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Review Article

MOLECULAR TARGET AND THERAPEUTIC ASPECTS OF RHEUMATOID ARTHRITIS: A REVIEW

BISWARANJAN DAS*, SUBIR SAMANTA

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi - 835 215, Jharkhand, India.
Email: dasbiswaranjan29@yahoo.com

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disease. Cytokine plays a significant role in modulating the immune system and specifically the inflammation. The role of cytokines in disease has led to new development in the treatment of inflammatory diseases. Different mediator cause inflammation, and degradation of joint cartilage in RA. Some of the novel target inhibitors, which are used to treat and control this disease are cyclooxygenase-2, tumor necrosis factor-α (TNF-α), TNF-α converting enzyme, interleukin-1, 6, 8, 10, 12, 15, 18, matrix metalloproteinase, nuclear factor κB, mitogen activated protein kinase, phosphodiesterase-4, janus kinase-signal transducer and activator of transcription, Bruton's tyrosine kinase, spleen tyrosine kinase, Phosphatidylinositol-3-kinases. The mode of action and their progress in research have been covered in this review.

Keywords: Disease-modifying anti-rheumatic drugs, Tumor necrosis factor-α converting enzyme, Tumor necrosis factor-α, Janus kinase, Spleen tyrosine kinase, Phosphatidylinositol-3-kinases (PI3K).

INTRODUCTION

Arthritis is a form of joint disorder that involves inflammation of one or more joints. Globally arthritis has affected different population of the world. Worldwide studies of rheumatoid arthritis (RA) suggest that, prevalence of arthritis is higher in Europe and North America than in other developing countries [1]. Arthritis and other rheumatic conditions are the leading cause of disabilityand most common chronic disease problems in US. It affects more than 52 million US adults and the number that's expected to increase to 67 million adults by the year 2030. The prevalence of arthritis is highest in older adults of age 65 years of age is expected to increase 7%, by 2030 raising to 20% of the population. Women will account for 61% (40.9 million) of the arthritis cases in 2030 [2].

As per American College of Rheumatology (2012) RA is affecting more than 1.3 million Americans, out of them 75% are women. Prevalence data for major arthritis disorders have been compiled in West for several decades. As per WHO and International League against Rheumatism collecting data for India, prevalence of arthritis in is scarce. The clinical features and laboratory data of India and some of the characteristics of the disease compared with Europe and North America, found quite similar to other developing countries (Fig. 1) [1].

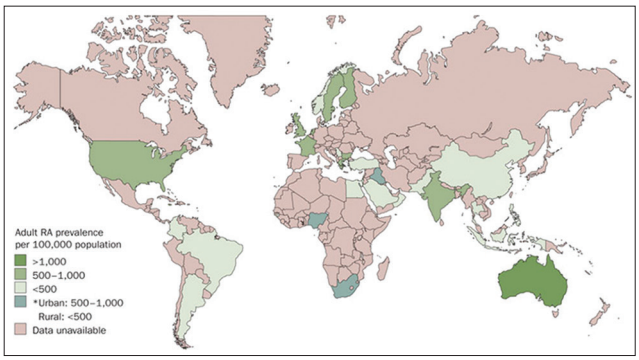

Fig. 1: Prevalence of rheumatoid arthritis in the adult population of various world regions [3]

TYPES OF ARTHRITIS

Depending on the part of organ affected arthritis are divided in to following: Osteoarthritis (OA), RA, psoriatic arthritis, gout and pseudogout, ankylosing spondylitis, juvenile RA, lupus, fibromyalgia, scleroderma, lyme disease. OA and RA are the two most common musculoskeletal conditions affecting individuals across the world. It is distinguished by cartilage degeneration and bony overgrowth.

OA

OA is "wear and tear" arthritis and also called degenerative joint disease, caused by the breakdown of joint cartilage. Cartilage acts as a cushion between the bones that form a joint. Loss of cartilage can cause bone to rub on bone in a joint and the condition that is very painful. Usually, OA begins in a single joint. OA affects approximately 13.9% of adults who are ≥25 years of age. The incidence of OA rises with age, estimates that OA affects 12.4 million adults ≥65 years of age. It occurs more frequently in women, after reaching 50 years of age have a greater risk for developing OA in the knee or hip [4].

RA

RA is chronic inflammatory autoimmune disease. In RA, the synovium is primarily affected and characterized by inflammation. RA typically affects the small joints of the hands, feet and usually both sides equally and symmetrically, although any synovial joint can be affected. It is a systemic disease and can affect the whole body, including the heart, lungs and eyes. It usually occurs in bilateral joints and have some systemic features such as fatigue or fever. Pain is often constant and may be localized to the affected joint. Pain is due to inflammation that occurs around the joint and damage of joint. Multiple joints are usually involved with RA. RA distributed worldwide with an estimated prevalence of 1-2%. Prevalence increases with age, approaching 5% in women over age 55. The average annual incidence in the United States is about 70 per 100,000 populations. The incidence of RA is 2-3 times greater in women than in men, with a higher life time risk (3.6%) women compared to men (1.7%) (Fig. 2) [4].

Diagnosis of RA

To diagnose RA, patient history is important. However, the physical examination (morning stiffness, warmth, swelling and pain in the joints), laboratory tests, radiographs, and other assessments (i.e., functional

status, disease activity) are frequently helpful in confirming a diagnosis. Some blood tests also can help confirm RA.

- Anemia (a low red blood cell count)
- Rheumatoid factor (an antibody or blood protein found in about 80% of patients with RA but in 30% at the start of arthritis)
- Antibodies to cyclic citrullinated peptides or anti-cyclic citrullinated peptide (60-70% of patients with RA)
- Elevated erythrocyte sedimentation rate in most patients with RA and confirms the amount of inflammation in the joints.

X-rays can help in detecting RA, but may not show anything abnormal in early arthritis. The first X-rays may be useful later to show if the disease is progressing. Magnetic resonance imaging and ultrasound scanning are done to help judge the severity of RA. There is no single test that confirms an RA diagnosis, for most patients with this disease. Doctor makes the diagnosis by looking at the symptoms and results from the physical examination, laboratory tests and X-rays [4].

RA treatment

Patient diagnosis of RA should begin their treatment with disease-modifying anti-rheumatic drugs (DMARDs). These drugs not only relieve symptoms but also slow progression of the disease. Doctors prescribe DMARDs along with non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose corticosteroids to lower swelling, pain, fever. DMARDs have greatly improved the symptoms. Common DMARDs include methotrexate (MTX), leflunomide, hydroxychloroquine and sulfasalazine. Older DMARDs includes auranofin, antibiotic minocycline, immune suppressants - azathioprine and cyclosporine.

