Introduction
1.1 Epidemiology

Rheumatic diseases were first recognized by Hippocrates in the fourth century B.C. In the first century A.D., the term ‘rheuma’ was first introduced to indicate a flow of pain through the joints of the body. The appearance and distribution of lesions in ancient skeletons suggest that rheumatoid arthritis (RA) may have existed in North America at least 3000 years ago (Goemaere S, 1990). The first clinical description of RA is credited to Augustin-Jacob Landre Beuvais in his thesis in 1800 (Sangha O, 2000). Sir Alfred Garrod first introduced the term ‘rheumatoid arthritis’ in 1876 (Garrod A, 1876). Paleopathologic studies have identified bone erosions consistent with RA in many Native American skeletons dating as far back as 6500 years ago in a circumscribed area of the Mississippi Basin (Rothschild BM, 1988). Rheumatic diseases have a major impact on individuals and societies, and economic costs in all countries.

Rheumatoid arthritis is prevalent worldwide among all races. Studies indicate a point prevalence of between 0.5 to 1% (Spector TD, 1990). Throughout the world, there are pockets of ethnic groups that have a much higher incidence of rheumatoid arthritis. North American Indians are one of such groups. In one geographic area, for instance, non-Indian populations had an RA prevalence of 0.9 to 1.1% between 1986 and 1994, whereas the prevalence in Algonquian Indians in the same region ranged from 2 to 2.1% and the disease onset was 12 years earlier in the Indian population (Peschken CA, 1998).

Environmental Factors

Gender: The prevalence of RA is clearly higher in females, the estimated ratio being ~2.5:1 (Mitchell DM, 1985). It is assumed that the risk for the higher incidence of RA among women is related to sex hormones (Spector TD, 1990). Estrogens have a generally
stimulatory effect on the immune system, and this may be a factor in the increased female-to-male ratio (Masi AT, 1995). The relative risks of developing RA in women appear to fluctuate with different stages of the reproductive cycle throughout their lives, from menarche to menopause. Oral contraceptives may protect women from developing more severe disease (Spector TD, 1990, Van Zeben D, 1990).

**Age:** An age associated increase in the prevalence of RA has also been observed in both males and females (Engel A, 1993).

**Education level:** There is an increased mortality and morbidity from RA in patients, particularly women, who have had less formal education (Pincus T, 1985).

**Climatic conditions:** In the Northern hemisphere, the onset of RA is more frequent in winter than in summer. In several seasons, the onset of RA from October to March in the Northern hemisphere was found to be twice as frequent as in the other 6 months (Jacoby RK, 1973).

**Infectious Agents:** Epstein-Barr virus (EBV) has been linked to RA for more than 25 years. Eighty percent of the patients with RA have a circulating antibody directed against antigens specific for EBV (Alspaugh MA, 1981) and the autoantibody response in RA enhances the response to these antigens (Venebles P, 1988). Epstein-Barr virus is well established as a polyclonal activator of B lymphocytes, resulting in the overproduction of immunoglobulins including rheumatoid factor (Slaughter L, 1978). Mycobacteria have often been linked to rheumatoid arthritis. Patients with RA have elevated levels of antibodies to heat-shock proteins from recombinant mycobacteria (Tsoulfa G, 1989).

**Endogenous Factors:** Cartilage may be invaded and destroyed by the proliferative synovitis and an immune response mounted against the epitopes on degraded portions of
collagen (Jasin HE, 1983). The collagen-antibody complexes, along with rheumatoid factor-IgG complexes, can precipitate within superficial layer of cartilage and serve as chemoattractant for the invasive tissue (Rowley M, 1986, Choi EK, 1988). Collagen and IgG are the endogenous proteins implicated in rheumatoid arthritis (Stuart JM, 1984). One of the hypotheses for RA states that RA is not caused by the development of antibodies to collagen (Type II) found in articular cartilage, but rather synovitis and the centripetal polarization of destructive arthritis leads to the disease (Mottonen T, 1988). Elevated titers of antibody to both naïve and denatured forms of Type II collagen are found in the serum of patients with rheumatoid arthritis (Jasin HE, 1985).

**Genetic Factors**

Studies have indicated a genetic predisposition for rheumatoid arthritis (Gregersen P, 1999, Cornelis F, 1998). Severe RA is found at approximately four times the expected rate in first-degree relatives of individuals with disease associated with the presence of rheumatoid factor, and ~10% of patients with RA have an affected first-degree relative (Silman AJ, 1993). In addition to age and sex-related predisposing factors, a number of other factors, including socio-economic status, education and stress have been suggested to play predisposing roles (Sangha O, 2000).
1.2 Etiopathogenesis

Rheumatoid arthritis is a chronic, multisystemic, autoimmune disorder of unknown cause. The major characteristic feature of RA is the chronic, symmetrical and erosive synovitis of peripheral joints (Sangha O, 2000). The diagnosis of RA is based on the presence or absence of combinations of clinical, laboratory and radiological abnormalities in individual patients. Earlier, the most widely used criteria to estimate the prevalence of RA have been those of the 1958 American Rheumatoid Association (Ropes MW, 1958). A modified definition of RA referred to as the American College of Rheumatology (ACR) 1987 revised criteria for the classification of RA, was published in 1988 (Arnett FC, 1988). These criteria distinguish RA from other rheumatic conditions, with a specificity of 89% and sensitivity between 91% and 94%.
Table 1.2.1: 1988 revised American Rheumatism Association criteria for classification of Rheumatoid Arthritis

<table>
<thead>
<tr>
<th>Criterion</th>
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<tr>
<td>1. Morning Stiffness</td>
<td>Morning stiffness in and around the joints lasting at least 1 hour before maximal improvement</td>
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<td>2. Arthritis of three or more joint areas</td>
<td>At least three joint areas simultaneously having soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician (the 14 possible joint areas are (right or left) PIP, MCP, wrist, elbow, knee, ankle, and MTP joints)</td>
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<td>3. Arthritis of hand joints</td>
<td>At least one joint area swollen as above in wrist, MCP, or PIP joint</td>
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<td>4. Symmetric arthritis</td>
<td>Simultaneous involvement of the same joint areas (as in criterion 2) on both sides of the body (bilateral involvement of PIP, MCP, or MTP joints is acceptable without absolute symmetry)</td>
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<tr>
<td>5. Rheumatoid Nodules</td>
<td>Subcutaneous nodules over bony prominences or extensor surfaces, or in juxta-articular regions, observed by a physician</td>
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<tr>
<td>6. Serum rheumatoid factor</td>
<td>Demonstration of abnormal amounts of serum “rheumatoid factor” by any method that has been positive in less than 5 percent of normal control subjects</td>
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<tr>
<td>7. Radiographic changes</td>
<td>Changes typical of RA on PA hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized to or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)</td>
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* For classification purposes, a patient is said to have RA if he or she has satisfied at least four of the seven criteria. Criteria 1 through 4 must be present for at least 6 weeks. Patients with two clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is not to be made.

Abbreviations: MCP: Metacarpophalangeal; MTP: Metatarsophalangeal; PA: Posteroanterior; PIP: Proximal interphalangeal

The majority of patients have elevated titers of serum rheumatoid factors (RF). The course of RA can be quite variable, and can differ in different patients. Some patients may experience only a mild oligoarticular illness of brief duration with minimal joint damage, whereas others may have a relentless progressive polyarthritis with marked functional impairment and disability (Sangha O, 2000).

No specific etiological agent has been identified as the causative agent and there are no unique clinical or laboratory features that can be used to define the disease clearly (Paul Wordsworth, 2001).

Rheumatoid arthritis is a multifactorial systemic autoimmune disease. Rheumatoid arthritis can be broadly classified into various phases, based on its pathobiologic, clinical, diagnostic and therapeutic stages (Choy E, 2001).
Figure 1.2.1: Cross-section of a normal joint and joint with early and established Rheumatoid arthritis (Choy E, 2001)
The symptoms and physical signs start with mild joint stiffness, swelling of small joints, fever, malaise and weakness leading to deformity of the joints in the last stages (Edward D Harris Jr, 1990).

**Stages of Rheumatoid Arthritis are:**

1. Presentation of antigen to the T-cells
2. Proliferation of the T & B cells followed by angiogenesis in the synovial membrane (SM)
3. Accumulation of the neutrophils in synovial fluid (SF). Synovial cell proliferation without the invasion of the cartilage
4. Polarization of the synovitis into the centripetally invasive pannus with the activation of the chondrocytes and initiation of enzyme degradation of cartilage
5. Erosion of subchondrial bone, invasion of the cartilage by the pannus, chondrocytes proliferation and stretched ligaments around the joints.

The presentation of a relevant antigen to an immunogenetically susceptible host is believed to trigger rheumatoid arthritis. Antigen-presenting cells ingest, process and present foreign protein antigens to T lymphocytes, which initiate a cellular immune response and stimulate the differentiation of B lymphocytes into plasma cells that secrete antibody. The relevant receptors on antigen-presenting cells, the class II major histocompatibility complex (MHC) locus is known to be associated with susceptibility in rheumatoid arthritis. The next step early in the human immune response to RA is the presentation of antigen-presenting cells to helper T cells. The initiation of the cellular immune response in stage 1 of RA is unlikely to produce symptoms.
The increase in the number of T cells leads to proliferation and differentiation of B cells and leads to a more organized immune response in the perivascular areas in the synovial membrane. Macrophages from rheumatoid synovial tissue can induce angiogenesis (Koch AE, 1986) which may be mediated by cytokines (Folkman J 1987). Macrophages act as amplifiers of local and systemic inflammation, with a direct contribution to matrix degradation. Locally, macrophages are involved in recruitment and activation of inflammatory cells, cell contact, or cytokine-mediated activation/differentiation of neighboring cells, secretion of matrix-degrading enzymes, and neovascularization. At a systemic level, macrophages amplify disease via the acute phase response network, production of tumor necrosis factor-alpha (TNF-α), development of bone marrow differentiation anomalies, and chronic activation of circulating monocytes (Raimund W Kinne, 2000).

A principal characteristic of patients with RA is the high titer of rheumatoid factor, an immunoglobulin directed against the Fc region of IgG. Rheumatoid factor amplifies the inflammation in rheumatoid arthritis (Harris Ed Jr, 1989). Increased number of neutrophils is found in the synovial fluid which is rapidly activated by the aggregates of immune complexes. Activation of neutrophils (Korchak HM, 1984) results in degranulation, with the release of proteinases and production of leukotriene B₄, products of arachidonic acid metabolism and reactive oxidants (Henson PM, 1987, Hibbs MS, 1984). The inflammation in the joint cavity may occur in this stage of the rheumatoid arthritis.

In the next stage, the proliferating synovial membrane becomes organized in an invasive front that invades cartilage, tendons and subchondral bone. In addition to the proliferation
of blood vessels, matrix proteins and immunocytes, there is extensive growth of synpivial lining cells. The proteinases released by rheumatoid synovial cells and chondrocytes are capable of destroying almost all the matrix proteins present in articular cartilage and bone. The irreversible destruction of cartilage occurs at this stage of rheumatoid arthritis. In the last stage, the irreversible destruction of cartilage is well underway finally resulting in progressive destruction of joints.

