Chapter 2

Review of Literature
2. Review of literature

2.1. Inflammation: an overview

Inflammation, a very important response of the immune system can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. It is essential to keep us alive and help recover from injuries and illnesses. When the inflammation process continues for a prolonged period it is known as chronic inflammation.

Chronically it can be related to persistent infection, ulceration, mechanical or chemical irritation, or autoimmune diseases. Wherever inflammation occurs there are certain common local mechanisms, despite differences in the causal factors and also in the relative prominence of the four cardinal features. Wherever inflammation is located, the condition is given a name ending in –itis, prefixed by the traditional name of the body part, such as arthritis for the joints, gastritis for the stomach, etc.

The inflammatory response directs the immune system components to the site of injury or infection and is manifested by increased blood supply and vascular permeability which, allows the chemotactic peptides, neutrophils, and mononuclear cells to leave the intravascular compartment. Microorganisms are engulfed by phagocytic cells (e.g., neutrophils and macrophages) in an attempt to contain the infection in a small-tissue space. The response includes attraction of phagocytes in a chemotactic gradient of microbial products, movement of the phagocyte to the inflammatory site and contact with the organism, phagocytosis (ingestion) of the organism, development of an oxidative burst directed toward the organism, fusion of the phagosome and lysosome with degranulation of lysosomal contents, and death and degradation of the organism (Weiss et al., 2009).
2.2. Rheumatoid arthritis

2.2.1. Background

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease, which primarily affects the joints and is characterized by inflammation of synovial joints in a symmetrical pattern. It also involves synovitis secondary to hyperplasia of synovial cells, synovial fluid accumulation, and pannus formation (Feldmann et al., 1996a). Active disease is characterized by pain, swelling and stiffness of joints in combination with raised levels of inflammatory markers. The inflammatory process can lead to destruction of cartilage and bone, resulting in joint deformities, permanent functional impairment and disability. The most commonly affected joints are the small joints of the fingers, wrists, feet, and ankles. In RA, the joints are often affected in a quite symmetrical fashion, although this is not specific, and initially it may be asymmetrical. RA can also produce inflammation in the pericardium, lungs, sclera, and...
pleura, and formation of nodular lesions, under the skin. The disease follows a variable course with remissions and exacerbations. Due to large inter-individual variability in disease activity, degree of joint destruction and systemic involvement, RA is a very heterogeneous disease. This is also a chronic illness that can last for years. Patients may experience long symptom free periods also.

Afflicting more than 1% of the world population, it commonly starts in the middle age (40-60 years) but can also develop at any other age group. RA is associated with various genetic and environmental factors and known to affect women about three times as frequently as men; however its prevalence is not race specific.

![Image of normal hand and intermediate stage of RA](image1.png)

**Figure 2.2.** Normal hand and the progressive deformities in small joints of rheumatoid arthritis (RA) (Top). Schematic diagram compares among normal and the joints affected during the intermediate and late stages of RA in the cellular level (below). The number of joints affected in RA (Top right).

### 2.2.2. Etiology

The etiology of RA is unknown but it is suggested that a genetic susceptibility in
combination with the influence of environmental factors are involved.

2.2.2.1. Environmental factors

Several infectious agents such as viruses, bacteria, and fungi have long been suspected as the cause for this disease. Certain infections or factors in the environment as well as smoking tobacco has been reported to increase the risk of developing rheumatoid factor (RF) positive RA (Manfredsdottir et al., 2006). Smoking also increases the risk of developing anti-cyclic citrullinated peptide antibodies (ACPA) in individuals with the shared epitope allele. However, none of these environmental factors have been proven as the cause for this disease. Gender and hormonal factors are also important with higher prevalence in women than in men, a difference that is less obvious after menopause.

2.2.2.2. Genetic factors

RA does not aggregate in families with very high frequency (Gregersen, 1999). In addition, concordance rates in identical (monozygotic) twins are relatively low compared with other autoimmune disorders, which generally have monozygotic twin concordance rates in the range of 30%. Nevertheless, the prevalence rates of RA in first-degree relatives of probands with RA are considerably higher than in the general population as a whole.

The description of the human leukocyte antigen (HLA) associations with RA over two decades ago has been a major source of support for the hypothesis that genetic factors are important for susceptibility to RA. The contribution of HLA to the overall genetic risk has been variously estimated at between 30 and 50%.

The major histocompatibility complex in susceptibility to RA: Since the late 1980s, a consensus has developed around susceptibility to RA being due to a closely related set of polymorphic sequences (the ‘shared epitope’) on several DRB1 alleles, especially certain subtypes of the DR4 and DR1 allelic families (Nepom, 1998). The
unifying concept of the shared epitope has considerable appeal for understanding the MHC class II associations with RA, but it is clearly an oversimplification to consider the shared epitope as the only relevant MHC polymorphism for susceptibility to RA. There are important haplotypic influences on the degree of risk conferred by the shared epitope alleles. Certain shared epitope alleles, such as DRB1*0401, confer much greater risk than others, such as DRB1*0101. In addition, homozygosity for particular combinations of haplotypes, such as DRB1*0401/0404, appear to confer especially high risk or influence disease severity as well as risk (Gregersen, 1999).