Patients with more serious disease may need medications called biologic response modifiers or "biologic agents." They can target the parts of the immune system and the signals that lead to inflammation, joint and tissue damage. These medications are also DMARDs. FDA - Approved drugs of this type include abatacept, adalimumab, anakinra, certolizumab, etanercept, golimumab, infliximab, rituximab and tocilizumab. Patients take these drugs with MTX, as the mix of medicines is more helpful [4].

Pathophysiology

RA is an autoimmune disease; own body system attacks the synovial tissue and other connective tissues, cannot differentiate self from non-self-tissues. That means certain cells of the immune system do not work properly and start attacking healthy tissues of the joints. Once the initial immune response is triggered, cells of the immune system produce autoantibody and inflammatory cytokines, creating a cascade of inflammation. Chronic inflammations of synovial tissue lining of the joint capsule result in the proliferation of this tissue. The inflamed proliferating synovium in RA is called as pannus. This pannus invades the cartilage and bone surface, producing erosion of bone and cartilage leading to destruction of the joint [5].

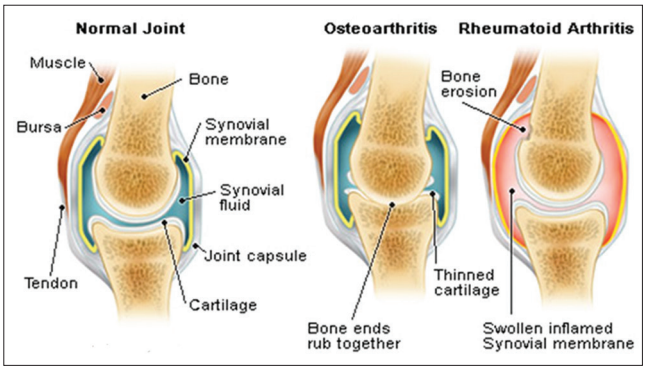

Fig. 2: Normal and arthritic joint [4]

The immune system has both humoral and cell-mediated functions. The humoral component is necessary for the formation of antibodies. These antibodies are produced by plasma cells. Most patients with RA form antibodies called rheumatoid factors. Immunoglobulin can activate the complement system. The complement system amplifies the immune response by encouraging chemotaxis, phagocytosis, and the release of lymphokines by mononuclear cells, which are then presented to T lymphocytes. The processed antigen is recognized by major histocompatibility complex (MHC) proteins on the lymphocyte, which activates to produce T and B-cells.

Lymphocytes may be either B-cells (derived from bone marrow) or T-cells (derived from thymus tissue). T-cells may be either T-helper (which promote inflammation) or T-suppressor cells (which attenuate the inflammatory response). Activated T-cells produce cytokines, which is toxic to tissues and stimulate further activation of inflammatory processes by attracting cells to areas of inflammation. Macrophages are stimulated to release prostaglandins (PGs) and cytokine. Activated B-cells produce plasma cells, which form antibodies. These antibodies in combination with complement result in the accumulation of polymorphonuclear leukocytes (PMNs). These PMNs release cytokine, oxygen free radicals, and hydroxyl radicals that promote cellular damage to synovium and bone. The pro inflammatory cytokines tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6 are key substances in the initiation and continuance of rheumatoid inflammation (Fig. 3).

Patients with RA appear to have an excessive amount of T-helper cell activity in synovial tissues. Vasoactive substances also play a role in the inflammatory process. Histamine, kinins and PGs are released at the site of inflammation. These substances increase blood flow to the site of inflammation and the permeability of blood vessels. These substances cause the edema, warmth; erythema and pain associated with joint inflammation and make it easier for granulocytes to pass from blood vessels to the site of inflammation. Loss of cartilage may result in a loss of the joint space. The formation of chronic granulation or scar tissue can lead to loss of joint motion or bony fusion (ankylosis). Laxity of tendon structures can result in a loss of support to the affected joint, leading to instability. Tendon contractures also may occur, leading to chronic deformity.

MOLECULAR APPROACHES IN DEVELOPING RA

Inflammation is a normal and vital protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents. Inflammation results in the activation of cellular elements and various biochemical mediators. Inflammation is a part of complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants [7]. Acute inflammation is characterized by physiological signs such as pain, heat, redness, swelling and loss of organ functions. Chronic inflammation leads to the pathological condition such as RA and cancer [8]. The development of RA has following stages.

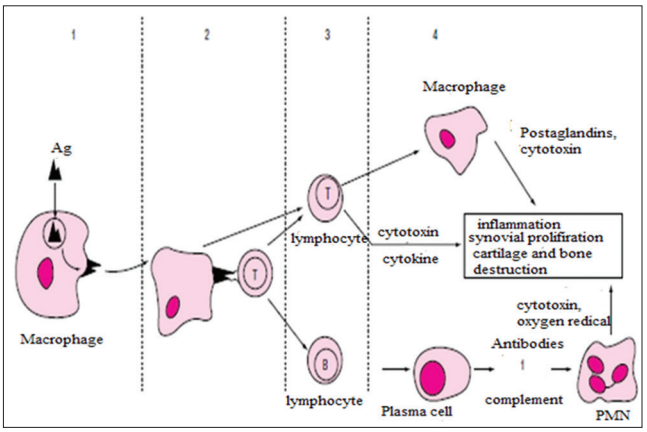

Fig. 3: Pathogenesis of inflammatory response [6]

Induction phase
Initial activation of the autoimmune system leads to an inflammatory cascade. Possible triggers are injuries, infections and exposures to toxic substances (smoking). These involves antigen-presenting cells (APCs) and the citrullination of relevant proteins, might occur outside of the joints as well as within them. Along with monocyte or macrophage infiltration into the synovium, and local synovial cells. Fibroblasts and macrophages are activated leading to the secretion of proinflammatory cytokines of both the innate and adaptive immune systems.

Inflammation phase
Self-antigens, notably citrullinated proteins, are presented in the context of Human leucocyte antigen Class II molecules that are characteristic of RA. This leads to polyclonal activation of T-cells and B-cells and formation of germinal-like centers in the synovial tissue. This process is insufficiently controlled by regulatory T (cell) cells.

Self perpetuation
Cartilage autoantigens, which are not normally accessible to the immune system, become exposed by damage and activating the immune system against cartilage tissue with further infiltration of pannus into the joints resulting in further destruction.