**Joint affected by Rheumatoid arthritis**

![Figure 1.2.2: Joint affected by rheumatoid arthritis. The inflammation of the synovium and the invasion of synovium by the pannus lead to destruction of cartilage.](image)

A local lymphoid tissue (pannus) with a strong germinal center reaction and macrophage activation in the RA synovium is the hallmark of the disease. The RA pannus consists of a hypertrophic synovial membrane composed of hyperplastic synoviocytes and
inflammatory cells that infiltrate the synovial membrane. T cells, B cells, CD68+ macrophage-like cells, mast cells and endothelial cells are present in the RA synovium and contribute to the inflammatory process (Weyand CM, 2000). Macrophage-derived cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor (TNF) are found in high concentrations in the synovial compartment of patients with RA, in contrast to many T lymphocyte cytokines (example, interleukin-2). B lymphocytes and plasma cells are also present in large numbers and synthesize rheumatoid factors locally in large amounts. Enhanced local dendritic cell - T-cell interactions, probably involving locally trapped microbial antigens of the gut environment, may be essential for the early pannus formation. The excessive cytokine, autoantibody and rheumatoid factor production in the pannus are probably the factors leading to cartilage and bone destruction via further local macrophage activation, complement activation and polymorphonuclear cell infiltration (Rosmalen JGM, 2000).

**Interaction between Innate and Adaptive Immunity**

Innate immunity, which is a primitive pattern-recognition system that can lead to rapid inflammatory responses, has been implicated through the engagement of Fc receptors by immune complexes and perhaps Toll-like receptors by bacterial products. Antigen-driven T cell and B cell responses may also participate. Cytokine networks clearly participate, with paracrine and autocrine loops that maintain cellular activation in the synovial intimal lining. Finally, permanent alterations in some cell types might occur during the evolution of disease that can accelerate destruction.

An induction phase, initiated by innate immunity, can “prepare” the joint for subsequent recruitment of inflammatory and immune cells (Firestein GS, 2002). Once the cell
recruitment begins, a genetically susceptible host in the appropriate environment can develop a primary response to an arthrotropic antigen, secondary immune responses to articular antigens or overactive cytokine networks due to gene polymorphisms that increase the production of pro-inflammatory factors. The typical clinical picture of RA ensues, driven by lymphocytes, macrophages and other antigen-presenting cells. Ultimately, a destructive phase proceeds, which can be antigen dependent and independent and supported by mesenchymal elements such as fibroblasts and synoviocytes. Bone erosions are subsequently caused by osteoclasts, whereas cartilage dissolution results from proteolytic enzymes produced by synoviocytes in the pannus or synovial fluid neutrophils.

The heterogeneity of mechanisms provides an explanation for the unpredictable response to therapeutic agents and also follows clinicians to envision new therapeutic targets to either prevent RA or interfere with the immunologic, inflammatory or destructive components as separate but interrelated entities.
Figure 1.2.3: Schematic diagram of disease mechanisms that likely occur in various phases of Rheumatoid Arthritis.

Innate immunity activates mesenchymal cells and macrophages in the earliest phases, which can focus a subsequent immune response to the synovium. Antigen-specific responses, although not proven, are probably most important in the inflammatory phases, even though macrophage and fibroblast-like synoviocyte (FLS) cytokines dominate. Direct T cell contact can also activate other cells in an antigen-independent manner. In
the latter phases of disease, many cell types activate osteoclasts through receptor activator of NFκB/receptor activator of NFκB ligand (RANK/RANKL) system, although FLSs likely provide the greatest stimulus. Autonomous activation of FLS might contribute to this process.

**Autoimmunity in Rheumatoid arthritis**

In 1948, the studies of Rose and colleagues confirmed the findings of Waaler linking a factor (Rheumatoid factor, RF) in sera of patients with RA to agglutination of normal and sensitized sheep red blood cells (Rose HM, 1948, Waaler, 1940) which is considered to be the first evidence of autoimmunity in rheumatoid arthritis. The identification and characterization of RF as a self antibody was the first evidence that autoimmunity might play a role in RA. The presence of RF and its resultant pathogenic consequences are considered a cardinal feature of rheumatoid arthritis.

The role of RF in the initiation, amplification and perpetuation of the process is sufficiently supported by experimental evidence. Although some patients with virtually no circulating IgG develop RA, patients with a positive test result for RF in blood have more severe clinical disease and complications than seronegative patients (Bonagura VR, 1989).

Rheumatoid factor is also able to fix and activate complement by the classic pathway, and there is clear evidence of local complement production and consumption in the rheumatoid joint. Large quantities of IgG RF are produced by rheumatoid synovial tissue and form complexes through self-association. RF-containing immune complexes are readily detected in RA synovial tissue as well as the surface layers of cartilage (Rawson AJ, 1969).
The driving force behind RF production still remains unclear. Enhanced helper T cell function has been correlated with the spontaneous production of mainly IgM isotype RF (Patel V, 1984). The natural killer (NK) cells and the cytokines of the joint possessing B-cell stimulating properties can mediate nonspecific B cell activation. Also, terminally differentiated plasma cells capable of spontaneously secreting RF are present in RA synovial fluid.

Several hypotheses have been proposed to explain the immunogenic characteristics of IgG.

- New determinants on IgG might be exposed after polymerization of molecules or formation of IgG complexes with specific antigens.
- Structural anomalies in the IgG of RA patients may render it immunogenic. Eg: Defect in the hinge region of rheumatoid IgG could increase the binding affinity to membrane fragment crystallizable (Fc) receptors on B lymphocytes.
- Autoantigenic reactivity of IgG may be a result of the change in relative extent of galactosylation.
- Genetic influence may play a role since first-degree relatives of seropositive patients with RA frequently are seropositive themselves.

Generally, three quarters of patients with RA are seropositive using standard tests for RF, although the percentage can be as high as 90% when assayed specifically for IgM RF. IgG and IgM RFs are known to be the most abundant and most pathogenic in RA. IgE RF has been detected in some patients, especially those with extra-articular manifestations. IgE RF can potentially complex with aggregated IgG in synovial tissues and the
subsequent complexes could then degranulate synovial mast cells through activation of Fc receptors in the synovium.

Rheumatoid factors may form large complexes that are capable of activating the complement cascade (Pope RM, 1974). One potentially important characteristic of RF is its propensity to precipitate out with IgG in superficial layers of cartilage, forming complexes that may well be attractants for the invasive and destructive pannus that occurs in rheumatoid arthritis (Jasin HE, 1985, Shiozawa S, 1980). Rheumatoid factor (RF) is useful prognostically, especially since the presence of RF in high titer is associated with more severe rheumatoid arthritis (Combe B, 2001). Rheumatoid factor can be used to predict the radiological progression of rheumatoid arthritis. Rheumatoid factor-positive RA patients are more likely to have progressive, erosive arthritis with loss of joint function and extra-articular complications (Vittecoq O, 2003).

Besides the rheumatoid factor (RF), another group of autoantibodies has recently been detected in serum of RA patients, the anti-cyclic citrullinated peptide antibodies (anti-CCP) (Schellekens GA, 1998). Citrulline is formed by deamination of arginine residues in several proteins by the action of enzyme peptidylarginine deiminase (PAD). PAD2 and PAD4 isoenzymes are abundant in the inflammatory RA aynovium and cause the local citrullination of synovial proteins, such as fibrin. Citrullinated extracellular fibrin in the RA synovium may be one of the major autoantigens driving the local immune response, suggested by the discovery of local production of anti-CCP and anti-citrullinated filaggrin antibodies in the joint (Khosla P, 2004). Anti-CCP antibodies show a great promise as a diagnostic marker of RA as they can detect RA in early stages (Lee DM, 2003). Citrullinated peptides fit better in the HLA DR4 (DRB1*0401 or *0404) antigen
binding grooves than the corresponding arginine containing peptides. HLA class II RA susceptibility alleles have been shown to be associated with production of anti-CCP antibodies in the joint (Van Gaalen FA, 2004).

Since the disease course of RA is highly variable in terms of aggressiveness, even after having established the diagnosis of RA, it is of value to determine criteria for prognosis of the most probably course of articular destruction and of functional deterioration. In response to most types of acute inflammation, hepatic protein synthesis undergoes stereotypical changes that are collectively known as the "acute-phase response". This response is triggered by cytokines such as IL-6 and leads to elevated plasma concentrations of acute-phase reactants such as fibrinogen (Gauldie J, 1987). In a study performed by Behzad Heidari et al, alterations in the levels of plasma proteins in chronic inflammation caused by infections and in many autoimmune diseases have been reported (Behzad Heidari, 2007). Changes in plasma protein concentrations, especially in fibrinogen, also alter the rate, at which erythrocytes in plasma fall in the gravitation field i.e., the erythrocyte sedimentation rate (ESR). The ESR is sensitive and reliable measure to screen for RA (Combe B, 2001). Erythrocyte sedimentation rate is expected to decrease concomitant with accompanying clinical improvement, or increase in accordance with deterioration of clinical variables (Behzad Heidari, 2007). In RA, measurement of the acute phase response by ESR or C reactive protein (CRP) concentration is generally used to monitor the disease activity (Van Leeuwen MA, 1994). Wolfe et al have stated in their study that ESR can definitely be used as a measure of disease activity in rheumatoid arthritis (Wolfe F, 1994). The ESR depends on the aggregations of red blood cells (RBCs), which is influenced by large asymmetrical
plasma proteins such as fibrinogen, alpha-2 macroglobulin and immunoglobulins (Pincus T, 2000). Bull et al have reported ESR as the single most useful test to monitor disease severity (Olsen N, 1989, Bull BS, 1986). Erythrocyte sedimentation rate and RF may be used as indicators for progression of articular erosions and functional impairment (Rudolf Mierau, 2006).

**Blood vessels in arthritis**

The microvasculature plays an active role not only as the means of selecting which cells should enter the tissue but also as a determinant of tissue growth and nutrition through the proliferation of new capillaries.

**Feeding the starved synovium:** From the vantage of new blood vessel proliferation in the synovium, synovitis in RA resembles both tumor growth and wound healing (Rooney M, 1988). The absolute number of blood vessels is increased in RA synovium, with a rich network of sublining capillaries and post capillary venules. However, the mass of tissue outstrips angiogenesis in RA as determined by the number of blood vessels per unit area and causes local tissue ischemia (Colville-Nash PR, 1992). Vascular endothelial growth factor (VEGF) is a specific endothelial cell mitogen which is present in high concentrations in rheumatoid synovial fluid and tissue (Rooney M, 1988). VEGF is also able to stimulate the expression of collagenase, which can degrade the extracellular matrix to make room for the advancing vasculature and pannus (Koch AE, 1994). VEGF expression is especially high in the synovial intimal lining.

In addition to the hypoxia-driven stimulus for blood vessel growth, the inflammatory cytokine milieu of the joint also encourages angiogenesis (Koch AE, 1986, Koch A, 1996). Several proinflammatory factors expressed by the rheumatoid joint, including IL-
8, FGF and TNF-α are angiogenic. Additional angiogenesis factors, such as soluble E-selectin and soluble VCAM, are released by activated endothelium in RA synovium and contribute to vascular proliferation (Dayer JM, 2003). Endothelial proliferation is especially prominent in synovial tissue regions containing VEGF (Unemori EN, 1992). Angiogenesis is essential for the establishment and progression of inflammatory arthritis, because of the need for blood vessels either to recruit leukocytes or to provide nutrients and oxygen to starved tissue.