2.2.3. Pathogenesis of rheumatoid arthritis

The inflammation usually starts in the synovial joints and more specifically in the synovium. Current hypothesis assumes an inappropriate immune response, triggered by a yet undiscovered exogenous or endogenous antigen. Different antigens activate macrophage-like and fibroblast-like synoviocytes, resulting in synovium hyperplasia followed by infiltration by macrophages and lymphocytes. This inflamed tissue, called pannus, extends over and invades the cartilage and bone. Antigen presenting cells (tissue macrophages or dendritic cells) activate CD4+ T-cells, which stimulates macrophages, monocytes and fibroblasts to produce a number of cytokines, including TNF-α. The cells in the pannus also produce excessive amount of pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin 1-beta, IL6 and receptor activator of nuclear factor-κB ligand (RANKL). Neutrophils from the circulation are attracted to the synovial fluid by chemo attractants. Fibroblasts, macrophages and neutrophils produce proteolytic enzymes and oxygen metabolites that contribute to matrix degradation. Besides resulting cartilage and bone degradation, pro-inflammatory cytokines are also found in the circulation leading to systemic symptoms and manifestations. This inflammatory response remains active due to disequilibrium between pro-inflammatory and anti-inflammatory cytokines, and is assumably driven
by dendritic cells or CD4+ T-cells (Zink et al., 2005). Earlier studies have indicated TNF-α being at the top of a cascade of pro-inflammatory cytokines and that blockade of TNF-α would suppress the production of other inflammatory mediators. Today we use biologic agents targeting TNF-α, IL1 and IL6.

2.2.4. Signs and symptoms of rheumatoid arthritis

The most prominent symptom of RA is the pain in the joints. These joints may have complete inflammation associated with redness, heat, swelling and an obvious pain. The most commonly affected joints are the small joints of the fingers, wrists, feet, and ankles. In RA, the joints are often affected in a quite symmetrical fashion, although this is not specific, and initially it may be asymmetrical too. The inflammation of RA can also occur in tissues around the joints, such as the tendons, ligaments, and muscles.

The characteristic symptoms of RA include the stiffness of affected joints which arises in the morning after waking up, or after long durations of rest, such as sitting in a particular position. Loss of sensation in the joints, weakness of muscles attached to the particular joints leading to inability to do heavy work. There is a constant feeling of tiredness, which may become quite extreme with a sharp bout of pain and relapse of fevers with acute pains. The symptoms of RA appear and disappear, depending on the degree of tissue inflammation. The disease is active with the presence of tissue inflammation and becomes inactive (in remission) after the inflammation subsides. Remissions may occur spontaneously or with treatment, and can last for weeks, months, or even years. During remissions, symptoms of the disease disappear, and patients generally feel well. When the disease relapses, symptoms reappear. The return of the disease and associated symptoms is called a ‘flare’. The course of RA varies from patient to patient, and periods of flares and remissions are typical (Wright et al., 1998). The extra-articular symptoms of RA include small painless lumps or 'nodules' developed under the skin at the palms, forearms, elbows, knees and at the Achilles'
tendons. Generally these nodules are not painful, occasionally they can become infected and cause deformities. Inflammation around tendons may occur. This is because the tissue which covers tendons is similar to the synovium around the joints.

As RA does not confine itself to the bones and joints alone, several complications can arise in different organs of the body such as fibrosis can be caused in the lungs, amyloidosis in the kidneys and xerophthalmia, i.e. the dry eye and dry mouth condition referred to as Sjogren’s syndrome. A rare, serious complication, usually with long-standing RA, is blood vessel inflammation (vasculitis) which in turn impairs blood supply to tissues and lead to tissue death. Other complications include endocarditis, pericarditis, fibrosis, valvulitis, and even failure of the left ventricle. The joint responsible for the tightening of vocal cords to change the tone of our voice, the cricoarytenoid joint, is also affected by RA, but rarely, and cause hoarseness of voice. RA induced anemia with reduced number of red blood cells and white blood cells can be associated with an enlarged spleen (referred to as Felty’s syndrome) and make the patient susceptible to infections.

2.2.5. Diagnosis of rheumatoid arthritis

An accurate diagnosis only enables appropriate treatment of RA. With several types of arthritis, early symptoms can overlap and diagnosis of RA can be difficult. As no single diagnostic symptom, sign or test exists, the diagnosis is often based upon the revised classification criteria adapted by the American College of Rheumatology (ACR) in 2010 (Aletaha et al., 2010). There is no single test which clearly diagnoses early RA. Medical history, physical examination, blood tests, and imaging studies are generally required.

When a patient first develops joint pains, it may be difficult for a doctor to say that he definitely has RA. This is because there are many other causes of joint pains. The
rheumatologist examines the patients’ joints for inflammation and deformity, the skin for rheumatoid nodules, and other parts of the body for inflammation.