Destruction phase
Synovial fibroblasts and osteoclasts are activated by proinflammatory cytokines such as TNF-α and IL-6. It may cause destruction of bone and cartilage (Fig. 4).

Treatment of inflammatory diseases particularly chronic inflammatory diseases such as RA, inflammatory bowel disease, etc., has been a big challenge for scientists as there are no safe drugs available for cure. Pathophysiological studies indicate the presence of chemical mediators such as IL-1, 6 and 8, TNF-α, mutagen activated protein kinase (MAPKs), transcription factors and zinc dependent matrix metalloproteinase (MMP) etc., play a vital role in the progression of inflammation and inhibition of such inflammatory mediators can relieve the inflammatory diseases.

MOLECULAR TARGETS IN RA

Cyclooxygenase (COX)
NSAIDs are the most important class of widely used therapeutics for the treatment of inflammation and pain. The pharmacological effects of conventional NSAIDs are to inhibit COX. COX is a key enzyme responsible for the biosynthesis of prostanoids, biologically active substances that are involves in several physiological process and pathological conditions, like inflammation [10]. Membrane phospholipids with the help of enzyme phospholipase A_2 converted to arachidonic acid. The enzyme COX metabolized arachidonic acid to generate PGs. COX exists in two isoforms namely constitutive COX-1 and inducible COX-2. COX-1 is responsible for the biosynthesis of PGs, has a physiological role in cytoprotection of the gastrointestinal tract and platelet aggregation [11,12]. COX-2 is induced by pro-inflammatory mediator such as IL-1, TNF-α, lipopolysaccharide (LPS) and carrageenan. COX-2 plays a major rule in the biosynthesis of PGs in the inflammatory cell (macrophages and monocytes). The interruption of COX-1 activity may lead to gastrointestinal toxicity such as ulceration, bleeding and perforation [13]. Selective COX-2 inhibitors afforded alternative approach to arthritic treatment (Fig. 5).

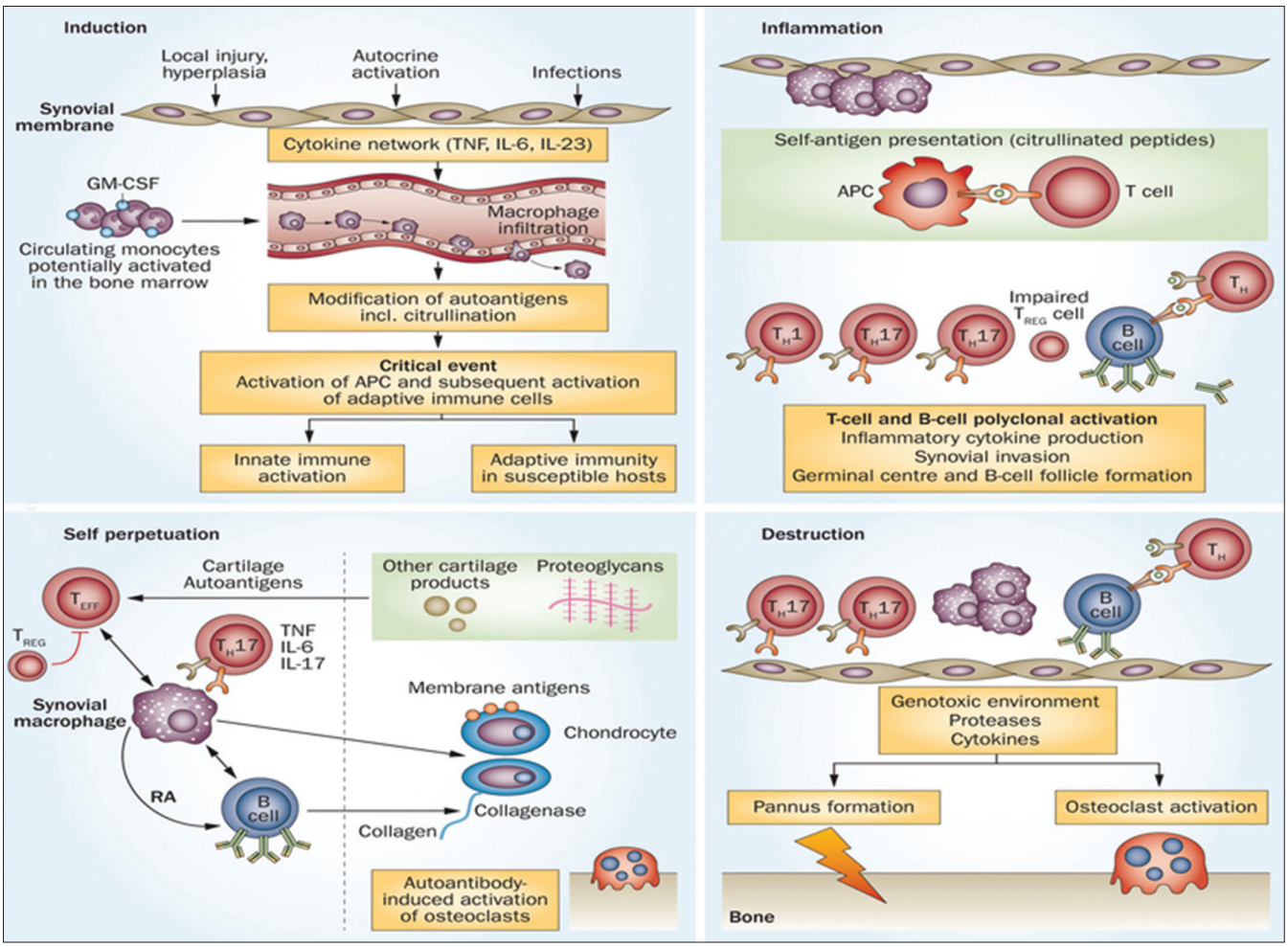

Fig. 4: Stepwise development of arthritis in rheumatoid arthritis [9]

Cytokines

Advances in the understanding of the role of cytokines in disease have led to new developments in the treatment of inflammatory diseases. Cytokine activities were first identified during the 1960s, and these small secreted proteins play a significant role in modulating the immune system and specifically the inflammation [16]. Although cytokines have pleiotropic activities, they are generally either pro-inflammatory, e.g., TNF-α and IL-1 or anti-inflammatory, e.g., IL-10 and transforming growth factor-β. Cytokine at low concentration stimulating expression of membrane proteins, proliferation of synoviocytes and secretion of effectors molecules.

