Cytokine antagonists are indicated in patients of resistant RA. Resistant RA is defined as the failure of at least two DMARDs used in optimal doses for an adequate period (at least 6 months). One of the DMARDs should have been MTX, unless contraindicated. Cytokine antagonists may also be useful in early RA (duration less than 3 years). There is little information regarding head-to-head comparisons between various TNF antagonists or between TNF antagonists and other DMARDs. The positioning of biologicals will emerge more clearly in times to come as these issues get addressed in newer trials. Apart from RA, biologicals are also being used in other diseases (Table 1).

The onset of action is rapid and occurs within 2-4 weeks. Infliximab cannot be used as monotherapy and has to be combined with MTX while other agents can be used alone or in combination with MTX. Nearly 20-40% patients may not respond to these agents. Cytokine antagonists should be withdrawn in face of adverse events or lack of adequate response (DAS improvement less than 1.2 at 3 months or more). There is data to suggest that if one TNF blocker fails, substitution with another agent may be helpful. The efficacy and tolerance of the second agent is not compromised irrespective of the reason for the discontinuation of the first anti-TNF agent. Recent data indicates that combination of biologicals do not provide added benefit. This is possibly true because currently available drugs act on essentially the same target. However, combinations of biological agents in future, that target different disease processes, like anti-TNF and CTLA4Ig, may yield more promising results. The major deterrent to the use of cytokine antagonists is their cost.

Cardiovascular disease due to accelerated atherosclerosis is the major cause of excessive mortality in RA. Cytokine antagonists have shown a favourable response in endothelial cell dysfunction in these patients.

**Safety issues**

The cytokines play an important role in protective immunity and the risk of infections increases with the use of these agents. Worldwide, over 5,00,000 patients have received biologicals to date, and over 320 cases of tuberculosis have been reported, mostly with infliximab. Most infections occurred earlier with infliximab (6-8 months) than with etanercept (11 months). The majority of affected patients had extra-pulmonary or disseminated forms of tuberculosis. This has specific implications regarding usage of biologicals in India due to the high prevalence of tuberculosis. Use of TNF inhibitors has been complicated by the occurrence of opportunistic infections with atypical mycobacteria, histoplasma, listeria, pneumocystis, coccidioides, aspergillus, nocardia, candida, and CMV in subjects receiving these agents. Lymphoma has been reported in association with all three TNF antagonists. The incidence of lymphoma is increased among patients with RA and it increases with the severity of the condition. The Standardised Incidence Ratio (SIR) for lymphomas was reported to be increased in 18,572 patients with RA (baseline for RA=1.9, Infliximab-2.6, Etanercept-3.8) though a causal relationship could not yet be established and needs further long-term observation. Demelinating disorders have been reported with all biologicals except anakinra. These include relapses of multiple sclerosis (MS) and development of other disorders like optic neuritis, encephalitis, transverse myelitis, seizures, leukoencephalopathy and demelinating polynuropathy. Nearly all of these cases improved or resolved with the discontinuation of TNF inhibitor therapy.

The most common side-effect seen with self-administered biologicals was injection site reactions (ISRs), ranging from 18.5% to 71% for adalimumab and anakinra respectively. However, drug discontinuation due to ISRs is uncommon (up to 7% with anakinra. ISRs are mild to moderate in severity and are primarily seen in the first few weeks of use, and become less prominent over time. ISRs from etanercept or adalimumab usually last for 3 to 5 days but may last up to 10 days with anakinra. Infusion reactions may occur in up to 20% of patients with infliximab infusions, usually during or within 2 hours after the infusion. This rarely necessitates cessation of therapy. Rare cases of bone marrow aplasia have been reported with both etanercept and infliximab. Periodic monitoring...
The common side-effects associated with the use of TNF inhibitors are mild, well-tolerated, self-limiting, and seldom enough to warrant discontinuation. Serious and potentially life-threatening adverse events are rare and proper patient selection and preventive measures may limit this risk further.

**Upcoming therapies**

Numerous other biological therapies are in various stages of development. TNF-α converting enzyme (TACE) cleaves cell-bound TNF-α to release it into circulation. Agents that block this enzyme reduce the TNF production by up to 95% and are under development. Many IL-1 inhibiting agents are being developed. These include a recombinant form IL-1 receptor Type II (IL-1RII), IL-1 Trap (recombinant molecule consisting of IL-1RI and IL-1 receptor accessory protein fused to human IgG Fc) and an inhibitor of IL-1 converting enzyme. Clinical trials are underway evaluating the safety and efficacy of monoclonal antibody to IL-6 receptor (MRA), IL-15 (HuMax-IL-15) and CD2 receptor (Alefacept).

Small molecular inhibitors of intracellular signalling are under development and have shown promising results in animal models. Corticosteroids act by interfering with the NF-κB pathway. Small molecule inhibitors of NF-κB and associated activator molecules are the focus of numerous clinical and preclinical research endeavours. Agents blocking the chemokines and adhesion molecules too are under trial. These include antibodies to IL-18, humanized anti-integrin αvβ3 monoclonal antibody (MEDI-522) and anti-VCAM antibodies.

There are several sets of T cell co-stimulatory molecules like CD40-CD40 ligand (CD-40L) and CD28-CTLA4-B7 etc. Blockade of some of these like CTLA4 immunoglobulin, anti-CD40 ligand antibody and anti-CD11a monoclonal antibody (efalizumab) are under various stages of development. CTLA4 immunoglobulin and MTX combination has shown a significantly greater ACR-20, 50 and 70 response than MTX alone in Phase II and Phase III trials are currently in progress. Rituximab (anti-CD20 antibody) is a monoclonal antibody that selectively depletes B cells bearing the CD20 surface marker. Widely used in the treatment of B-cell lymphomas, it has been shown to be surprisingly effective in RA. In patients with active RA despite MTX, a single course of two infusions of rituximab, alone or in combination with either cyclophosphamide or continued MTX, provided significant improvement in disease symptoms at both, 24 and 48 weeks. The rituximab/MTX combination represents a potential therapeutic option for RA patients.

**Conclusions**

Although the cause of rheumatoid arthritis still eludes us, improved understanding of the pathogenesis of the disease has opened the door to innovative therapies. TNF blocking agents and IL-1 blocking agents have proved to be at least as effective as DMARDs, if not better. These agents retard the disease progression and are a major advance in the treatment of RA. By targeting molecules that are directly involved in the pathogenesis, these therapies may be more efficacious, more specific and less toxic in the short- and long-term than standard therapies. The major deterrent is their cost. Newer biologicals targeting specific pathways are being studied. These are exciting times in Rheumatology as the gap between the bench and the bedside is narrowing, slowly but surely.

**References**