Disease activity score (DAS) (calculated using DAS-28 disease score calculator, (Prevoo et al., 1995) is generally used for the diagnosis of the disease on the basis of the number of tender joints involved, number of swollen joints, erythrocyte sedimentation rate (ESR) and visual analogue score (VAS) for general health as subjectively estimated by the patients. 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 DAS (Sato et al., 2006). In time, X-rays of joints may begin to show typical erosions (early damage) and other features of RA, makes the diagnosis more certain (Scott et al., 1984). The diagnosis will be based on the pattern of symptoms, the distribution of the inflamed joints, and the blood and X-ray findings. The distribution of joint inflammation is important to the doctor in making a diagnosis (Arnett et al., 1988). In RA, the small joints of the hands, wrists, feet, and knees are typically inflamed in a symmetrical distribution (affecting both sides of the body). When only one or two joints are inflamed, the diagnosis of RA becomes more difficult. The doctor may then perform other tests to exclude arthritis due to infection or gout. The detection of rheumatoid nodules, most often around the elbows and fingers, can help the diagnosis. Erythrocyte sedimentation rate (ESR) is used as a crude measure of the inflammation of the joints which is usually faster during disease flares, and slower during remissions. Another blood test that is used to measure the degree of inflammation present in the body is the C-reactive protein (CRP). The RF factor, ANA, ESR and C-reactive protein tests can also be abnormal in other systemic autoimmune conditions. Therefore, abnormalities in these blood tests alone are not sufficient for a firm diagnosis of RA (Skogh et al., 2003). Analysis of the joint fluid, in the laboratory (arthrocentesis), can help to exclude other causes of arthritis, such as infection and gout (Norberg et al., 1983). Blood tests can detect inflammation, characteristic antibodies, and
anemia which may suggest the presence of RA. These tests include those for IgM and IgG RF, anti-CCP antibodies. Presence of these characteristic autoantibodies like RF factors and anti-citrullinated protein antibodies (ACPA) has been implicated in disease diagnosis and are widely studied in patients with RA. A test for anti-CCP antibodies is most helpful in looking for the cause of previously undiagnosed inflammatory arthritis when the traditional blood test for RA, i.e. RF, is not present. Citrulline antibodies have been felt to represent the earlier stages of RA in this setting (Nielen et al., 2004). Another antibody called “the antinuclear antibody” (ANA) is also frequently found in patients with RA (Chellingworth et al., 1984). Earlier, our laboratory reported a significant increased level of anti-MBL-antibodies in the RA patients as compared to healthy controls (Gupta et al., 2006).

Figure 2.3. Cytokine pathways involved in RA (Choy and Panayi, 2001)

2.2.6. Role of cytokines in rheumatoid arthritis

Early studies suggested an unrestricted abundance of cytokines in the rheumatoid joint (Firestein and Zvaifler, 1990). However, later experiments
demonstrated a relative paucity of many T cell-derived cytokines, including IL-2, IL-4, and TNF-β. One exception is IL-17, which can regulate cartilage metabolism and may be produced by CD4+ T cells in the joint. T cells can also potentially contribute to macrophage and synoviocyte activation by inducing metalloproteinase gene expression via direct cell-cell contact.

T helper cells can be divided into subsets that mediate distinct functions of the immune system. T helper type 1 (Th1) cells produce interferon gamma and IL-2 but not IL-4, IL-5, or IL-10; T helper type 2 (Th2) cells produce the opposite cytokine profile. Th1 over activity predominates in most animal models of autoimmunity, whereas Th2 cytokines mediate disease suppression. The small amounts of T cell cytokines that can be detected in RA are biased toward the Th1 phenotype, including IL-17. In contrast, Th2 cytokines (especially IL-4) are virtually absent from the joint. Some IL-10 is present but is derived mainly from macrophages, and the amount is not sufficient to suppress Th1 cytokine production. The relative lack of suppressive Th2 cytokines may contribute to the pathogenesis of rheumatoid synovitis. Levels of other suppressive cytokines, such as the natural IL-1 receptor antagonist (IL-1ra), are also low in RA joint tissues.

Macrophage-and fibroblast-derived cytokines, e.g., IL-1, IL-6, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are abundantly expressed in the rheumatoid joint (Feldmann et al., 1996b). Although many of these cytokines are involved in the pathogenesis of RA, TNF-α and IL-1 are major pathogenic factors: both can induce synoviocyte proliferation, collagenase production, and prostaglandin release; over expression of either TNF-α or IL-1 can induce arthritis in animal models (Bingham, III, 2002). IL-15 is produced by macrophages but shares many activities of the T cell-derived cytokine IL-2. It increases the ability of T cells to induce TNF-α production by macrophages through an antigen-independent mechanism that involves cell-cell contact. IL-18 is also present in the RA joint and can bias T cell responses towards Th1 or directly activate macrophages to produce proinflammatory mediators.
Cytokine networks can potentially establish paracrine or autocrine networks that can perpetuate arthritis long after the etiologic agent has been cleared. Studies have suggested that anti-cytokine therapy (including therapy with IL-1, TNF-α, and IL-6) is effective in severe RA and demonstrates the importance of fibroblast and macrophage products in chronic synovitis (Edwards, 2005).