TNF-α

In inflammatory diseases, the activation of macrophages causes the excessive release of pro-inflammatory cytokines such as TNF-α, IL-1β. The cytokine TNF has an important role in inflammatory processes in RA and in other immune-mediated disorders [17].

TNF-α is predominantly produced by macrophages but also by other immune cells including lymphocytes, natural killer cells and mast cells [18]. It activates macrophages, endothelial cells, synovial fibroblasts, chondrocytes, osteophytes stimulating cell proliferation, MMPs and adhesion molecule expression and release of other cytokines (Fig. 6) and PGs. TNF-α is found at high concentrations in rheumatoid synovial fluid and synovial tissue and aggravate the damage that is associated with RA [19].

TNF-α has emerged as an important target for the development of therapeutic strategies for treating RA. Two soluble forms of the TNF receptor (TNFR; namely p55 and p75) naturally occur in synovial fluid and can inhibit the action of TNF-α by competing with the cell surface receptors [20]. The TNF inhibitors that have been approved for clinical use to treat RA are infliximab, adalimumab and etanercept. Infliximab is a chimeric mouse human monoclonal antibody, whereas adalimumab is a fully humanized monoclonal antibody; both agents are specific for TNF. Etanercept is a fusion protein comprising the ligand-binding portion of the human p75 TNFRII and the Fc fragment of human immunoglobulin G (IgG1). The TNF inhibitors cause their primary effect by blocking the interaction of TNF with cell-surface receptors.Celltech has generated a polyethylene glycosylated (PEGylated) humanized antibody fragment that binds with high affinity to TNF-α (CDP 870) [21]. CDP 870 Phase III trials have suggested a promising response in RA, with a significant 20% reduction in the American College of Rheumatology score (ACR20) at week 1, which was maintained for the duration of the 24-weeks study. Amgen has developed a PEGylated soluble TNFR Type I (PEG sTNF-R1) that has shown efficacy in animal models and is now in Phase II trials [22,23]. ISIS Pharmaceuticals is producing ISIS 104838, an antisense oligonucleotide that reduces the production of TNF-α [24]. In Phase II trials have shown low toxicity, and further trials to determine dose and treatment duration are planned. Some TNF-α inhibitor drugs, which are FDA approved for the treatment of this disease. These drugs are biological response modifier (monoclonal antibody and fusion protein) they also produce some side effect. Infliximab-tuberculosis (TB), and sepsis. Adalimumab - TB and upper respiratory tract infection. Etanercept-chronic obstructive pulmonary disease (COPD), and respiratory tract infection [25].

TNF-α converting enzyme (TACE)

The complexity of TNF-α signaling and the various mechanisms associated with the regulation of TNF-α expression; there are many potential targets for the inhibition of TNF-α related activities (Fig. 7). Some protein-based inhibitors target the TNF-α molecule or its receptor, which prevents the resultant signaling pathways.

In addition targets include TACE, which processes the 26-kDa membrane form of TNF-α to the soluble 17-kDa form preventing its release into the circulation. Molecules are targeting intracellular TNF-α related signaling pathways have also been identified, including inhibitors of phosphodiesterase-4 (PDE-4) and p38. In addition, nuclear

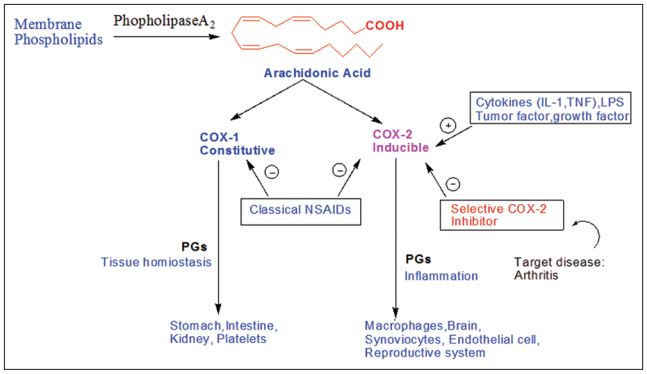

Fig. 5: Schematic representation of cyclooxygenase action [14,15]

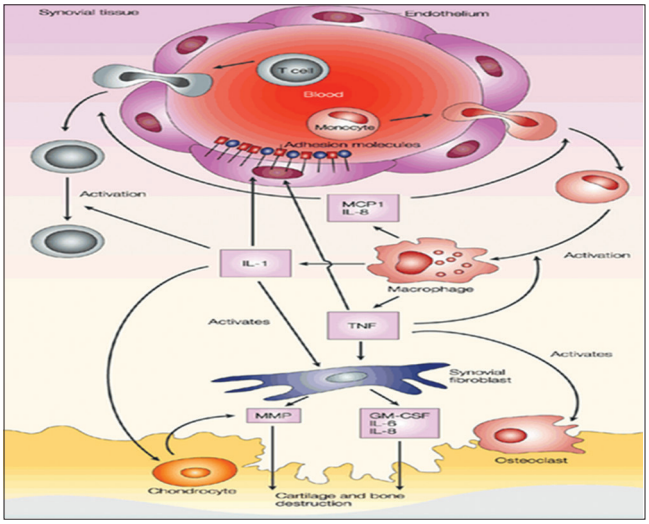

Fig. 6: Pathogenesis of tumor necrosis factor-α [5]

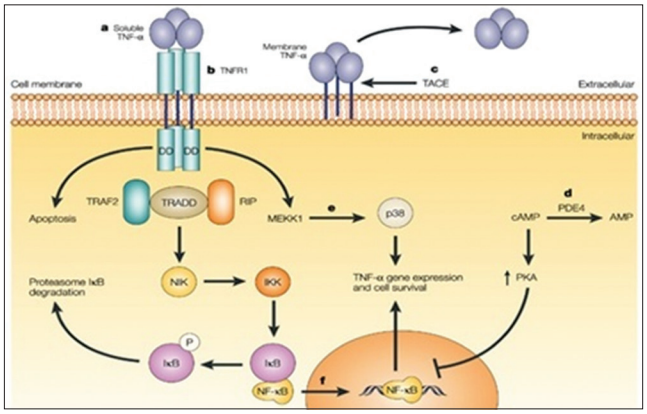

Fig. 7: Tumor necrosis factor-α signalling and the various mechanisms [26]

factor κB (NF-κB) inhibitors include molecules targeting the activities of upstream signaling events involved in the initial activation of NF-κB (NF-κB-inducing kinase [NIK], IκB kinase [IKK], IB degradation). Inhibition of the activation of NF-κB prevents the synthesis of NF-κB inducible genes, which include many proinflammatory cytokines, as well as other important inflammation-related proteins [26].