**Adhesion molecule regulation:** The formation of new capillaries is only one aspect of blood vessel involvement in the rheumatoid process. Endothelial cells are also activated by cytokines to express adhesion molecules that bind to counter receptors on mononuclear cells and neutrophils from the circulation and facilitate their transfer from the blood into the subsynovial tissue (Brown RA, 1983).

There are 2 main categories of vascular adhesion molecules,

i) **Selectins (E-, L-, & P-selectin):** family of adhesion molecules whose primary ligands are carbohydrates (Bevilacqua MP, 1993)

ii) **Integrins:** Heterodimers that include an α- and a β-chain (Loeser RF, 1993)

Adhesion molecule expression is increased in the RA synovium (Cronstein BN, 1993). This is due to exposure of the vasculature to the rich cytokine milieu, especially IL-1 and TNF-α (Cicuttini F, 1994). Immunohistochemical techniques localize high levels of ICAM-1 to sublining macrophages, macrophage-like synovial lining cells and fibroblasts, compared to normal tissue (Pober JS, 1986). Cultured fibroblast-like synoviocytes also constitutively express ICAM-1, which can be markedly increased by TNF-α, IL-1 and IFN-γ (Koch AE, 1991). Moderate amounts of VCAM-1 are expressed in RA synovial
blood vessels. Cultured fibroblast-like synoviocytes constitutively express small amounts of VCAM-1, and the level is increased by IL-1, TNF-α, IFN-γ and IL-4. VCAM-1 on synoviocytes is functionally active and can support T cell binding (Ziff M, 1992). E-selectin expression is also detected in rheumatoid synovium, although the increase is less dramatic than for the integrins and their counter receptors (Bevilacqua MP, 1993).

**Rheumatoid Synovitis**

Proliferation of macrophages and synovial lining cells dominates early rheumatoid synovitis. These cells are activated in the beginning and the proliferation of the synoviocytes is observed. Subintimal mononuclear cells are numerous than lymphocytes (Soden M, 1989). Neutrophils are rarely seen as they quickly traverse the area between synovial vasculature and their destination, the joint fluid (Soden M, 1991). New blood vessels proliferate along with this nonspecific synovitis (Kulka PJ, 1964). The endothelial cells become full, deep and tall, consistent with their active state. After these changes appear, synovial lining cells as well as the sublining cells proliferate progressively. As the volume of cells increases in the synovium, the matrix structural proteins (eg: collagen) of the subsynovial layers and joint capsule begin to accumulate (Remmers EF, 1990). Mast cells, multinucleate giant cells and macrophages accumulate along with the foci of lymphocytes, proliferating synovial lining cells, and new blood vessels. The mature active synovitis is a heterogeneous mixture of mesenchymal and marrow-derived cells.

The non-immune cells of the synovial lining are a mixture of highly activated macrophages that have transmigrated from the marrow, and proliferating resident fibroblast-like cells that have a slow but steady doubling rate and become – when
activated – the aggressive metalloprotease-producing cells of invasive synovitis (Lafayatis R, 1989, Goto M, 1987). Monocytes and macrophages are attracted to the joints early in rheumatoid arthritis and are of central importance in driving proliferation in rheumatoid synovitis. There is an increase in the expression of adhesion antigens on synovial tissue lymphocytes which have a role in lymphocyte function, help in mediating cytotoxicity and antigen recognition by T cells, as well as help them fix in tissues after transmigration from vessels to the synovium (Cush JJ, 1988, Haynes BF, 1988). The activated fibroblasts and macrophages in sublining areas of rheumatoid synovium generate substantial numbers of cytokines.

**Cartilage and Bone destruction**

In RA, the cartilage is often covered by a layer of tissue composed of mesenchymal cells, which might represent the progenitor of the aggressive, mature pannus. In the established lesion, numerous areas are seen in which macrophage-like and fibroblast-like cells penetration into the cartilage matrix far from lymphocytes (Annefeld M, 1983). Invasive pannus is more commonly found in metatarsophalangeal joints, compared to hip and knee joints in which a layer of resting fibroblasts appeared to separate pannus from cartilage, perhaps explaining why erosions occur more often around small joints. In contrast, joint-space narrowing without erosions is more common in knees (Allard SA, 1991). Fibroblast-like synoviocytes from the intimal lining exhibit some characteristics of transformed cells but are not necessarily alone in their ability to degrade articular structures (Mohr W, 1978). Other cells in the joint, especially from the pannus that erodes directly into cartilage, could also be responsible for cartilage, whereas osteoclasts mediate bone erosions (Harris ED, 1970). Despite differences in morphologic
appearances and cellular content, direct comparisons of erosion and non-erosion synovial tissue reveal more similarities than differences with regard to cytokine and protease expression (Kingsley-Mills WM, 1970). Primitive mesenchymal cells isolated directly from the cartilage-pannus junction express phenotypic and functional features of both synoviocytes and chondrocytes and have been referred to as “pannocytes” (Zvaifler NJ, 1997). They exhibit a distinctive rhomboid morphology and can grow in culture for a prolonged time without becoming senescent. In addition, VCAM-1 surface expression is constitutive and very high compared with synoviocytes or chondrocytes (Alsalameh S, 1990). Pannocytes exhibit some features of chondrocytes in that both express inducible lymphocyte antigen, and inducible nitric oxide synthase (Bromley M, 1984). Interestingly, pannocytes, like synoviocytes, do not produce nitric oxide synthase even though they contain nitric oxide synthase mRNA. Pannocytes are more fibroblast-like in that they produce type I but not type II collagen (Harris ED, 1977, Dodge GR, 1991).

The enzymes induced by factors such as IL-1, TNF-α, phagocytosis of debris by synovial cells and mechanic trauma cause the joint destruction (Woolley DE, 1977). Early in synovitis, proteoglycans are depleted from the tissue, most likely due to the catabolic effect of cytokines such as IL-1 in chondrocytes with the production of matrix metalloproteinases (MMPs) and aggrecanases and this leads to mechanical weakening of cartilage (Werb Z, 1993). As proteoglycans are depleted, cartilage loses the ability to rebound from a deforming load and thereby becomes susceptible to mechanical fragmentation and fibrillation. Eventually the tissue loses functional integrity concurrent with its complete dissolution by collagenase and stromelysin (Brinckerhoff CE, 1990). Some of the MMPs responsible for this process are also derived from the chondrocytes.
themselves (Evasnton JM, 1968). Hence, the cartilage is under attack from a multitude of sources. Enzymes released from PMNs in synovial fluid also contribute to cartilage loss (Cawston TE, 1989).

**Polymorphonuclear leukocytes:** The articular cavity serves as a depository for PMNs; they enter the synovial fluid by direct passage from postcapillary venules in the synovium (Yanni G, 1993). Few PMNs are seen in the pannus itself and subsynovial tissue; once in the synovium they move rapidly to the synovial fluid, drawn by the activated component of cleavage of the fifth component of complement (C5a), leukotriene B₄ (LTB₄), platelet-activating factor and chemokines (Yanni G, 1994).

In the synovial fluid, PMNs come in contact with immune complexes and particulate material (i.e, fibrin, cell membranes, cartilage fragments). Phagocytosis occurs, especially to particles coated with IgG, and the PMNs are activated (Zvaifler NJ, 1994). The neutrophils degranulate, generate products of oxygen metabolism, metabolize arachidonic acid and develop the capacity for aggregation (Henderson B, 1988, Mohr W, 1975). In addition, PMNs from synovial fluid in RA release de novo synthesized proteins, including fibronectin, neutral proteinases and IL-1 (Lafyatis R, 1989, Goto M, 1987).

**Macrophages:** The abundance and activation of macrophages in the inflamed synovial membrane/pannus significantly correlates with the severity of rheumatoid arthritis (Raimund WK, 2000). Macrophages possess widespread pro-inflammatory, destructive, and remodeling capabilities that can critically contribute to acute and chronic phases of rheumatoid arthritis (Kinne RW, 2000, Michaelsson E, 1995). The monocytes differentiate to mature macrophages in the RA synovial membrane. Locally, synovial macrophages also differentiate into stimulatory or inhibitory subpopulations, which are
known to influence T-cell reactivity differentially (van den Berg TK, 1996). In RA, macrophages may be responsible for the synthesis of pro-inflammatory (eg. IL-1 or TNF-α) or regulatory cytokines (eg. IL-10), the balance of which is critical to perpetuation of disease (Arend WP, 1997, Miossac P, 1997). A subset of synovial macrophages may also exert a predominant role in angiogenetic processes (Koch AE, 1998). The degree of macrophage infiltration/activation correlates not only with the joint pain and general inflammatory status of the patient (Tak PP, 1997), but also with the radiological progression of permanent joint damage (Mulherin D, 1998).

Myeloid-related dendritic cells are also enriched in RA synovial compartments. Their efficacy as antigen-presenting cells and their interdigitating location in perivascular lymphoid aggregates are optimal prerequisites for the presentation of putative arthrogenic antigens to T cells and for the regulation of B cells (Petit AR, 1999).

**Peripheral Blood:** The activation of circulating monocytes and other molecules in RA is evidenced by the spontaneous production of prostanoids and prostaglandins (Bomalaski JS, 1986), cytokines (Hahn G, 1993, Schulze-Koops H, 1997), soluble CD14 (Liote F, 1996), increased production of the matrix-degrading enzyme gelatinase B and the metalloprotease inhibitor tissue inhibitor of metalloproteinase (TIMP-1) (Ahrens D, 1996), expression of superoxide dismutase (SOD), a critical enzyme for the control of oxygen radicals (Heller RA, 1997), increased phagocytic activity (Steven MM, 1984), increased integrin expression and monocyte adhesiveness (Liote F, 1996, Mazure G, 1995) and gene activation (Stout RD, 1997).

**T cells:** T cells at the site of inflammation in RA patients are known to regulate the inflammatory process either by releasing cytokines or by affecting the function of
contiguous cells involved in inflammation by direct cell-cell contact (Breedveld FC, 1997). Studies have indicated a definite role of T cells in rheumatoid arthritis (Gregersen PK, 1987). Owing to the lack of evidence for T-cell proliferation in synovial tissue, it is supposed that T cells are continuously recruited. This recruitment is facilitated by the high expression of adhesion molecules on synovial endothelium and the local production of chemotactic cytokines. Evidence exists that the direct contact between T cells with an activated phenotype and macrophages or fibroblasts may be important in the pathogenesis of rheumatoid arthritis (Chizzolini C, 1997). The disturbed balance between T helper 1 (Th1) and T helper 2 (Th2) cells may be held responsible for a pathogenic role of T cells. Studies have indicated that the balance between Th1 and Th2 is critical (Dolhain RJEM, 1996) and a preponderance of Th1 cells in rheumatoid synovial tissue is important for the persistence of inflammation in RA synovium (Cope AP, 1994).