2.2.7. Role of oxidative stress in rheumatoid arthritis

The antioxidant defense system is compromised in RA patients. There is a shift in the oxidant/antioxidant balance in favor of lipid peroxidation, which could lead to the tissue damage observed in the disease. Generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is an important factor in the development and maintenance of RA in humans. ROS are produced in cells by several physiological and environmental stimulations, such as infections, ultraviolet radiation and pollutants, known collectively as oxidants. Interestingly, ROS have also been considered as risk and enhancer factors for autoimmune diseases as there is a significant relation between the oxidative stress and such diseases.

Several lines of evidence suggest a role for oxidative stress in the pathogenesis of RA. Radical species with oxidative activity, which include RNS and ROS represent the mediators and effectors of cartilage damage (Vasanthi et al., 2009). ROS are involved in extracellular matrix degrading activity. RNS are derived from the oxidation of the guanido nitrogen of L-arginine, with production of a nitrogen-centred radical, nitric oxide (NO), by the enzyme nitric oxide synthase (NOS). Nitric oxide is known to have several physiological roles, including the regulation of platelet function, neurotransmission and the killing of intracellular pathogens (Droge, 2002). Growing evidence implicates NO in immune regulation, inflammation, autoimmunity and arthritis. Epidemiologic studies have shown an inverse association between dietary intake of antioxidants and RA incidence, and inverse associations between antioxidant
levels and inflammation have been found (Cerhan et al., 2003). Iron, a catalyst for hydroxyl radical production from hydrogen peroxide, is present in RA synovial tissue and is associated with poorer prognosis. Several groups have demonstrated increased oxidative enzyme activity along with decreased antioxidant levels in RA sera and synovial fluids (Hitchon and El-Gabalawy, 2004b). Because of the highly reactive nature of ROS, it is difficult to directly demonstrate their presence in vivo. It is considerably more practical to measure the ‘footprints’ of ROS and RNS, such as their effects on various lipids, proteins, and nucleic acids. Thus, evidence for oxidative stress in RA has in many cases been generated by approaches that detect oxidant-induced changes to these molecules (Henrotin et al., 2003c). Studies of RA synovial fluid and tissue have demonstrated oxidative damage to hyaluronic acid, lipid peroxidation products, oxidized low-density-lipoprotein (LDL), and increased carbonyl groups reflective of oxidation damage to proteins (Filippin et al., 2008). Evidence of oxidative damage to cartilage, extracellular collagen, and intracellular DNA has also been demonstrated (Ediz et al., 2011). Oxidative stress has been shown to induce T cell hyporesponsiveness in RA through effects on proteins and proteosomal degradation. Finally, antioxidants and oxidative enzymes have been shown to ameliorate arthritis in animal models (Bo et al., 2009; Cuzzocrea et al., 2000a).

ROS and RNS damage cellular elements in cartilage directly and damage components of the extracellular matrix either directly or indirectly by up regulating mediators of matrix degradation (Henrotin et al., 2003b). Modification of amino acids by oxidation, nitrosylation, nitration, and chlorination can alter protein structure and impair biological function, leading to cell death. ROS impair chondrocyte responses to growth factors and migration to sites of cartilage injury; RNS, in particular NO, interfere with interactions between chondrocytes and the extracellular matrix (Hitchon and El-Gabalawy, 2004b). NO can also increase chondrocyte apoptosis. Oxygen and nitrogen radicals inhibit the synthesis of matrix components including proteoglycans by
chondrocytes. In particular, NO and O\textsubscript{2} seem to inhibit type II collagen and proteoglycan synthesis and the sulfation of newly synthesized glycosaminoglycans. Oxygen radicals can cause low levels of collagen fragmentation and enhanced collagen fibril cross-linking. Oxygen radicals have also been shown to fragment hyaluronan and chondroitin sulfate and damage the hyaluronan binding region of the proteoglycan core protein, thereby interfering with proteoglycan – hyaluronan interactions (Henderson et al., 1991). In addition, ROS and RNS can damage the components of the extracellular matrix indirectly through the activation and up regulation of matrix metalloproteinases. Therefore, one therapeutic approach to treat RA is to remove these reactive oxygen species (ROS).

2.2.8. Current anti-arthritis drug regimen

Till date, the goal of treatment in RA is to reduce joint inflammation and pain, maximize joint function, and prevent joint destruction and deformity. Early medical intervention has been shown to be important in improving outcomes. Aggressive management can improve function, stop damage to joints as seen on x-rays, and prevent work disability. Optimal treatment for the disease involves a combination of medications, rest, joint strengthening exercises, joint protection, and patient (and family) education. Treatment is customized according to many factors such as disease activity, types of joints involved, general health, age, and patient occupation. Treatment is most successful when there is close cooperation between the doctor, patient, and family members. Medications are the cornerstone of treatment for active RA, which are mainly given symptomatically. Two classes of medications are used: fast-acting “first-line drugs,” and slow-acting “second-line drugs (also referred to as Disease-Modifying Antirheumatic Drugs or DMARDs).” The first-line drugs or NSAIDs, are the painkillers, such as aspirin, nimesulide, ibuprofen, etc., reduce inflammation. Additional medications are frequently recommended to protect the stomach from NSAID induced
ulcers, which include antacids, sucralfate, proton-pump inhibitors, and misoprostol. NSAIDs interfere with only a small cascade of the inflammatory steps, namely, prostaglandin production by cyclooxygenase (COXs), but not the underlying immuno-inflammatory reaction. Therefore, NSAIDs do not slow the progression of the disease. Furthermore, NSAIDs are relatively old and conservative treatments for RA. It has been reported that COX-2-specific drugs may have side effects that can increase the risk of cardiovascular diseases (Rho et al., 2009).