TACE promote the release active cytokine TNF-α from its membrane-bound precursor of the cell surface by enzymatic cleavage. Small molecular inhibitors of TACE that slow the progression of RA by preventing the release of soluble TNF from its membrane bound precursor [27].

Approach to TACE drug design will revolutionize this area of research and provide a novel therapy for TNF-α-based disorder. Dual MMP-TACE inhibitors GW3333 and GW4459 from GlaxoSmithKline (GSK), PKF 242 and PKF 484 from Novartis, and TMI-001 from Wyeth proved to be efficacious in various animal models ranging from collagen-induced arthritis (CIA) to the more severe adjuvant-induced arthritis [17]. However, preclinical studies were discontinued due to the development of liver toxicity.

Extensive efforts have been made to modify broad range MMP inhibitors (TMI-001) into selective compounds with drug-like properties. BMS-561392 by Bristol-Myers Squibb (BMS), and TMI-002 from Wyeth, showed increased affinity and able to decrease inflammation in the mouse CIA model. These results are important as they provide preclinical validation that TACE inhibition is beneficial for disorders associated with excessive TNF-α. In fact, a 10.5 mg/kg dose of BMS-561392 twice daily worked as well as Enbrel at reducing inflammation and a much lower dose, 2.8 mg/kg day, was more effective than remicade [28]. Two TACE inhibitors reached Phase II clinical trials but were not pursued due to lack of efficacy (TMI-005) and liver toxicity (BMS-561392) [29]. Both of these inhibitors showed some inhibitory activity against other MMPs, and it remains unclear whether selective inhibition of TACE would illicit liver toxicity. Compounds GW3333 and TMI-005 would have been considered beyond Phase II clinical trials. It remains unclear whether hepatoxicity is the direct result of the TACE inhibition or that of non-selective MMP inhibition (Table 1).

IL-1

IL-1 is a potent inducer of MMPs, eicosanoids, inducible nitric oxide synthase and receptor activator of NF-κB ligand, among many other factors, making it a key pro-inflammatory mediator. It is produced by macrophages, and its production is regulated by TNF-α in RA synovium. Therefore, IL-1 is also a potential therapeutic target in RA. IL-1 was the first cytokine to be identified in the synovial fluid of RA patients, contributing to the progression of inflammation and joint damage associated with RA [30]. In the mid-1980s, a naturally occurring inhibitor was identified, namely IL-1 receptor antagonist (IL-1RA). IL-1RA specifically blocks the effects of IL-1 without affecting TNF-α by binding the cell surface receptor IL-1R1 and preventing the activation of IL-1β. IL-1RA is also present in RA synovial fluid, but it appears that, in the disease state, there is an imbalance between IL-1 and IL-1RA. Recombinant non-glycosylated form of IL-1RA was tested in clinical trials kineret (anakinra) in 2001. Approval was given for treating the symptoms and joint destruction associated with RA. However, postmarketing surveillance has shown it to be much less effective than anti-TNF-α biological. It has a short half-life of 4-6 hrs.

Table 1: TACE inhibition, which under preclinical and clinical stage [17]

Name (former name)	Originator company (licensee company)	Mechanism of action	Highest development phase
GW3333	GSK	TACE and MMP inhibition	Preclinical
TMI-1	Amgen (Wyeth)	TACE and MMP inhibition	Preclinical
BMS-561392	BMS	TACE inhibition	Phase II
TMI-2	Wyeth	TACE inhibition	Preclinical
BMS-566394	BMS	TACE inhibitor	Preclinical
TMI-005	Wyeth	TACE and MMP inhibition	Phase II
DPC-A38088	BMS	TACE inhibitor	Preclinical
IK682	BMS	TACE inhibitor	Preclinical
DPH-067517	BMS	TACE inhibitor	Preclinical
R-618	Hoffmann-La Roche	TACE inhibitor	Phase I

TACE: Tumor necrosis factor-α converting enzyme, MMP: Matrix metalloproteinase

and is slower acting than the TNF biologicals [31]. Patients can undergo months of daily injections before any reduction in clinical disease activity is observed. Celltech has developed CDP 484, a PEGylated antibody fragment against IL-1. A Phase I trial was scheduled in 2003, but no results have been published. Caspase I IL-1β converting enzyme possible target aimed at decreasing the amount of IL-1 in RA, as it cleaves the precursor form of IL-1β, changing it to the mature active form. Vertex and aventis developed a caspase 1 inhibitor, called pralnacasan (HMR 3480/VX-740) but discontinued its Phase IIb trial after adverse toxicology results were received from a long-term animal study, although shorter trials are still being carried out [32].

IL-6

IL-6 is another cytokine that is found in abundance in both the serum and synovial fluid of patients with RA. IL-6 is produced by macrophages, monocytes, T lymphocytes, endothelial cells and synovial fibroblasts. The overproduction of IL-6 in RA might result in the production of autoantibodies owing to the differentiation of B-cells and activation of auto reactive T-cells [33]. IL-6 also activates osteoclasts, resulting in bone reabsorption, upregulates intercellular cell adhesion molecule 1 expression. Roche in collaboration with Osaka University has developed a humanized antihuman IL-6R monoclonal antibody (MRA). It was developed from the initial mouse anti-IL-6R monoclonal antibody (PM-1), which caused patients to generate antibodies to mouse immunoglobulin. An initial pilot study suggested that MRA was a safe and effective treatment for RA [34]. Tests were therefore continued to the level of Phase II trials, and again the results were positive: A clear dose-response relationship and good tolerance of the antibody were demonstrated. MRA has entered into a Phase III trial in Japan, and similar trials are planned for Europe and the USA. Neutralization of the receptor may have added benefits in the case of the IL-6 system.

IL-8

IL-8 has been found at increased levels in RA and is a potential therapeutic target in inflammatory diseases. Synovial fibroblasts, macrophages, endothelial cells and chondrocytes can all produce IL-8. An antibody to IL-8 has been shown to reduce joint swelling significantly in rabbits with monosodium-urate-crystal-induced arthritis [35]. Abgenix has completed a double-blind Phase IIa trial in RA patients using a human monoclonal antibody targeting IL-8 (ABX-IL8). However, the efficacy was disappointing, and there are no further plans for clinical development, Phase IIa and IIb trials with ABX-IL8 were also performed in patients with moderate to severe psoriasis and COPD, respectively, but these trials also proved unsuccessful.