**Perpetuation of Synovitis by macrophage-fibroblast cytokine networks**

To incorporate information on the cytokine profile into current concepts of RA, a variety of models have been proposed. One recurrent theme is that the chronic inflammatory process might achieve a certain degree of autonomy that permits inflammation after a T cell response has been downregulated. This could occur if the inflammation is sustained by factors produced by neighboring macrophages and synovial fibroblasts in the joint lining in paracrine or autocrine networks (Remmers EF, 1990, Hogg N, 1985). Several cytokines that have been identified in the synovium or synovial fluid can participate in this system and might explain lining cell hyperplasia, HLA-DR and adhesion-molecule induction, and synovial angiogenesis (Goto M, 1987).
The list of potential candidates in this highly redundant system is very long. One can assume that at least two, IL-1 and TNF-α play particularly central role. Both are produced by synovial macrophages and stimulate

- Synovial fibroblast proliferation and secretion of IL-6
- GM-CSF
- Chemokines
- Effector molecules such as MMPs and prostaglandins

GM-CSF, which is produced by both synovial macrophages and IL-1β or TNF-α stimulated synovial fibroblasts, can in turn induce IL-1 secretion to form a positive feedback loop (Haynes BF, 1988). GM-CSF, especially in combination with TNF-α, also increases HLA-DR expression on macrophages. Macrophage and fibroblast cytokines could also indirectly contribute to the evidence for local T cell and B cell activation, including RF production (Bucala R, 1991).

This model for the perpetuation of RA does not eliminate the requirement for an initiating event, perhaps involving a specific arthritogenic antigen. In fact, it certainly requires an external stimulus to initiate the process, along with periodic restimulation.

T-cell mediated responses, directed against either an inciting antigen or a secondary target-like type II collagen or proteoglycan can occur along with this macrophage-fibroblast cytokine network and might enhance the local inflammatory response (Koch AE, 1991). Although the factors released by macrophages and fibroblasts are reasonably well defined, the precise function of synovial T cells remains less well characterized and might involve antigen-independent processes (eg: cell contact with or without IL-15) or traditional antigen-specific stimulation (Hamilton JA, 1981).
Figure 1.2.4: Cytokine networks in Rheumatoid arthritis. Paracrine and autocrine pathways can lead to activation of fibroblast-like and macrophage-like synoviocytes in the synovial intimal lining. Both +ve and –ve feedback loops are present, although in RA the former predominate. T helper type1 (Th1) cytokines can potentially enhance the network, whereas Th2 cytokines are suppressive.

Cytokines

Cytokines play an important role in the normal immune response. In RA, they play an integral role in the initiation and perpetuation of synovitis. The balance between the pro-and anti-inflammatory cytokines plays an important role in determining the severity of
inflammation in rheumatoid arthritis (Feldmann M, 1996). The known activity of proinflammatory cytokines detected in inflamed synovium can account for many processes important in joint destruction: production of proteases and reactive oxygen intermediates, synovial fibroblast (SF) proliferation, cartilage degradation, influx of inflammatory cells, and angiogenesis (Shingu M, 1993). Immunosuppressive and anti-inflammatory cytokines, including transforming growth factor-beta (TGF-β), IL-10 and interleukin 1 receptor antagonist (IL-1Ra), are also highly and consistently expressed during RA synovitis (Feldmann M, 1996, Ivashkiv LB, 1996). Production of these cytokines has been proposed to reflect the patient’s attempts to contain or control inflammation and achieve homeostasis (Arend WP, 1995, Firestein GS, 1997)

**Tumor necrosis factor-alpha (TNF-α)**

TNF-α is a key proinflammatory cytokine in RA and detected in rheumatoid synovial fluid and serum. TNF-α is produced mainly by monocytes and macrophages, but also by B cells, T cells and fibroblasts. TNF-α is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines, including IL-1, IL-6, IL-8 and granulocyte monocyte-colony stimulating factor (GM-CSF) (Butler DM, 1995, Pallinti V, 2007). Tumor necrosis factor acts as a costimulator for natural killer cells, activated B and T lymphocytes, and it enhances the pathogen-directed cytotoxicity of monocytes, neutrophils and eosinophils. It has stimulatory effects on the vascular bed lining on the endothelial cells, which result in enhanced surface expression of adhesion molecules and induction of procoagulant activity. The adhesion of neutrophils, lymphocytes and monocytes to the endothelial cells is followed by transendothelial cell migration and
extravasation. Tumor necrosis factor has also been demonstrated to stimulate angiogenesis in animal models (Matthias Grell, 2001).

IL-1 and TNF-α have many similar activities

- Enhance cytokine production
- Adhesion-molecule expression
- Proliferation
- MMP production by cultured synoviocytes

TNF-α stimulates collagenase and PGE₂ production by human synovial cells, induces bone resorption, stimulates resorption of proteoglycan and inhibits its biosynthesis in explants of cartilage. TNF-α also promotes inflammation by stimulating fibroblasts to express adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) (Koch AE, 1995). TNF inhibition in RA significantly decreases extracellular matrix destruction as measured by radiographic progression (Lipsky PE, 2000). TNF-α, in combination with IL-1, is a potent inducer of synovitis (Henderson B, 1989). The TNF-α levels in the synovial fluid correlate with the number of lining macrophages and with the degree of radiologically assessed bone erosion (Neidel J, 1995).

**Interleukin-1β (IL-1β)**

Interleukin 1 (IL-1) family is a ubiquitous group of polypeptides with a wide range of biologic activity; they include IL-1α, IL-1β, IL-18 and IL-1Ra. The proinflammatory actions of IL-1α and IL-1β allow them to serve as major amplifiers and translators of the inflammatory response of RA into a destructive one.

IL-1 has been implicated in RA, and inhibition of this mediator using IL-1Ra has modest anti-inflammatory activities in humans. The macrophages, synovial fibroblasts, PMN and
endothelial cells can be induced to generate this powerful mediator within the rheumatoid joint. Synovial macrophages are the most prolific source of IL-1 gene expression in the joint and nearly half of all macrophages in the RA synovium contain significant amounts of IL-1β mRNA.

Interleukin-1 stimulates the release of pituitary hormones, increased the synthesis of collagenases, resulting in the destruction of cartilage, and stimulates the production of prostaglandins, leading to a decrease in the pain threshold. Interleukin-1 has also been implicated in the destruction of beta cells of the islets of Langerhans, the growth of myelogenous leukemia cells, inflammation associated with arthritis and colitis (Charles A Dinarello, 1993). Intraarticular injections of IL-1 induce leukocyte infiltration, cartilage breakdown, and periarticular bone remodeling in animals (Schwab JH, 1991). Evidence suggests that the role of IL-1 in autoimmune diseases is an effector-mediator rather than an initiator. Interleukin-1 plays an important role in the destructive processes of arthritis, presumably due to its extremely potent ability to inhibit the tissue repair process. In recent studies, interleukin 18 (IL-18), a member of the IL-1 superfamily of cytokines has been demonstrated to be an important mediator of both innate and adaptive immune responses (McInnes IB, 2005). Interleukin 18 has been discussed as a potential therapeutic target in rheumatoid arthritis by Dinarello et al (Dinarello CA, 2004). In RA, IL-18 is mainly produced by macrophages, which in turn activates T cells and macrophages to produce pro-inflammatory cytokines, chemokines, adhesion molecules and RANKL which, in turn, perpetuate chronic inflammation and induce bone and cartilage destruction (Sheng-Ming Dai, 2007, Liew FY, 2003).
Interleukin-1 levels in the synovial fluid significantly correlate with joint inflammatory activity (Arend WP, 1998). This cytokine is believed to act in sequence with TNF-α (Feldmann M, 1996) and appears to mediate most of the articular damage in arthritis (Arend WP, 1998), because it induces proteoglycan degradation and inhibition of proteoglycan synthesis (von den Hoff H, 1995). Interleukin-1 is also involved in inducing the production of the metalloproteases stromelysin and collagenase and enhancing bone resorption (Assuma R, 1996). In RA, the balance between IL-1 and its physiological inhibitor IL-1Ra is shifted in favor of IL-1, indicating a dysregulation that may be crucial in promoting chronicity. A broad range of stimuli are capable of inducing IL-1 production by macrophages

- Immunoglobulin Fc fragments: generate IL-1 production by rheumatoid synovial macrophages
- Immune complexes: generate IL-1 production by rheumatoid synovial macrophages
- Collagen fragments: induce IL-1 production
- Type IX collagen (found only in articular cartilage): potent inducer of IL-1 by monocyte

The IL-1 in the lining can subsequently activate type B synoviocytes to proliferate and secrete a variety of mediators. Within the rheumatoid joint, 

- IL-1 induces fibroblast proliferation
- IL-1 stimulates the biosynthesis of IL-6, IL-8 and GM-CSF by synovial cells
- IL-1 enhances collagenase and prostaglandin production
- IL-1 induces a number of adhesion molecules on fibroblast-like synoviocytes and endothelial cells, including VCAM-1, ICAM-1 and enhances bone resorption

**Table 1.2.2: The involvement of IL-1 in the inflammatory and destructive process of Rheumatoid arthritis**

<table>
<thead>
<tr>
<th>Inflammation / pain</th>
<th>Tissue destruction / Inhibition of repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte-macrophages and T- and B-lymphocyte activation</td>
<td>Increased synovial cell proliferation</td>
</tr>
<tr>
<td>Increased expression of cell adhesion molecules</td>
<td>Increased production of MMPs by chondrocytes and synovial cells</td>
</tr>
<tr>
<td>Increased expression of cytokine genes (eg. TNF-α, IL-6)</td>
<td>Increased cartilage degradation (mediated by MMPs)</td>
</tr>
<tr>
<td>Increased expression of chemokines and angiogenic factors</td>
<td>Inhibition of proteoglycan and type II collagen synthesis resulting in impaired cartilage repair</td>
</tr>
<tr>
<td>Increased expression of PGE₂, nitric oxide and COX-2</td>
<td>Resorption of bone by activation of osteoclasts</td>
</tr>
</tbody>
</table>

Studies of arthritis in animals have strong implicated IL-1 in joint damage, destruction of bone and cartilage. Similar to TNF-α, IL-1 may cause damage by stimulating the release of matrix metalloproteinases from fibroblasts and chondrocytes (Koch AE, 1995).
Interleukin-6 (IL-6)

Interleukin-6 is one of the most abundant cytokines found in both the joint and blood of patients with active rheumatoid arthritis (Houssiau FA, 1988). Initially, it was known as B cell stimulatory factor 2 because it stimulated B cell growth and maturation. It is also known as hepatocyte stimulating factor because it activates hepatocytes to produce acute phase reactants such as C reactive protein and amyloid A. It has effects on the adaptive and innate immune responses (Choy E, 2003). In the adaptive immune response, IL-6 alias B cell stimulatory factor stimulates B cells to differentiate into plasma cells to produce immunoglobulin. It also stimulates proliferation and differentiation of T lymphocytes into cytotoxic T cells. Interleukin 6 also stimulates the innate immune response through induction of the acute phase response by stimulating hepatocytes and activation of the hypothalamic-pituitary-adrenal axis (Navarra P, 1990) and the generation of fever (Kluger MJ, 1998, Choy E, 2003).