By contrast, DMARDs, immunosuppressive agents, retard or halt disease progression or delay disease onset but have multiple side effects, some of which are undesirable including toxicity over the long term. They do not suppress the progression of clinical disability (Lee et al., 2009). Pain killers are most frequently used which may cause many untoward effects such as kidney damage, gastritis and liver dysfunction.

A number of immunosuppressive drugs are also used to treat RA. They include methotrexate, azathioprine, cyclophosphamide, and cyclosporine. Because of potentially serious side effects, immunosuppressive medicines are generally reserved for patients with very aggressive disease.

Corticosteroid medications can be given orally or injected directly into tissues and joints. They are more potent than NSAIDs in reducing inflammation, and in restoring joint mobility and function. Corticosteroids are useful for short periods during severe flares of disease activity, or when the disease is not responding to NSAIDs. However, corticosteroids can have serious side effects too, especially when given in high doses for long periods of time.

2.3. Use of natural products in the treatment of RA and/or inflammation

2.3.1. Natural products in drug discovery

For thousands of years, natural products have played a very important role in healthcare and prevention of diseases. The ancient civilizations of the Chinese, Indians
and North Africans provide written evidence for the use of natural sources for curing various diseases. The earliest known written document is a 4000 year old Sumerian clay tablet that records remedies for various illnesses. For instance, mandrake was prescribed for pain relief, turmeric possesses blood clotting properties, roots of the endive plant were used for treatment of gall bladder disorders, and raw garlic was prescribed for circulatory disorders. These are still being used in several countries as alternative medicines. However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants. Friedrich Sertürner isolated morphine from *Papaver somniferum* in 1806, and since then natural products have been extensively screened for their medicinal purposes. Atropine obtained from *Atropa belladonna*, strychnine, a CNS stimulant, ziconotide, identified from a cone snail, *Conus magus*, and Taxol® obtained from the bark of the Pacific yew tree are a few examples of active components isolated from natural sources.

According to the studies conducted by the World Health Organization (WHO), about 80% of the world’s population relies on traditional medicine [WHO 2002]. About 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources. Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics. Between the years 1981-2006, about a hundred anticancer agents have been developed, of which, twenty five are natural product derivatives, eighteen are natural product mimics, eleven candidates are derived from a natural product pharmacophore, and nine are pure natural products (Khanna et al., 2007). Thus natural sources make a very significant contribution to the health care system.

The use of plants as medicines has a long history in the treatment of various diseases. The earliest known records for the use of plants as drugs are from Mesopotamia in 2600 B.C., and these still are a significant part of traditional medicine and herbal remedies. To date, 35,000-70,000 plant species have been screened for their medicinal use. Several
important drugs such as Taxol®, camptothecin, morphine and quinine have been isolated from plant sources. The first two are widely used as anticancer drugs, while the remaining are analgesic and antimalarial agents, respectively.

2.3.2. Natural products in the treatment of rheumatoid arthritis

Arthritis (both osteoarthritis (OA) and RA) is one of the foremost diseases for which patient seeks the complementary and alternative medicine (CAM) option (Ahmed et al., 2005); (Soeken et al., 2003b). Because there is little evidence about the long-term effects of currently available traditional medicines and its associated adverse effects, the American College of Rheumatology recommends the careful use of dietary supplements and herbal medicines during early stages of treatment or disease development to limit the degree of joint destruction. It is no surprise, then, that the use of alternative medicine, such as botanicals and nutritional supplements, has become popular with arthritis patients and is on the rise. An increasing number of people in the United States, as many as 42% use CAM approaches to meet their personal health problems (Soeken et al., 2003c).

However, despite optimal use of currently available anti-rheumatic agents, most RA patients live with chronic pain and severe functional decline because these therapies focus primarily on preventing joint inflammation and soft tissue swelling, but are not effective in preventing cartilage breakdown and the joint destruction associated with RA. Because of these and other limitations, the use of CAM therapies, such as acupuncture and extracts of medicinal herbs, is on the rise and according to reports 60–90% of dissatisfied arthritis patients are likely to seek the option of CAM therapy (Engel and Straus, 2002). While most of the rheumatologists and other clinicians are skeptical of CAM therapies, patients who use CAM appear to be satisfied with the self-care approach. This self-satisfaction is mostly based on the notion that since these herbs and plants are found in nature, remedies derived from them must be safe. However, the
long-term safety and efficacy of most of the herbal preparations commonly promoted as anti-arthritic have not been established by placebo-controlled randomized trials either in OA or RA patients and indeed some of these may even interfere with the ongoing treatments. Therefore, it is imperative that scientific evidence regarding the safety and efficacy of herbal preparations commonly used by arthritis patients be presented to both the physicians and the patients helping them in making informed decisions.