IL-12

IL-12 is a potential target in RA as it has been found in elevated levels in the synovial fluid of RA patients [36]. It is mainly produced by macrophages and dendritic cells. Experiments using an anti-IL-12 antibody have shown promising results in arthritis mouse models. In one study, an IL-12 antibody prevented the development of CIA in interferon γ (IFN-γ) receptor knockout mice. IL-12 antibody has also been reported to act in synergy with anti-TNF-α antibodies to inhibit the progression of CIA in mice, producing a greater effect than anti-TNF-α alone. IL-12 p40 has been shown to be shared with IL-12 and IL-23. Thus, it is not clear whether these results in animal models were due to blockade of the action of either IL-12 or IL-23. Collaboration between Cambridge Antibody Technology and Abbott has produced a human anti-IL-12 monoclonal antibody called ABT-874 (formerly J695), which has proved to have some potential for treating Crohn's disease and multiple sclerosis. A multi-center, dose-randomized, double-blind, placebo-controlled study of ABT- 874 is currently in progress to test its potential for treating RA.

IL-15

IL-15 has been detected in RA joint synovium. It is produced by monocytes and a variety of other cells. IL-15 activates T-cells and promotes the release of more IL-15 and stimulates macrophages in a cell-contact-dependent manner to release TNF-α. Pre-clinical data

have suggested that a soluble fragment of IL-15Rα may be useful as a therapeutic; when this fragment was administered to DBA/1 mice; it profoundly suppressed the development of CIA [37]. A human monoclonal antibody against IL-15 (HuMax-IL-15) has been tested in a Phase II trial for RA by Genmab and Amgen. The antibody was found to be safe (with no dose-limiting toxicity) and well-tolerated by the patients. The results showed HuMax-IL-15 to be more beneficial than a placebo in patients who had previously failed to respond to treatment with DMARDs.

IL-18

IL-18 was originally identified as an IFN-γ inducing factor. It is mainly produced by macrophages and is closely related to IL-1α and IL-1β. IL-18 is capable of enhancing the production of IL-1 and TNF and works in synergy with IL-12 and IL-15, which are both present in the synovium and increase the production of other cytokines [38]. Elevated levels of IL-18 have been observed in RA synovial fluid. A naturally occurring inhibitor to IL-18 is IL 18 binding protein (IL-18bp), a molecule that binds IL-18 in the fluid phase and prevents it from binding to cells. Pre-clinical data have suggested that the development of IL-18bp might produce a beneficial therapy for RA. In the murine CIA model, mice infected with an adenovirus expressing the murine gene encoding IL-18bp isoform showed less severe inflammation or bone erosions in their joints than mice, not expressing IL-18bp [39]. The pharmaceutical company Serono has put an IL-18bp (Tadekinig-α) into a Phase IIa trial for RA, the results of which are awaited.

MMP inhibitors

The MMPs are a family of 25 zinc- and calcium-dependent proteinases that are involved in the degradation of the extracellular matrix. MMPs degrade bone and cartilage in the RA joint; tissue inhibitors of metalloproteinases (TIMPs) occur naturally and regulate the activities of MMPs. A balance between MMPs and TIMPs is essential for the normal turnover of extracellular matrix components [40]. Low-molecular-weight molecules that bind to zinc or other parts of the catalytic site of MMPs have been pursued as inhibitors. The broad-range MMP inhibitors batimastat (MB94), marinastat (BB 2516) and CG 270323A are used to treat cancer patients but have all been ruled out as treatments for RA, because trials with marinastat revealed a drug-related toxicity causing upper-body musculoskeletal pain and stiffness of the joints that spread in a time-dependent manner but was reversible on withdrawal of the drug [41]. Trocade (Ro 32-3555), an oral MMP-1 inhibitor developed by Roche, appeared to be a strong candidate for testing in clinical trials after it was shown to be a potent inhibitor of cartilage resorption *in-vitro* and *in-vivo*, acting as a collagenase inhibitor. Trocade being well-tolerated by patients, trials were discontinued after 1 year because its efficacy was limited. Currently the class of antibiotics known as tetracyclines are the only form of treatment in clinical use against MMPs, and the tetracycline doxycycline has been shown to inhibit the activity of some MMPs [42]. Although antibiotics seem to have a beneficial effect for the treatment of RA, the tetracyclines can induce lupus. Thus, although targeting MMPs could theoretically be beneficial in RA, it is clear that a new approach to inhibiting these proteases needs to be developed.

NF-κB

NF-κB proteins are a family of transcription factors that play an important role in several physiological processes including cell survival, proliferation and activation. The NF-κB family comprises five members, relA (p65), relB, c-Rel, p105/p50 and p100/p52, all of which share the Rel homology domain that allows their dimerization and translocation to the nucleus. NF-kB dimers are bound to inhibitors of NF-κB (IκB) proteins, which retain NF-κB in the cytosol. In the so-called canonical or classical activation pathway, inflammatory mediators such as TNF-α and IL-1, activate IKK2 within the multisubunit IKK complex phosphorylates IκB inducing its ubiquitination and degradation by the proteasome, releasing NF-κB to translocate the nucleus [43]. An alternative or non-canonical pathway of NF-κB activation, under the control of NIK and IKK1. In response to stimuli such as lymphotoxin beta and CD40L, NIK has been shown to activate IKK1, leading to inducible processing of p100 with preferential nuclear translocation of p52-RelB dimers. Once activated, NF-κB is a transcription factor that regulates many inflammatory mediators, including cytokines, chemokines, adhesion and co-stimulatory molecules, MHC Class I and II antigen-presenting molecules, enzymes and antiapoptotic proteins [44]. In RA, NF-κB appears to be activated and localized in the nucleus of both macrophage and fibroblast like synoviocytes from both early and later stage patients. NF-κB can promote joint destruction by inducing osteoclasts maturation and increased bone-resorbing activity. By inhibiting chondrocytes differentiation and repair of damaged cartilage tissue. The inhibition of NF-κB through the over expression of IκBα in RA synovial membrane cultures down regulates the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-8. NF-κB inhibition also results in the down regulation of MMP 1 and MMP 3 without affecting the beneficial expression of TIMP, the natural inhibitor of MMPs. In animal models of arthritis, NF-κB inhibition has a beneficial effect and potential therapeutic target in RA. One strategy to block NF-κB activation involves targeting the 26s subunit of the proteasome, thus inhibiting IκBα degradation, NF-κB nuclear translocation, as well as preventing inducible p100 NF-κB processing [45]. One such proteasome inhibitor bortezomib (velcade; millennium), fast-track clinical development and has recently been approved by the FDA for the treatment of multiple myeloma and is under development for various other hematological malignancies. Moreover, in chronic inflammatory diseases such as RA, the side effects of bortezomib may not be acceptable for longer term treatment. Thus, the most promising approach to block NF-κB specifically appears to be through the targeting of IKK2, which phosphorylates IκBα, allowing it to be degraded. Although in RA synovial membrane cultures, the inhibition of IKK2 only marginally affects TNF-α production, it has profound inhibitory effects on the expression of most other cytokines (e.g. vascular endothelial growth factor, IL-1β, IL-6, IL-8) and MMPs, also inhibits the activation of the endothelium [46].