In the synovial joints, IL-6 is produced by lymphocytes, monocytes, fibroblasts, synoviocytes, and endothelial cells (Choy E, 2003). Interleukin-1 and TNF-α have been shown to be potent inducers of IL-6 synthesis (Arend WP, 1990). Increased levels of IL-6 and IL-6 receptor have been found in the serum of patients with RA compared to controls, and in synovial fluid than serum, reflecting local production by the rheumatoid synovium (Madhok R, 1993, Swaak AJ, 1988). The acute rise in the levels is consistent with the role of IL-6 in acute phase responses. IL-6 and soluble IL-6 receptors promote the generation of osteoclasts (Kotake S, 1996) and excessive bone formation in chronic disease condition (Van de Loo FA, 1997). The reported anti-inflammatory effects of IL-6 include induction of anti-inflammatory cytokines such as IL-1Ra (Taga T, 1997),
induction of acute phase reactants that sub serve anti-inflammatory functions, induction of glucocorticoid production, suppression of cytokine production (IL-1, TNF and IL-12) and adhesion molecule expression, suppression of proliferation of RA synovial fibroblasts, inhibition of protease expression and induction of protease inhibitors (such as TIMP-1) in RA synovial fibroblasts (Hirano T, 1991). The precise pathogenic role of IL-6 in RA is controversial because it has both pro-inflammatory and anti-inflammatory properties in vitro (Choy E, 2003).

**Interleukin-10 (IL-10)**

Interleukin-10 is produced by monocytes, macrophages, B cells and T cells. Interleukin-10 reduces HLA-DR expression and antigen presentation in monocytes and inhibits production of proinflammatory cytokines, GM-CSF and Fc receptors by synovial macrophages (Isomaki P, 1996). Interleukin-10 prevents the differentiation of the monocytes to dendritic cells, which play a key role in the pathogenesis of rheumatoid arthritis (Allavena P, 1998). The pro-inflammatory cytokines themselves appear to trigger the synthesis of IL-10 as evidenced by experiments in which adding TNF-α or IL-1 has been shown to augment IL-10 production (Katsikis PD, 1994). Interleukin-10 inhibits the production of inflammatory cytokines including IL-1 and tumor necrosis factor – alpha (Howard M, 1992). It can also reverse the cartilage degradation mediated by antigen-stimulated mononuclear cells from patients with rheumatoid arthritis (van Roon JAG, 1996). Interleukin-10 may act as an important enhancer of cartilage growth. Conditioned media from antigen stimulated synovial fluid mononuclear cells have been sown to inhibit proteoglycan synthesis by cultured cartilage explants, an effect largely dependent on TNF-α and IL-1 (van Roon JAG, 1996). This inhibitory effect on proteoglycan
synthesis is reversed by IL-10 (van Roon JAG, 1996). IL-10 suppresses pro-inflammatory cytokines and chemokines, deactivates macrophages and inhibits T cell proliferation (Mottonen M, 1998).

**Signal Transduction and Transcription factors**

Many of the inflammatory responses observed in RA synovium, including the activation of cytokine and adhesion-molecule genes, can be traced to specific transcription factors and signal transduction pathways (Rouse J, 1994, Firestein GS, 1999). These pathways play a role in normal cells and host defense, thereby increasing the issues of balancing efficacy with toxicity (Paul A, 1997, Lee JC, 1994, Krause A, 1998).

**Nuclear factor kappa B (NFκB):** NFκB is a ubiquitous transcription factor that plays a key role in the expression of many genes central to RA, including IL-1β in monocytes, as well as ICAM-1, TNF-α, IL-6 and IL-8 in rheumatoid synoviocytes (Takashi O, 2006). NFκB is abundant in rheumatoid synovium (Baldwin AS, 1996). NFκB activation is much greater in RA because of phosphorylation and degradation of IKB in RA intimal lining cells (Kopp EB, 1995). The relevance of NFκB to inflammatory arthritis has been tested in several animal models (Haddad EB, 1996). Synovial NFκB is rapidly activated, often long before clinical arthritis (Marok R, 1996). NFκB inhibition was associated with decreased synovial cellular infiltration, as well as increased apoptosis (Iwabuchi K, 1994, Ao Y, 2001).

One of the primary concerns with NFκB-derived therapy is that it participates in so many cellular functions, especially those involved in host defense. Blockade could potentially impair both innate and adaptive immune responses (Kopp EB, 1995). In addition, NFκB activation prevents apoptosis in many cell lineages, especially after exposure to
proinflammatory cytokine such as TNF-α. By interfering with this pathway, increased apoptosis can potentially damage major organs (Ao Y, 2001). Although, NFκB inhibition has great potential, balancing the risks and benefits will be crucial.

**Mitogen-activated protein kinases:** The MAP kinases are widely expressed in synovial tissue and are activated in rheumatoid synovium (Saccani S, 2002, Anest V, 2003). Phosphorylated ERK, p38 and JNK are constitutively expressed by cultured fibroblast-like synoviocytes and can be activated within minutes after exposure to cytokines such as IL-1 and TNF-α (Hu Y, 1997). Once phosphorylated, each initiates an interlocking series of additional kinases or transcription factors (Siebenlist U, 1994). Cytokine gene expression, especially IL-1 and TNF-α, are induced by p38, whereas JNK can activate AP-1 by phosphorylating c-Jun, which, in turn, increases MMP gene expression (Hu Y, 1997). The p38 and ERK pathways can also regulate MMPs in many cell types, such as synoviocytes and chondrocytes. As with the NFκB pathway, the ubiquitous expression and initial role of these kinases in normal homeostasis suggests that development of agents for clinical use will be challenging (Senftleben U, 2001).

**Activator protein-1 (AP-1):** Like NFκB, AP-1 regulates many genes implicated in RA, including TNF-α and the MMPs (Feldmann M, 2001, Okamoto T, 1997). AP-1 activity can be induced by extracellular signals including cytokines, growth factors, tumor promoters and the Ras oncoprotein (Jiang X, 2003). AP-1 includes members of the Jun and Fos families of transcription factors. AP-1 proteins and mRNA including c-jun and c-fos are expressed in RA synovium, especially in the nuclei of cells in the intimal lining layer (Bohuslav J, 2004). Localization of AP-1 to the intimal lining correlates with the site where most protease and cytokine genes are overexpressed in RA (Adcocok IM,
1994, Yang-Yen HF, 1990). AP-1 proteins are usually not detected in normal synovium. Cytokines such as IL-1 and TNF-α probably contribute to the activation of AP-1 in RA synovium (Wang P, 1995).

**Signal Transducers and Activators of Transcription (STAT):** STATs are a family of latent cytoplasmic transcription factors that are activated in response to cytokine stimulation of cells. STATs have been implicated in the expression of many proinflammatory genes. STAT3 is strongly phosphorylated in RA synovium and supports the hypothesis that IL-6 plays a pathogenic role in the disease.

**Oxidative Stress**

Oxidative stress refers to the situation of serious imbalance between production of reactive species and antioxidant defense. Sies, who introduced the term, defines it as "a disturbance in the pro-oxidant - antioxidant balance in favor of the former, leading to potential damage" (Sies H, 1991).

In principle, oxidative stress can result from:

i. Diminished antioxidants

ii. Increased production of ROS and RNS, for example, by exposure to elevated levels of toxins that are themselves reactive species (example - NO₂ gas, NO₂⁻) or are metabolized to generate such species, or by excessive activation of 'natural' ROS/RNS-producing systems (example - inappropriate activation of phagocytic cells in chronic inflammatory diseases, such as rheumatoid arthritis and ulcerative colitis) (Irshad M, 2002).
Oxidative stress plays an important role in tissue injury in rheumatoid arthritis, inflammatory bowel disease, acute respiratory distress syndrome (ARDS) and cancers related to chronic inflammation (Nalini G, 1997, Pallinti V, 2004).

Oxidative stress in the joints of RA patient results from a confluence of several stimuli – increased pressure in the synovial cavity, reduced capillary density, vascular changes, an increased metabolic rate of synovial tissue and locally activated leukocytes (Finkel T, 2000, Takashi O, 2006). An increased intra-articular pressure in RA joints due to decreased compliance of the joint wall due to synovial membrane swelling and fibrosis of the capsule was observed by Jayson and Dixon (Jayson MIV, 1970). Because of this elevated intra-articular pressure, the capillary flow rates of the inflamed joint tissues greatly fell and reperfusion was delayed, thus associated with a decrease in the synovial O$_2$ tension (pO$_2$), an elevated pCO$_2$, an increase in the concentration of synovial fluid lactate, and a decrease in pH (James MJ, 1990, Levick JR, 1990). The synovial hypoxia was shown to cause accumulation of adenosine and its breakdown products including hypoxanthine and xanthine (Herbert KE, 1988), which subsequently activates the xanthine oxidase system (Allen RE, 1989) leading to repeated episodes of oxidative injury in the rheumatoid joints. Reactive oxygen species production was detected in the joints of RA patients including the direct measurement of superoxide anion by electron spin resonance (Lunee J, 1985), ROS-modified IgG, increase in lipid peroxidation product (Mapp PI, 1995), depletion of ascorbate (Lunee J, 1985), and ROS-mediated fragmentation of glycosaminoglycans such as synovial hyaluronic acid (Grootveld MC, 1991). The degradation of hyaluronic acid is considered responsible for the decreased viscosity of joint fluid and the increase in intra-articular pressure, and the oxidatively
damaged IgG accounts for the generation of reactive epitopes for the production of rheumatoid factor (Grootveld MC, 1991). The generation of reactive oxygen species can also be facilitated by repetitive ischemia reperfusion injury in the joint (Irshad M, 2002). Tissue injury releases iron and copper ions and heme proteins that are catalytic for free-radical reactions (Kurien BT, 2006). Electron transport chains are also disrupted in the mitochondria and endoplasmic reticulum, leading to leakage of electrons to form superoxide.

Evidence for increased production of reactive oxygen species in RA patients includes

- Elevated levels of lipid peroxidation products (Mapp PI, 1995)
- Decreased levels of ascorbic acid in serum and synovial fluid (Lunee J, 1985)
- Degradation of hyaluronic acid (Grootveld MC, 1991)
- Increased breath pentane excretion (Holmgren A, 2000)

The levels of thioredoxin, which is a marker of oxidative stress, are significantly higher in synovial fluid of RA patients (Maurice MM, 1998, Holmgren A, 1985, Holmgren A, 1989). Peripheral blood lymphocyte DNA from RA patients contains significantly increased levels of the mutagenic 8-oxohydrodeoxy guanosine (Bashir S, 1993), which is a product of oxidative damage to DNA, pointing to the genotoxic effects of oxidative stress.
Figure 1.2.5: An overall picture of the mechanism of ROS and the mechanism of oxidative tissue damage leading to pathological conditions (Uday B, 1999)
Arachidonate metabolites and Nitric Oxide

Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. Peroxidation of lipids exposed to oxygen is responsible for damage to tissues in vivo, in cancer, inflammatory diseases, atherosclerosis and aging (Rowley D, 1984). Lipid hydroperoxides (LOOH) are prominent nonradical intermediates of lipid peroxidation. Lipid peroxidation can also result in formation of several toxic byproducts that can attack other cellular targets, including DNA, away from the site of generation. They can also alter cell signaling or act as 'toxic second messengers' that amplify damage. Such byproducts include 4-hydroxynonenal and malondialdehyde (Thomas PA, 2002). Malondialdehyde is the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (PUFA) (Tavazzi B, 1992). Malondialdehyde, a secondary product of lipid peroxidation, is used as an indicator of tissue damage by a series of chain reactions (Taysi S, 2002). The potential cellular and plasma antioxidants that protect against singlet oxygen include carotenoids, tocopherols, thiols and small molecular compounds such as carnosine and bilirubin (Irshad M, 2002, Thomas PA, 2002).
Fatty acid with double bonds

Hydrogen abstraction by a lipid radical or OH generated from O₂

Molecular Rearrangement

Conjugated diene with absorbance at 234nm

Lipid radical abstracts H from another fatty acid causing an autocatalytic chain reaction

Fragmentation to aldehydes (including malondialdehyde) & polymerisation products

Lipid hydroperoxide

Cyclic hydroperoxide

Cyclic endoperoxide

Figure 1.2.6: Lipid Peroxidation
Nitric oxide (NO) is an effector molecule in macrophage-mediated host defense and is known to play a part in chronic and acute inflammation (Ialenti A, 1992, Ialenti A, 1993) leading to tissue damage (Moncada S, 1992). Peroxynitrite and other reactive nitrogen species cause damage to membrane proteins and lipids as well as intracellular constituents through their potent oxidizing activity and protein nitration. Nitric oxide may be directly involved in the cartilage destruction in arthritic joints, acting to increase the activity of metalloprotease enzymes and to suppress the formation of novel matrix components induced by pro-inflammatory cytokines (Farell AJ, 1992). Nitric oxide has also been implicated in the regulation of osteolysis caused by osteoclasts. In addition, NO acts as a local regulator of the complex network of cytokines and other inflammatory mediators produced in inflamed joints. Nitric oxide production is high in rheumatoid synovial tissue (Sakurai H, 1995). There is a substantial increase by inducible NO synthase after stimulation by cytokines or bacterial products. The nitrite levels in synovial fluid are elevated in RA patients, indicating local NO production (Farell AJ, 1992). In addition, the urinary nitrate-creatinine ratio is increased and inducible NO synthase is present in the synovium.