The majority of naturally occurring phenolics found in plants possess tremendous anti-oxidative and anti-inflammatory activities (Yadav et al., 2003). Anti-inflammatory properties of various phytochemicals are mediated through the inhibition of nitric oxide (NO), prostaglandins, leukotriene synthesis, and production of cytokines such as interleukin IL-1β, IL-6, IL-12, IFN-γ and TNF-α. Antioxidants such as epigallocatechin-3-gallate (Lin and Lin, 1997), resveratrol and naturally occurring flavonoids, including apigenin and kaempferol (Liang et al., 1999) have been reported to suppress NO production through inhibition of NF-κB.

With this perspective the present study is designed to screen a large number of medicinal plants already used in traditional system of medicine for treating inflammatory diseases such as arthritis, inflammatory bowel disease, asthma, atherosclerosis etc in murine macrophage cells. The rationale behind the use of most of these plants is still unknown. Therefore, the present study is designed to elucidate the signaling mechanism underlying such anti-inflammatory effects. This attractive hypothesis encourages further studies but demands rigorous experimentation. Once the underlying molecular mechanism(s) for the observed anti-inflammatory and chondroprotective effects of nutraceuticals are elucidated, their health benefits may be exploited to develop new and better modalities for treating degenerative and inflammatory joint diseases.

2.3.3. Role of antioxidants in the treatment of rheumatoid arthritis
Antioxidants are the compounds of exogenous or endogenous in nature which either prevent the generation of toxic oxidants or intercept any that are generated and inactivate them and thereby block the propagation of chain reaction produced by these oxidants. These can be classified as enzymatic antioxidants, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, non-enzymatic antioxidants like (nutrient antioxidants) beta-carotene, α-tocopherol, ascorbic acid, bioflavonoids and (metabolic antioxidants) like glutathione, ceruloplasmin, albumin, bilirubin, ferritin, transferrin, uric acid and lactoferrin. Increasing experimental and clinical data has provided compelling evidences for the involvement of FR/ROS in large number of pathophysiological states (Pillai and Pillai, 2002) including RA. This has led to increased interest amongst the researchers globally to evaluate role of antioxidant therapy in RA.

Furthermore, another study suggested that therapeutic co-administration of antioxidant along with conventional drugs to RA patients result in statistically significant increase in the post-treatment concentration of these antioxidants, decrease in the concentration of MDA along with improved symptoms (Jaswal et al., 2003). Similarly antioxidants and few fatty acids have been suggested to ameliorate RA and related disorders (Darlington and Stone, 2001). The difference with RA treatment is that more antioxidants are needed. Not only there is a need of antioxidants to stop the free radical damage and inflammation, but also to help in the improvement of the immune system. It is very essential to get the amount of required nutrients and antioxidants to stop the damage and correct the immune system is to supplement and eating a healthy diet is still important.

2.4. TNFα and anti-TNF antagonists in the treatment of rheumatoid arthritis

2.4.1. TNF: role in the pathogenesis of chronic inflammatory diseases and anti-TNF immunotherapy.
The central role of TNF-α in the pathogenesis of several immunomediated and inflammatory disorders has been described. The pathogenetic role of TNF in chronic inflammatory diseases has been initially suggested by the finding that high levels of TNF were present in the synovium of joints in RA (Matsuno et al., 2002), where TNF-α mediates both inflammatory synovitis and articular matrix degradation (Keystone et al., 2004). TNF-α-directed biologic immunotherapy is a successful tool for the treatment of many inflammatory diseases (Patel and Gordon, 2004). In particular, among the best studied are RA, Crohn’s disease, and psoriasis/psoriatic arthritis.
2.4.2. Anti-TNF agents inhibit TNFα

Targeting of pro-inflammatory cytokines has become a strategic basis for developing therapies to treat RA. Approaches that have been investigated include soluble TNF receptors, soluble IL-1 receptors, IL-1 receptor antagonists and monoclonal antibodies to TNFα (Koopman and Moreland, 1998). TNFα activity can be inhibited at various steps. The FDA has approved TNF-α blocking agents and IL-1 antagonists for clinical use. The TNF blockers include etanercept, infliximab and adalimumab. Anakinra is the recombinant form of IL-1ra (Sautner and Leeb, 2003; Weaver, 2004). Metalloproteinases are also one of the preferred therapeutic targets in RA (Bigg and Rowan, 2001). Natural and synthetic MMP inhibitors have also been identified and some are in early clinical testing (Black et al., 1997); (Parsons et al., 1997)

2.5. Suramin a pleiotropic drug is anti-TNF, thus can be anti-arthritic

Suramin, the hexasodium salt of 8, 8’-(carbonyl-bis (imino-3, 1-phenylene-carboxylimino (4-methyl-3,1-phenylene) carbonylimo) bis-1,3,5-naphthalenetrissulfonic acid is a polysulfonated naphthylurea which has found clinical use as an anti-parasitic
agent since the 1920s. Suramin also has demonstrated an ability to inhibit the activity of various growth factors in vitro and has therefore been used clinically to treat various cancers. The compound has also been noted to bind to a variety of cytoplasmic and intranuclear enzymes but the exact mode of its action is still not fully understood. People have also attempted to use suramin and related compounds for several other purposes such as a collagenase inhibitor and for inhibition of the complement activators associated with angioneurotic edema (Quinke’s Disease). Some studies have speculated on the use of suramin to treat RA and related diseases and autoimmune diseases such as experimental autoimmune uveoretinitis (EAU).