MAPKs

The MAPKs are a group of related kinase proteins that require dual phosphorylation of tyrosine and serine/threonine. The three major ones are p38, p42/44 (ERK) and p46/54 (JNK), which are activated by a kinase cascade. Activated p38, p42/44 and p54 MAPKs are all present in synovial tissue of RA patients [47]. p42/44 MAPK activation is localized around the synovial microvessels, p54 MAPK activation is found around and within the mononuclear cell infiltrates. p38 MAPK activation is mostly seen in the synovial lining layer and in synovial endothelial cells. The importance of p38 MAPK in inflammation was demonstrated by, inhibition of this enzyme have effect on TNF-α production in LPS stimulated macrophages. In human monocytes/macrophages, p54 MAPK (JNK) has been proposed to control TNF-α production at the translational level, whereas p42/44 MAPK (ERK) affects TNF-α production at the transcriptional level [48]. The expression of other inflammatory cytokines such as IL-1, IL-6 and IL-8 is also regulated by MAPKs. In mouse or rat models of arthritis, p38 MAPK inhibitors such as SB 203580, SB 220025, SB 242235, RWJ 67657 or RPR200765A, some of which are also orally effective and reduce the incidence of arthritis. The p54 MAPK inhibitor SP600125 prevents radiological joint destruction and moderately decreases paw swelling in mouse arthritis [49]. The inhibition of p38 MAPK demonstrated the importance of this enzyme to TNF-α synthesis, and the p38 MAPK has been seen as a key target for the treatment of RA.

PDE-4 inhibitors

The PDE-4 enzyme inactivates cAMP and is expressed in inflammatory cells such as monocytes and dendritic cells. Inhibitors of PDE-4 are useful in controlling inflammation because they bring about a sustained elevated level of cAMP that leads to activation of protein kinase A and subsequent inhibition of transcription factors like NF-κB that transcribe inflammatory genes (e.g., TNF-α). The main areas of investigation for PDE-4 inhibitors have been in the treatment of COPD and asthma. PDE-4 inhibitors have proved to have a therapeutic benefit in animal

models. One of the inhibitor rolipram manufactured by AG Scientific has proved beneficial in a mouse model of asthma. Rolipram has also been tested in a rat arthritis model where it was observed to have anti-inflammatory actions in suppressing TNF-α and inhibition of cellular infiltration, as well as suppressing bone and cartilage destruction. Rolipram has also been tested in a clinical trial but was found to have a dose limiting side effects of nausea and emesis (vomiting). Another inhibitor, ariflo (cilomilast) manufactured by GSK, has also been tested in clinical trials and was given FDA approval for the treatment of COPD. Daxas (roflumilast), which is being developed by Altana Pharma, is one of a new generation of PDE-4 inhibitors that are better tolerated and, therefore, show more potential for clinical approval. Multinational Phase III clinical studies around Europe involving patients who have asthma and COPD have shown positive results.Celgene is also developing a group of PDE-4 inhibitors, the selective cytokine inhibitory drugs, which incorporate the phthalimide fragment of thalidomide. The PDE-4 inhibitors under investigation by Celgene have the advantage of lower emetic effects compared with inhibitors that are currently in trials. The most potent of these inhibitors, CDC-998, was well tolerated at the doses administered in a Phase I clinical trial [50].

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)

Class II MHC molecules present antigens to CD4+ T-cells that activate them and have been associated with susceptibility to RA. Activated T-cells have also been present in the RA synovium. Full activation of T-cells requires the activation of CD28 as well as the engagement of the T-cell receptor with an MHC peptide complex on an antigen presenting cell. CTLA4 is transiently expressed on the cell surface of T-cells and can bind CD80 and CD86 on APC as well as CD28 on T-cells, but interacts with higher avidity to CD80 and CD86 than to CD28. CTLA4 blockade of CD28 engagement inhibits the activation of T-cell and has been suggested to be a useful therapy in RA. Human CTLA4 fused to the constant region of IgG1, to increase its half-life, has been used in clinical trials involving RA patients. Initial results indicated that abatacept (CTLA4-Ig) (along with MTX) is a promising therapy for RA, because the trial patients experienced a significant improvement in their symptoms [51]. A Phase III clinical trial involving RA patients has shown positive results, suggesting that CTLA4-Ig may be useful in the treatment of patients who do not respond well to conventional treatments.

IL-10

IL-10 produced by monocytes, macrophages, B-cells and T-cells acts *in vitro* to decrease the production of pro-inflammatory cytokines and can increase the production of IL-1RA. IL-10 is a potential therapeutic strategy in RA. Mature B-cells contribute to autoimmunity by their ability to produce cytokines, present antigens and secrete autoantibodies. Autoantibodies such as anti-cyclic citrullinated peptide antibodies have become a useful diagnostic tool for RA [52]. The interest in B-cells as a major factor in the pathogenesis of RA has been generated by the success with anti-CD20 antibody in clinical trials. Rituximab has been well tolerated by RA patients in clinical trials when given either on its own or in combination with glucocorticoids, MTX or cyclophosphamide. The greatest benefit is achieved when rituximab is used in combination with MTX, including in those patients who previously were non-responsive to MTX. In a Phase IIb trial, B-cell depletion was evident for 6 months after two initial infusions of rituximab.