Several studies suggest that tissue injury in inflammation involves NO production (Stefanovic-Racic M, 1993, Farell AJ, 1992). Growing evidence implicates NO in immune regulation, inflammation, autoimmunity and arthritis (Stefanovic-Racic M, 1993). Raised levels of NO in serum and synovial fluid have been reported in patients with rheumatoid arthritis (Stefanovic-Racic M, 1993, Ersoy Y, 2002). Since it is difficult to measure NO directly because of its short half-life, nitrates (NO$_3^-$) or nitrites (NO$_2^-$) are measured normally as indices of NO production.
Antioxidants can be primarily classified into two classes:

I) preventive antioxidants, which reduce the rate of chain initiation

II) chain-breaking antioxidants, which interfere with chain propagation.

In vivo, the principal chain-breaking antioxidants are superoxide dismutase, which acts in the aqueous phase to trap superoxide free radicals (O$_2^-$); vitamin E, which acts in the lipid phase to trap ROO$^-$ radicals.

Vitamin E is an efficient lipid soluble antioxidant that functions as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including low density lipoprotein (LDL) (Irshad M, 2002, Thomas PA, 2002, Traber MG, 1997). This is one of the most important and least toxic of all lipid soluble antioxidant vitamins. It appears to be the first line of defense against peroxidation of PUFAs contained in cellular and subcellular membrane phospholipids. The phospholipids of mitochondria, endoplasmic reticulum and plasma membranes possess affinities for α-tocopherol and the vitamin appears to concentrate at these sites (Halliwell B, 1994). It scavenges peroxyl radical intermediates in lipid peroxidation and is responsible for protecting PUFA present in cell membrane and LDL against lipid peroxidation. They can prevent genetic changes by inhibiting DNA damage induced by the reactive oxygen species (ROS), which include superoxide anions (O$_2^*$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (●OH), MDA and nitric oxide (NO) (Ashour M, 2000, Cimen MY, 2000).
The tocopherols act as antioxidants, breaking free-radical chain reactions as a result of their ability to transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid (PUFA). The phenoxy free radical formed may react with vitamin C to regenerate tocopherol, or it reacts with a further peroxyl free radical so that the chromane ring and the side chain are oxidized to the non-free-radical product.

The antioxidant action of tocopherol is effective at high oxygen concentrations, and thus it tends to be concentrated in lipid structures exposed to the highest O\textsubscript{2} partial pressures, example: the erythrocyte membrane, the membrane of the respiratory tree and the retina.

\[
\text{ROO}^- + \text{TocOH} \rightarrow \text{ROOH} + \text{TocO}^-
\]

\[
\text{ROO}^- + \text{TocO}^- \rightarrow \text{ROOH} + \text{non-free radical product}
\]

Figure 1.2.8: The chain-breaking antioxidant activity of tocopherols (TocOH) toward peroxyl radicals (ROO\textsuperscript{-}).
The resultant radical is relatively stable and in normal circumstances, insufficiently reactive to initiate lipid peroxidation itself; an essential criterion of a good antioxidant.

**Prostaglandins**

Accompanying activation of PMNs is the increased mobilization of membrane phospholipids in these cells to arachidonic acid and its subsequent oxidation by cyclooxygenases (COX) to prostaglandins and thromboxanes, or by lipoxygenases to leukotrienes.

Although the stable prostaglandins, especially prostaglandin E\(_2\) (PGE\(_2\)),

- Produce vasodilation
- Cause increased vascular permeability
- Involved centrally in fever production,

there is increasing evidence that they have significant anti-inflammatory activities as well. Physiologic concentration of PGE\(_2\) inhibits interferon-gamma (IFN-\(\gamma\)) production by T cells, HLA-DR expression by macrophages and T cell proliferation (Kojima F, 2005).

Production of prostaglandins in RA is dependent on two distinct COX enzymes, COX-1 and COX-2 (McCoy JM, 2002). COX-1 is constitutively expressed and is responsible for the normal endogenous production of prostaglandins in the joint as well as in other tissue. COX-2, on the other hand, is an inducible enzyme responsible for increased prostaglandin synthesis in inflamed tissue. The prostaglandins and thromboxanes are local hormones that are synthesized rapidly when required and act near their site of synthesis.
Among the prostaglandins, PGE\(_2\) exists in a wide variety of cells and tissues, and it plays an important role in various physiological functions. It is one of the major mediators of inflammation (Akogi J, 2006). High concentrations of PGE\(_2\) have been detected in the synovial fluid of patients with rheumatoid arthritis (Fumiaki Kojima, 2003). It has been reported that cytokine-activated cells such as synovial cells, chondrocytes and macrophages/monocytes are the primary source of PGE\(_2\) in arthritic joints (Egg D, 1980).

**Figure 1.2.9: Conversion of arachidonic acid to prostaglandins and thromboxanes via the cyclooxygenase pathway and to leukotrienes and lipoxins via the lipoxygenase pathway**
Figure 1.2.10: Synthesis of the clinically relevant prostaglandins and thromboxanes from arachidonic acid. Numerous stimuli (e.g. epinephrine, thrombin and bradykinin) activate phospholipase A2 which hydrolyzes arachidonic acid from membrane phospholipids. The prostaglandins are identified as PG and the thromboxanes as TX. Prostaglandin PGI2 is also known as prostacyclin. The subscript 2 in each molecule refers to the number of double present.

In response to various stimuli including pro-inflammatory cytokines, arachidonic acid is liberated from membrane phospholipids to the cytosol by several phospholipase A2. The arachidonic acid is converted to prostanoids by the action of cyclo-oxygenase (COX). The COX-1 is constitutively produced and functions to maintain homeostasis, whereas COX-2 acts as a stress response gene and is responsible for high levels of prostaglandin production during inflammation (Goetzl EJ, 1995). The COX converts arachidonate to an unstable endoperoxide intermediate, PGH2, which in turn, is metabolized to PGD2, PGE2, PGF2alpha, PGI2 and thromboxane A2 by cell-specific isomerases and synthases.
Both oxidative injury and inflammation have been proven to play a vital role in chronic inflammatory diseases (Halliwell B, 1995). Non-enzymatic free radical and enzymatic cyclooxygenase catalyzed oxidation of arachidonic acid occur in various types of rheumatic diseases including rheumatoid arthritis. High levels of both free radical mediated F_2-isoprostanes and the cyclooxygenase derived PGF_2α metabolite have been found to be elevated in blood and synovial fluid of patients with rheumatoid arthritis (Morrow JD, 1995).

The mediator PGE_2 is produced during inflammatory responses and increased levels of PGE_2 mediate some of the cardinal features of inflammation, including pain, edema and fevered (Witt DL, 1991). Several studies suggest that in addition to its pro-inflammatory actions, PGE_2 may also exert strong anti-inflammatory effects such that PGE_2 suppresses the production of pro-inflammatory cytokines and enhances the synthesis of anti-inflammatory cytokines (Kambayashi T, 1995, Strassmann G, 1994). Prostaglandin E_2 also promotes humoral and Th2-type immune responses and inhibits Th1-type immune responses (Harris SG, 2002). Hence, PGE_2 may be regarded as modulator of immune responses.

Prostaglandin E_2 (PGE_2) is one of the important mediators of inflammation associated with rheumatoid synovitis and the ligand-activated cells in synovial tissue are likely to be a primary source of this prostaglandin in the joints of patients with rheumatoid arthritis (Crofford LJ, 1994, Crofford LJ, 1999). Increased PGE_2 production in response to stimulation by pro-inflammatory cytokines (Cheon H, 2006) coincides with the up-regulation of COX-2 expression in synovial cells, and COX-2 is considered to be the rate-limiting enzyme for PGE_2 production at sites of inflammation (Fumiaki Kojima,
Prostaglandin E\textsubscript{2} has been associated with the edema and the erosion of cartilage and juxta-articular bone commonly found in rheumatoid arthritis (Davies P, 1984, Griffiths R, 1999, Dayer JM, 1976).

**Genetic aspects in the etiopathogenesis of rheumatoid arthritis**

Genetic and environmental factors are involved in the etiology of rheumatoid arthritis (Edward D Harris Jr, 1990). Cumulative studies suggest that RA occurs in patients whole genetic background includes multiple common genetic risk factors that have been inherited (Cuenca J, 2003). Genetic analysis of RA susceptibility suggests a polygenic inheritance with the largest contribution from the major histocompatibility complex (MHC) (Winchester R, 1994, Cornelis F, 1998). Part of the MHC genetic contribution to RA is provided by several alleles of the HLA-DRB1 gene located in the MHC class II region (6p21) (Wordsworth P, 1995). Specifically the HLA-DRB1*04 and HLA-DRB1*01 alleles of the HLA-DRB1 gene have been associated with RA, while the HLA-DRB1*02 was not (Nepom GT, 1992). An explanation for the association of HLA-DRB1*04 and HLA-DRB1*01 alleles with RA has been proposed in the ‘shared epitope’ (SE) hypothesis (Gregersen PK, 1987). However, the HLA-DR SE does not account for the total MHC genetic contribution to RA (Genin E, 1998) and may be primarily associated with severe forms of the disease (Burmester GR, 1997). Another MHC contribution to RA is provided by the TNF locus (Mulcahy B, 1996, Mu Hua, 1999), in a region recently re-classified as the class IV region of the MHC (previously class III) (Ruuls SR, 1999).