Suramin has anti-TNFα activity. It specifically promotes dissociation of the biologically active trimeric form of TNF-α into inactive subunits, thus inhibiting the binding of TNF-α to its cellular receptors as TNF-α exerts its effects by binding to two receptors, TNFR1 or TNFR2 (Alzani et al., 1993; Alzani et al., 1995). Suramin also blocks the activity of IL-6 as it interferes with the binding of IL-6 to its cell surface receptor implying it to be very effective in inhibiting inflammation (Mancini et al., 1999; Strassmann et al., 1993). Suramin has been recently reported to be an effective treatment of rheumatological disorders.

2.6. Use of peptides in drug discovery

Drug designing and development using suitable synthetic and natural peptides has been proved to be a promising field of drug discovery. The basis for choosing peptide is the one or more number of sequences which may be the parts of the target proteins. These peptides may be responsible for molecular recognition and other biological processes. The main objectives of the field include the designing of peptides in such a way that it will inhibit the protein-protein interactions and to mimic the small molecule inhibitors of the target proteins as ligands. The targeted mode of action makes the peptides as an ideal candidate for drug development.
To look for a peptide mimetic, it is very important to identify a peptide or a peptide sequence within the target protein that is considered for the assay. The structural constraints are added to check the effects of these features.

The interaction of a peptide with a biological target may occur via a direct binding of a linear sequence in any number of conformations accessible to a peptide. The modern peptide mimetics approach incorporates a production of small molecules which mimic peptides in order to overcome their ineffectiveness as drugs when administered orally. The small molecular mimetics retain the desired biological properties of the peptide lead, but are metabolically stable, have unlimited diversity, and can be designed to provide the new drugs.

The increasing importance of proteins and peptides can be attributed to three main developments. First, improved analytical methods have promoted the discovery of numerous hormones and peptides that have found applications as biopharmaceuticals. Second, molecular biology and genetic engineering have enabled the large-scale production of polypeptides previously available only in small quantities. Lastly, there is a better understanding of the role of regulatory proteins/peptides in the pathophysiology of human diseases. Simultaneously, pharmaceutical companies around the world have endeavored to develop the processes for producing therapeutically active entities at commercial scales.

Peptides are very specific towards their targets. Also, small peptides have advantages over proteins for therapeutic application, due to their stability, solubility, increased bio-availability and lack of immune response in the host cell. Small peptides have advantages over proteins for therapeutic application, due to their stability, solubility, increased bio-availability and lack of immune response in the host cell. However, instability of peptide is due to the proteolytic cleavage of the peptide backbone. Low bioavailability of the peptides is also a major drawback associated with peptide drugs which is partly due to the poor membrane transport capability of its
amide backbone structure. Designing and formulating a polypeptide drug delivery through the gastro intestinal tract has been a persistent challenge because of their unfavorable physicochemical properties, which includes enzymatic degradation, poor membrane permeability and large molecular size.

2.7. Glycosylation alteration of proteins in rheumatoid arthritis

Structural modifications of proteins are an essential feature of living cells, and yet such modifications when aberrantly regulated are often the basis of disease. Two of the most abundant forms of protein modification, phosphorylation and glycosylation, are topologically restricted from each other. Phosphorylation, which takes place in the cytoplasm and nucleus of cells, involves the reversible addition of phosphate molecules. In contrast, glycosylation, which is mostly confined to the secretory pathway, involves the addition and removal of different monosaccharide linkages (termed glycans) to proteins and lipids that are bound to the cell surface and extracellular compartments.

Although glycans are the most abundant and diverse of nature’s biopolymers, much less is known about their functions than the other basic components of cells - nucleic acids (DNA and RNA), lipids, and proteins. Nevertheless, one percent of the mammalian genome encodes enzymes that produce and modify glycan structures among all cell types. Glycans bearing multiple monosaccharide linkages may exist as single or multi-antennary branched oligosaccharide structures. During embryogenesis, normal adult physiology, and in many disease states, the appearance of different glycan linkages at the cell surface occurs in part by altered gene expression involving glycosyltransferases and glycosidases. Oligosaccharide variants thus occur on glycoproteins, can lead to alterations in protein activity and functions. Human plasma is rich in glycoproteins, many of which exist in different glycoforms. Aberrant glycosylation of cell surface glycoconjugates and serum glycoproteins are known to be involved in a variety of biological phenomena such as inflammation, cell
differentiation, infection, tumor progression, and metastasis. Changes in protein glycosylation are early indicators of cellular changes in many such diseases, providing useful diagnostic markers and insights into disease progression and pathogenesis (Raghav et al., 2006d). Protein glycosylation is a posttranslational event which depends on the protein core and biosynthetic cell type and results in a set of microheterogeneous forms (glycoforms) of an individual glycoprotein. Under pathological conditions an alteration of the glycosylation pattern of plasma glycoproteins may occur. Thus, degalactosylated IgG and IgA1 detected in RA and IgA nephropathy respectively, are implicated in the pathogenic mechanisms. Alteration of transferrin, alpha 1-acid glycoprotein and alpha-fetoprotein glycosylation (reduced sialylation and increased branching of oligosaccharide chains) occurs in liver diseases. In inflammations and infections the alteration is dependent on the disease studied, while increased sialylation and fucosylation of acute-phase proteins are detected in cancer sera. Lectin-based methods have been developed for clinical purposes, in order to improve the diagnosis, prognosis evaluation, or treatment monitoring.