Janus kinase (JAK)-Signal transducer and activator of transcription (STAT)

JAKs are a family of non-receptor protein tyrosine kinase that affect intracellular signaling through their association with transcription factors known as STATs, otherwise known as the JAK-STAT pathway. JAKs are constitutively bound to their associated receptors and are activated when the corresponding cytokine or growth factor binds to its receptor. Activated JAKs phosphorylates the tyrosine residues on the receptor, causing a conformational change, allowing the binding of a STAT protein in the SRC2 homology (SH2) domain. JAKs then phosphorylates the tyrosine residues on the STAT proteins, allowing for dimerization of the STATs, which then migrate into the cytoplasm and translocate into the nucleus, allowing for transcription of their target genes [53]. Within the family of JAKs, there are four members: JAK1, JAK2, JAK3 and tyrosine kinase 2 (Tyk2). JAK1, JAK2 and Tyk2 are fairly ubiquitously expressed in mammalian cells, JAKs is associated with cytokine receptors and is activated when a cytokine binds to its cognate receptor. Therapeutic targets in the JAK-STAT pathway, specifically JAK inhibitors, are currently under investigation for myeloproliferative neoplasms (JAK1 and JAK2), transplant rejection and autoimmune diseases including RA [54,55].

Spleen tyrosine kinase (SYK)

SYK is another cytoplasmic tyrosine kinase, and is a member of the ZAP70 and family of non-receptor protein kinase SYK is expressed on most hematopoietic cells, including B-cells, immature T-cells, macrophages, neutrophils and mast cells, and therefore plays a role in both the innate and adaptive immune response. It is also expressed on platelets and in some non-hematopoietic cells, including osteoclasts and lymphatic vessels [56]. Several SYK inhibitors are currently being investigated in clinical trials for human diseases, particularly RA.

Bruton's tyrosine kinase (BTK)

BTK is another key intracellular kinase under investigation. It is a member of the Tec family of cytoplasmic protein tyrosine kinase. It is predominantly expressed in hematopoietic cells, including B-cells, but also in cells of myeloid lineage such as macrophages, two cell lineages believed to be quite important in the treatment of inflammatory arthritis and other autoimmune diseases. Animal models of arthritis suggest that BTK inhibition can result in inhibition of B-cell receptor-dependent cell proliferation and a reduction of inflammatory cytokine production from myeloid cells (including TNF, IL-1 and IL-6) by preventing signaling through the FCyRIII receptor [57]. More recently, BTK has been shown to be a key element in the signaling pathways induced in macrophages by LPS stimulation of toll-like receptor 4 leading to the production of TNF-α, a key cytokine in RA pathogenesis.

Phosphatidylinositol 3-kinases (PI3K)

PI3Ks are a group of intracellular signaling molecules that phosphorylates the 3-hydroxyl group of the inositol ring of phosphatidylinositol lipid substrates. Their functions are diverse, affecting cell growth, survival, proliferation and metabolism in a variety of cell types. Inhibitors of Class, I PI3K p110 isoforms are being studied in inflammatory diseases such as asthma, RA, systemic lupus erythematous and certain hematological malignancies [58].

Clinical complications

TNF-α and IL-1 have been the main clinical target for RA, but these proteins are also major pro-inflammatory cytokines in the role against infection. Inhibiting their effects can, therefore, increase the risk of infection. Patients who were treated with anti-TNF-α therapies have a lupus-like syndrome, demyelination syndrome and serious infections including bacterial sepsis and incidence of TB. TNF-α is known to play an important role in host defense against TB in a TNF-α knockout mouse model anti-TNF-α therapy infliximab may be due to the reduced IFN-γ production. IFNγ activates phagocytosis and resulted in the killing of intracellular bacteria (Table 2) [59].

SUMMARY AND CONCLUSION

- As most of the world population get affected by RA, and it is a chronic inflammatory autoimmune disorder, presently there are no specific drugs available for the curability of the disease
- The drug of choice available are NSAIDs, DMARD, corticosteroid, immunomodulatory agent. These drugs have some undesirable side effect-NSAID-cardiovascular risk and gastrointestinal bleeding, MTX - liver damage, sulfasalazine-gastrointestinal events and allergy to sulfur, leflunomide-liver toxicity
- Some TNF-α inhibitor drugs, which are FDA approved for the treatment of disease. These drugs are biological response modifier (monoclonal antibody and fusion protein) they also produce some side effect: Infliximab- TB and sepsis, adalimumab - TB and upper

Table 2: Summary of RA therapeutics in development [59]

Name	Type/target	Manufacturer	Clinical stage
Infliximab	Monoclonal antibody/ TNF-α	Centocor Inc.	FDA approved
Etanercept	Receptor/TNF-α	Immunex/ Wyeth	FDA approved
Adalimumab	Monoclonal antibody/ TNF-α	Abbott	FDA approved
CDP 870	Monoclonal antibody/ TNF-α	Celltech	Phase III
PEGylated	Soluble receptor/ TNF-α	Amgen	Phase II
ISIS 104838	Antisense oligonucleotide		Pre-clinical
TMI-1	Small molecule/TACE	Wyeth	Pre-clinical
Anakinra	Protein/IL-1	Amgen	FDA approved
IL-1	TRAP receptor/IL-1	Regeneron	Phase Iib
CDP 484	PEGylated antibody	Celltech	Phase I
Pralnacasan	Small molecule/ Caspase I	Vertex/aventis	Discontinued
MRA	Monoclonal antibody/ IL-6	Chugai/Roche	Phase III
ABX-IL8	Monoclonal antibody/ IL-8	Abgenix	Discontinued
ABT-874	Monoclonal antibody/ IL-12	CAT/Abbott	Phase II
HuMax-IL-15	Monoclonal antibody/ IL-15	Genmab/ Amgen	Phase II
IL-18bp	(Tadekinig-α) protein/ IL-18	Serono	Phase IIa
Trocade	Small molecule/MMP-1	Roche	Discontinued
Abatacept	Fusion protein/CTLA4	Repligen	Phase III
Rituxan	Monoclonal antibody/ CD20	Genentech	Phase III

TNF-α: Tumor necrosis factor-α, IL: Interleukin, MMP: Matrix metalloproteinase, TACE: Tumor necrosis factor-α converting enzyme, TRAP: Thrombin receptor activating peptide, PEGylated: Polyethylene glycosylated

respiratory tract infection, abatacept - COPD and respiratory tract infection

- Similarly, some TACE inhibitor drug, which is in Phase-II clinical trial like (TMI-005) and (BMS-561392) have liver toxicity
- As the production of TNF-α, a key cytokine in RA pathogenesis, so there is need of some anti-TNF molecule an oral small molecule that regulates TNF-alpha biology could either replace the injectables or provide better disease control when used alone or in conjunction with existing therapies.

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