Although its etiology remains poorly understood, the association of some HLA-DR alleles with the susceptibility to (Statsny P, 1978), or severity of (Gregersen PK, 1987)
the disease has been known for over two decades. A study performed by Miterski et al proves the existence of a genetic effect in rheumatoid arthritis. Miterski et al observed a 4–6 fold higher concordance rate in identical (monozygotic) twins compared with non-identical (dizygotic) twins (Miterski B, 2004). It has been well established that certain HLA-DRB1 alleles play a major role in disease susceptibility/severity (Devries N, 1997). However, the contribution of the HLA complex represents no more than 50% of the genetic background (Kilding R, 2004). Therefore, identification of other genetic markers associated with susceptibility to or severity of RA is being aggressively pursued by researchers worldwide (Sylke KH, 2001, Newton JL, 2004). Cytokines with polymorphic gene sequences are potential markers of disease severity. Since their gene products are involved in RA pathogenesis, differences in severity between individuals could be related to different levels of cytokine production or functional differences resulting from polymorphism in their genes (Waheba A Zarouk, 2005). The importance of the unbalanced production of cytokines such as TNF-α, IL-1β and IL-6 in affected tissues (Feldmann M, 1996) and the successful introduction of a monoclonal anti-tumor necrosis factor α (anti-TNF α) monoclonal antibody into therapeutic use (Elliott MJ, 1994) have suggested that TNF is involved in the pathogenesis of the disease (Feldmann M, 1996, Elliott MJ, 1993). It has been suggested that variability in the promoter and coding regions (single-nucleotide polymorphisms [SNPs]) of the TNF gene may modulate the magnitude of the secretory response of this cytokine (Bouma G, 1996, Wilson AG, 1997, Kroeger K, 1997).
**Tumor necrosis factor-alpha:** The TNF gene is tightly regulated at several levels i.e., at the transcriptional, post-transcriptional, translational and post-translational levels. Transcriptional regulation of the TNF gene is mediated via the 5’ promoter while post-transcriptional regulation is via the 3’ untranslated region (3’ UTR) (Aggarwal BB, 1996). A recent study of TNF 3’ UTR in RA patients and unaffected individuals failed to show any polymorphisms (Waldron-Lynch F, 1999). Thus, it appears that the human TNF 3’ UTR is highly conserved and does not influence susceptibility to rheumatoid arthritis. Studies have also indicated that a few polymorphisms at the TNF region (polymorphic positions at the TNF promoter and microsatellites in its proximity) could be associated with the disease (Udalova IA, 1993, Vinasco J, 1997).

A potential predisposing factor for RA is the tumor necrosis factor – alpha (TNF-α) gene (Brinkman BMN, 1997). Persistent expression of TNF-α alone may be sufficient to induce arthritis, as demonstrated in a transgenic mouse model in which mice that ‘over’-expressed a human TNF-α transgene developed a chronic arthritis resembling rheumatoid arthritis (Keffer J, 1991). TNF-α production was shown to vary between individuals and the variation was associated with certain HLA-DR alleles (Jacob CO, 1990). Present knowledge suggests that the highest genetic variability is concentrated in the promoter area of the TNF-α gene, where at least eight different single-nucleotide polymorphisms (SNPs) are concentrated, with the potential to affect the binding of transcription factors and thus to control the activity of the promoter and resulting mRNA and protein levels (Bayley JP, 2004). An association was found between some SNPs of TNF-α (-857, -308 and -238) and systemic manifestations, radiological progression, work disability and joint surgeries. There may be a possible influence of the -308 and -857 TNF-α promoter
positions on the production of TNF-alpha (Joao Eurico Fonseca, 2007). A study of fifty
two Spanish families clearly shows that TNFa/b microsatellites are associated with
rheumatoid arthritis. It also indicated that TNFa6.65 not only confers susceptibility, but
also increases the susceptibility conferred by haplotypes with shared epitope (Alfonso M,
2000). This study also supports the notion that TNF α is primarily involved in the
pathogenesis of the disease, as suggested from animal models of RA (Thorbecke GJ,
1992) and by the successful introduction of a monoclonal antibody anti-TNFα in clinical
use. In a study of thirty three multiplex families performed by Waldron-Lynch et al,
significant associations were found between RA susceptibility and the -308A and -857T
alleles in individuals possessing the shared epitope (Waldron-Lynch F, 2001). These
experimental data provide evidence that TNF promoter SNPs may play an independent
role in RA susceptibility in specific immunogenetically-defined groups of RA patients,
thus clearly indicating that TNF plays a pivotal role in the pathogenesis of rheumatoid
arthritis. They also demonstrate the potential for genetic heterogeneity even within the
TNF promoter.

**Interleukin-1 beta:** Interleukin-1 (IL-1) is one of the most potent pro-inflammatory
agents and it has a central role in joint inflammation and destruction (Buchs N, 2001).
The loci for human IL-1α, IL-1β, IL-1R1 and IL-1Ra genes are found as a cluster on
chromosome 2q12 to 2q13 (Nicklin MJ, 1994). Biallelic polymorphisms at positions IL-
1α – 889, IL-1β + 3953 and IL-1R1 + 970 have been described in literature.
Polymorphisms of the IL-1 gene complex, including IL-1 α – 889 and IL-1β + 3953 are
known to influence IL-1 expression (Hulkkonen J, 2000, Buchs N, 2001). Positive
associations have been observed between alleles from this gene cluster and rheumatoid
arthritis (Kaijzel EL, 2002). An Egyptian study showed that the homozygous allele E2 of IL-1β exon5 was increased in RA patients who developed erosions. Their study also indicates that the presence of allele E2 of IL-1β exon5 along with a shared epitope allowed prediction of early identification of erosive disease and could serve as a prognostic marker (Waheba A Zarouk, 2005). In this study, we investigated the polymorphism in the IL-1β exon5 region.

**Drug Therapy**

The ultimate goal of treating RA is to induce sustained remission or to cure the disease. Owing to the multifactorial nature of the disease, the more realistic aims of current therapy are to alleviate pain, suppress inflammation, prevent joint damage and loss of joint function, forestall disability, and improve mortality rates (Raphaela GM, 2003). Non-steroidal anti-inflammatory drugs (NSAIDs) are known to be helpful in the first few weeks in which the patient has symptoms, because the drugs provide partial relief of pain and stiffness until a definitive diagnosis of RA can be established (James RO Dell, 2004). NSAIDs have not been shown to slow the progression of the disease, in long-term care, NSAIDs should be used together with disease-modifying antirheumatic drugs (DMARDs) (ACR Guidelines, 2002). Corticosteroids are potent suppressors of the inflammatory response in rheumatoid arthritis (Moreland LW, 2002). Recent studies clearly establish that corticosteroids decrease the radiographic progression of rheumatoid arthritis (Weinblatt ME, 1999, Lipsky PE, 2000, Cohen S, 2002). The side effects associated with the use of drugs need to be considered on an individual basis before deciding the mode of treatment in these patients. Clinical evidence has supported the conclusion that patients should be treated early and aggressively (Raphaela GM, 2003).
Evidence that early treatment with DMARDs produces better short-term outcome, with tolerable toxicity profiles comes from a number of trials that tested the effects of DMARDs (Australian Multicentre Clinical Trial group, 1992, Hannonen P, 1993, HERA Study group, 1995, van der Heide A, 1996). Combination therapy is used in patients with other diseases, such as hypertension, diabetes, and malignancies, when no single agent is sufficiently effective (Olsen NJ, 2004). In RA treatment, methotrexate is used in most combinations (Mottonen T, 1999, Dougados M, 1999, Proudman SM, 2000, Kremer JM, 2000, O’Dell JR, 2002). Clinical evidence supports the use of combination therapy in patients with both early and established RA who have prognostic features of aggressive disease or have failed therapy with a single DMARD (Raphaela GM, 2003).

**Biological Therapy:** Biological therapies represent one of the major advances in the treatment of RA in the past decade (Feng-chun Zhang, 2006, Sandra VN, 2006). The TNF-α inhibitors represent a major breakthrough in the treatment of RA (Jin-Wuk Hur, 2006, James Cheng CW, 2006). This mode of therapy is based on the neutralization of the excess amount of cytokines to control inflammation in rheumatoid arthritis (Pallinti V, 2007). Several lines of treatment may be devised to neutralize cytokines. Recombinant soluble cytokine receptors may be used to help suppress inflammation. Another approach is to use antibodies against cytokines. The type of antibody needs to be designed carefully to achieve clinical efficacy. Receptor antagonism may also be used as an alternative strategy to intercept signal transduction. Such molecules are grouped under a broad category called as “biological agents”.

‘Biological’ technically means a substance that is a product of biological system and functionally as an agent that targets specific biological molecule (Mahajan A, 2006).
Currently there are several biological agents used clinically in the therapeutic approach to RA (Peter N, 2006, Ashok Kumar, 2006). The available licensed drugs for anti-TNF-α therapy are Etanercept, Infliximab and Adalimumab. These drugs differ not only in structure and mechanism of action, but also in pharmacokinetics and mode of delivery. Studies have demonstrated dramatic improvement in synovial inflammation in RA patients after treatment with neutralizing anti-TNF-α Abs or soluble TNF receptors, and decreased joint destruction after treatment with IL-1Ra (Weaver AL, 2004). Immunosuppressive and anti-inflammatory cytokines, including TGFβ, IL-10 and IL-1Ra are highly and consistently expressed during RA synovitis (Arend WP, 2001). Production of these cytokines has been proposed to reflect the patient’s attempts to contain or control inflammation and achieve homeostasis (David Deon, 2001).

**Gene Therapy:** As the human genome project progresses, it is believed that gene therapy may change the course of rheumatoid arthritis (Abraham Simon, 2001). However, the onset of this disease does not depend on a single gene (Bridge SL, 1999, Chemajovsky Y, 1999). Research in this area may lead to interesting and exciting results in the next decade (Evans CH, 1996).
1.3 Global Relevance of the Study

Rheumatic diseases are a huge burden on the health care systems of countries worldwide and account for significant disability, lost productivity and reduction in quality of life (Sangha O, 2000). Depression, anxiety and poor self-esteem are common consequences which must be addressed if adequate rehabilitation is to be achieved. RA patients are best managed within the context of a multidisciplinary team including medical specialist, nursing, occupational therapy, physiotherapy and surgical specialists. As appropriate, advice from psychologists, social workers and employment officers will be required to ensure maximal rehabilitation of the patient into an active role in the community (Paul Wordsworth, 2001).

Studies have suggested that musculoskeletal conditions are among the most prevalent chronic conditions, accounting for a high proportion of those with disability in the workforce as well as in the elderly (Verbrugge L, 1995). Rheumatoid arthritis (RA) is the most common inflammatory musculoskeletal disease, affecting approximately 0.5 – 1% of the population, and frequently leading to structural and functional damage, impairment of daily activities and loss of quality of life (Emery P, 2002). Early and correct diagnosis is of great importance because on this basis, early and effective treatment can be initiated, which may not only suppress inflammatory activity, but also prevent disease progression and improve outcome (Scott DL, 2000). In the USA, arthritis and rheumatism have been demonstrated to be the leading causes of disability in persons >15yr of age (Center for Disease Control, 1994). It has been estimated that >43 million Americans are affected by arthritis; and as the population ages, this number is expected to increase to >60 million by the year 2020 (Center for Disease control, 1995).
The World Health Organisation (WHO) has reported the figures for burden due to musculoskeletal disease and shown that not only are they significant in terms of absolute disability-adjusted-life years (DALYs) but that this burden is seen (and is growing) in both the developed and developing world (Peter M Brooks, 2006).