Alteration in the glycosylation of IgG (agalactosylated IgG/IgG0) has been directly implicated in the pathogenesis of RA (Bond et al., 1996). The IgG0 levels correlate with the disease severity in patients with RA. Rheumatoid factors, the characteristic autoantibodies which bind to the Fc region of agalactosylated IgG molecule, are increased in the sera of patients with RA (Carson et al., 1987). Another plasma protein alpha-1-acid glycoprotein (AGP produced by the liver) is known to be present in different glycoforms, and alteration in its glycosylation was reported in several pathological conditions including RA (Smith et al., 2001).

In RA, cytokines produced at inflammatory lesions are believed to travel via the circulation to the liver where they induce the production of acute phase proteins by hepatocytes (Moshage, 1997). Some proteins are over-expressed whereas the levels of some others are decreased, termed as positive and negative acute phase proteins.
respectively (Gabay and Kushner, 1999). Acute phase proteins are not merely associated with inflammation, rather they are contributing to the disease pathogenesis as well, and for example, C-reactive protein has been implicated in the atherosclerotic disease (Hak et al., 1999). Particularly in the area of joint inflammation acute phase proteins related to complement cascade have been found in excess. One such protein, namely, mannose-binding lectin, due to its strong affinity for the exposed GlcNAc of IgG₀ (agalactosyl IgG), has been found to stimulate the complement pathway following its binding with IgG₀. Such complexes have been recovered from the synovial fluid of RA patients (Malhotra et al., 1995). Hence, it appears that differential expression pattern of proteins, as acute phase response, may be significant in the pathogenesis of RA.

The oligosaccharide profile of acute phase proteins is modified in inflammatory disorders (Turner, 1995), without any relation to the serum concentrations of these glycoproteins during acute inflammation. In several serum glycoproteins, such as AGP, TRF, and α2-HS glycoprotein, during acute inflammation the number of branches is reduced, whereas during chronic inflammation, the number increases.

2.8. Experimental animal models of rheumatoid arthritis

Animal models are usually used by pharmaceutical companies and other research labs for evaluation of new therapeutic agents, analysis of genetic susceptibility factors and search for biomarkers of RA and other inflammatory diseases (Bendele, 2001c). In the area of RA, there are excellent models that have good track records for predictability. This is in large part due to the fact that numerous agents have been evaluated in clinical trials of this disease and criteria for assessment of efficacy are measurable. It is believed that these animal models, regardless of the method of induction, will predict the aetiopathogenetic mechanisms involved in the human disease. Some of these animal models resemble the human disease with respect to
clinical features and histopathology; but, unfortunately this resemblance does not apply to the entire spectrum of manifestations found in RA.

2.8.1. Collagen-induced arthritis (CIA)

This is the well established *in vivo* model that has been used for studies of new drugs for therapeutic intervention of RA. Both CIA and RA share many clinical, histological and immunological features. This animal model of RA has been utilized extensively for elucidating pathogenetic mechanisms and for evaluating novel therapies. CIA can be induced in both male and female rats (Sprague-Dawley, Wistar, Lewis, etc) in 5-6 weeks’ age-group, mice (DBA/1 and B10) and primates (monkeys) following immunization with type II collagen (CII). CII is the principal protein found in the articular and hyaline cartilage, it constitutes about 50% of all cartilage proteins and 80-90% of collagen of articular cartilage. Intradermal injection of heterologous (porcine, chick, bovine) CII in Freund’s complete adjuvant (FCA) induces a chronic polyarthritis in rats (Bendele, 2001b). Primary immunization is followed by a booster dose of CII dissolved in acetic acid and emulsified in FCA. The onset of CIA usually starts around 12-16 days after primary immunization with variable incidence of CIA. The disease progression is asymmetrical and any combination of joints can be affected up to varying degrees. The onset of disease is characterized by marked cartilage destruction, bone resorption, moderate to marked synovial cell proliferation, polymorphonuclear cell infiltration and periarticular inflammation.
Figure 2.6. The comparative morphology of the hind paws of the healthy control and CIA rats (Sahu et al., 2012).

Apart from CIA, there are some other animal models which are comparatively less features with RA, namely, adjuvant-induced arthritis (AIA), Carrageenan induced paw edema, formaldehyde induced arthritis, cotton pellet induced granuloma, etc.