From a social perspective, the burden of disease is generally measured in terms of dollars. For countries for which data are available – the USA, Canada, UK, France and Australia – the cost of rheumatic diseases has been estimated to account for 1-2.5% of the gross national product of these countries (Yelin E, 1995). A 1999 paper demonstrated clearly that in persons with RA, poor and declining function is associated with much higher cumulative costs of care (Yelin E, 1999). Preliminary results from an Indian setting clearly reflect the economic burden of this disease directly on the family and indirectly to the nation due to loss of work and productivity (Sukhpreet, 2007).

**Mortality:** In well-established RA, the median life expectancy is less than in control populations (Pinals RS, 1987, Vandenbroucke JP, 1984). The disability develops most rapidly during the first two years of rheumatoid arthritis (Sherrer YS, 1986, Kirwan JR, 1997). A 25-year prospective follow-up study of 208 patients shows that the median life expectancy was shortened by 7 years in males and 3 years in females. (Reilly PA, 1990).
Table 1.3.1: Expenditure involved in managing RA patients (Kobelt G, 2006)

<table>
<thead>
<tr>
<th>Details of items included (non-exhaustive)</th>
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</thead>
<tbody>
<tr>
<td><strong>Direct medical costs</strong></td>
</tr>
<tr>
<td>Inpatient admissions (including surgery and protheses)</td>
</tr>
<tr>
<td>Outpatient admissions (including surgery and protheses)</td>
</tr>
<tr>
<td>Rehabilitation (in- and outpatient)</td>
</tr>
<tr>
<td>Medical consultations (rheumatologist, general practitioner, gastroenterologist, surgeon, psychiatrist)</td>
</tr>
<tr>
<td>Paramedical consultations (physiotherapist, psychologist, podiatrist, acupuncturist)</td>
</tr>
<tr>
<td>Examinations (blood analyses, X-rays, scans)</td>
</tr>
<tr>
<td>Drugs (DMARDs, anti-inflammatory and symptomatic drugs)</td>
</tr>
<tr>
<td>Home care</td>
</tr>
<tr>
<td><strong>Direct non-medical costs</strong></td>
</tr>
<tr>
<td>Devices (wheel chairs, walking aids, technical devices)</td>
</tr>
<tr>
<td>Investments (changes to the house or the car)</td>
</tr>
<tr>
<td>Community help (home help, meals in wheels)</td>
</tr>
<tr>
<td>Transportation (ambulance, taxi, private vehicle)</td>
</tr>
<tr>
<td>Informal care (help from family and friends due to the disease)</td>
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<tr>
<td><strong>Production losses</strong></td>
</tr>
<tr>
<td>Short and long-term sick leave</td>
</tr>
<tr>
<td>Early retirement (invalidity)</td>
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<tr>
<td>Premature mortality</td>
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</tbody>
</table>
Rheumatoid arthritis presents a major socioeconomic burden that has and will continue to attract major research and development efforts aimed at elucidating the basis of the disease as well as developing effective therapies. Until there is a fundamental advance in our understanding of the etiology and pathogenesis of the disease, treatment is likely to remain palliative rather than curative.

It will be interesting to study the disease with respect to the biochemical changes occurring in the peripheral blood related to the inflammation and the genetic polymorphisms of pivotal genes to understand the disease in our population. Such study may prove to be helpful in providing further insights into the pathogenesis of the disease and may be helpful in monitoring treatment.
1.4 Review of Literature

It has been observed that majority of the arthritic conditions characterized by chronic inflammation result in secondary changes in serum biochemistry.

A study by Feldman et al states that the balance between the pro- and anti-inflammatory cytokines plays an important role in determining the severity of inflammation in rheumatoid arthritis (Feldmann M, 1996).

Studies have reported that TNF-α is a potent stimulator of hyaluronan synthesis by synovial cells and there is an increased serum level of TNF-α and IL-6 levels in patients with rheumatoid arthritis (Manicourt DH, 1993).

Recent study has linked the serum tumor necrosis factor-alpha concentrations with vitamin D levels and has demonstrated a negative correlation between them (Peterson CA, 2008).

Tumor necrosis factor-alpha serum levels were found to be higher in active with bone erosion in comparison with inactive without bone erosion rheumatoid arthritis patients (Rostamian AR, 2007).

Interleukin-1 stimulates prostaglandin synthesis and the destruction of bone and cartilage and contributes to pannus formation in rheumatoid arthritis (Dayer JM, 1991, Krane Sm, 1990).
Interleukin-1 acts in sequence with TNF-α (Feldmann M, 1996) and appears to mediate most of the articular damage in arthritis (Arend WP, 1998).

Bresnihan et al recently reported raised serum levels of serum interleukin 18 consistent with the pathophysiological role of IL-18 in rheumatoid arthritis (Bresnihan B, 2002).

Blocking the effects of IL-1 in rheumatoid arthritis has been known to protect the bone and cartilage (Abramson SB, 2002).

Kay et al reported elevated levels of IL-1 in RA patients and correlation of IL-1 with parameters of disease activity supporting the role of IL-1 in the pathogenesis of rheumatoid arthritis (Kay J, 2004).

Eastgate et al report increased plasma levels of IL-1 in RA patients (Eastgate JA, 1991).

Navarra et al reported that IL-6 stimulates the innate immune response through induction of the acute phase response by stimulating hepatocytes and activation of the hypothalamic-pituitary-adrenal axis (Navarra P, 1990).

Interleukin 6 is proven to be involved in the generation of fever in rheumatoid arthritis (Kluger MJ, 1998, Choy E, 2003).
Interleukin 6 is produced by lymphocytes, monocytes, fibroblasts, synoviocytes, and endothelial cells in the synovial joints (Choy E, 2003).

Interleukin-1 and TNF-α have been shown to be potent inducers of IL-6 synthesis (Arend WP, 1990). Danis et al have stated in their study that IL-1, IL-6 and TNF-alpha are pleiotropic cytokines produced predominantly by macrophages and have been implicated in the pathogenesis of rheumatoid arthritis (Danis VA, 1992).

Serum IL-6 concentration correlates with disease activity and radiological damage (Miek A van Leeuwen, 1995).

Katsikis et al indicated in their study that the pro-inflammatory cytokines themselves appear to trigger the synthesis of IL-10 as evidenced by experiments in which adding TNF- α or IL-1 has been shown to augment IL-10 production (Katsikis PD, 1994).

Interleukin 10 has been proven to reverse the cartilage degradation mediated by antigen-stimulated mononuclear cells from patients with rheumatoid arthritis (van Roon JAG, 1996).

Cush et al have stated in their study that there is an increased production of IL-10 by non-T cells in patients with RA which may contribute to the diminished T cell function and increased antibody and RF production in these patients (Cush JJ, 1995).
Malondialdehyde is a by product of lipid peroxidation which may be measured for estimating oxidative damage (Thomas PA, 2002).

Elevated levels of NO in serum and synovial fluid have been reported in patients with rheumatoid arthritis (Stefanovic-Racic M, 1993, Ersoy Y, 2002).

Studies have suggested the involvement of NO production lead to tissue injury in inflammation (Stefanovic-Racic M, 1993, Farell AJ, 1992).

Research conducted on the functions of NO indicates to the evidence implicating the role of NO in immune regulation, inflammation, autoimmunity and arthritis (Stefanovic-Racic M, 1993).

Karatas et al have reported an increased oxidative stress and a low antioxidant status in patients with rheumatoid arthritis (Karatas F, 2003).

Pallinti V et al have recently reported increased plasma pro-oxidant levels and decreased lipophilic antioxidant levels in rheumatoid arthritis (Pallinti V, 2004).

Witt et al have reported that the mediator PGE\textsubscript{2} is produced during inflammatory responses and increased levels of PGE\textsubscript{2} mediate some of the cardinal features of inflammation, including pain, edema and fevered (Witt DL, 1991).
Increased PGE₂ production in response to stimulation by pro-inflammatory cytokines coincides with the up-regulation of COX-2 expression in synovial cells, and COX-2 is considered to be the rate-limiting enzyme for PGE₂ production at sites of inflammation (Fumiaki Kojima, 2003).

Prostaglandin E₂ (PGE₂) is one of the important mediators of inflammation associated with rheumatoid synovitis and the ligand-activated cells in synovial tissue are likely to be a primary source of this prostaglandin in the joints of patients with rheumatoid arthritis (Crofford LJ, 1994, Crofford LJ, 1999).

Korotkova et al have reported that microsomal prostaglandin E synthase (mPGES)-1 is upregulated in experimental arthritis and is markedly expressed in synovial tissue from patients with rheumatoid arthritis (RA), suggesting its important role of in the pathogenesis of inflammatory arthritis (Korotkova M, 2005).

Brinkman et al have suggested the tumor necrosis factor – alpha (TNF-α) gene to be a potential predisposing factor for RA (Brinkman BMN, 1997).

Studies have indicated that a few polymorphisms at the TNF region (polymorphic positions at the TNF promoter and microsatellites in its proximity) could be associated with the disease (Udalova IA, 1993, Vinasco J, 1997).
Significant associations were found between RA susceptibility and the -308A and -857T alleles in individuals possessing the shared epitope in a study of thirty three multiplex families performed by Waldron-Lynch et al (Waldron-Lynch F, 2001).

Kaijzel et al reported positive associations between alleles from IL-1 gene cluster and rheumatoid arthritis (Kaijzel EL, 2002).

An Egyptian study showed that the homozygous allele E2 of IL-1β exon5 was increased in RA patients who developed erosions (Waheba A Zarouk, 2005).
1.5 Lacunae in current literature

Rheumatoid arthritis is a disease characterized by changes in the functional activities of cells such as macrophages, polymorphonuclear leukocytes, T cells and B cells resulting in varying degrees of pain, inflammation, swelling and destruction of joints. Extensive literature survey indicates that research that has been done so far in RA tend to concentrate on only single/specific aspect of the disease. An ideal study would be to study the disease by analyzing a wide range of parameters in the same patients, which has not been attempted by many researchers. Literature and data on Asian, especially Indian communities is minimal and research is still in primitive stages with respect to this population.

1.6 Significance of this study

The aim of this study is to identify the status of a panel of markers encompassing both biochemical and genetic and attempt to correlate them with the disease severity in RA patients and compare with healthy controls. The ultimate scope of this study is to achieve a better prospect of biochemical and genetic monitoring of the disease activity and to learn more about the mechanism of the destructive pathological processes to evaluate the action of potential drugs.
1.7 Objectives

- To measure Rheumatoid factor (RF) and Erythrocyte Sedimentation Rate (ESR) to establish the severity of the disease
- To estimate the levels of Malondialdehyde (MDA), vitamin E and total nitric oxide to get a measure of oxidative balance
- To measure the levels of prostaglandin E$_2$ and prostaglandin F$_{2\alpha}$ to estimate the metabolic changes and associated oxidative injury
- To study the levels of pro- and anti-inflammatory cytokines: TNF-α, IL-1β, IL-6 and IL-10
- To elucidate the restriction fragment length polymorphic (RFLP) pattern of TNF-α and IL-1β genes and to relate it to the protein levels. Level of risk and severity of disease with respect to these alleles will also be studied
- To determine whether known polymorphisms in TNF-α and IL-1β gene loci influence susceptibility to rheumatoid arthritis