Chapter II

Review of literature
2.1. Introduction:
Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown aetiology (Wang et al, 2011). The disease is characterised by articular inflammation and by the formation of an inflammatory and invasive tissue, rheumatoid pannus that eventually leads to the destruction of joints (Fig. 1) (Lee & Weinblatt 2001). The genetic background predisposes to the disease, but also person-related and environmental factors, such as age, gender, infectious agents, smoking and dietary factors are thought to play a role in the disease pathogenesis (Malaviya et al 1993; Malviya et al 2010).

![Figure 1: A schematic representation of rheumatoid joint indicating major cell types and sites of joint destruction.](image)

The prevalence of RA globally is around 1%. However, the occurrence of the disease varies among populations (Akhter et al 2011). RA is rare in less developed rural parts of the world and more common in the industrialized countries. A fall in the incidence, especially in women, and a rise in the peak age of onset of RA has been observed over the last few decades. Possible explanations for these phenomena are an increased use of oral contraceptive pill among women and higher life expectancy.

2.2. History of Rheumatoid arthritis:
Examination of skeletal remains from antiquity in Europe and North Africa shows various forms of arthritis, including osteoarthritis, ankylosing spondylitis and gout (Aceves-Avila et al 1998). But characteristic rheumatoid lesions with marginal erosions at the bone-cartilage interface of the small joints are strikingly absent. In contrast,
palaeopathological studies of specimens dating back several thousand years show clear evidence of rheumatoid arthritis (RA) in Native American tribes in North America (Rothschild et al 1988). The prevalence of RA in the same regions today remains extraordinarily high, with over 5% of individuals affected in some groups.

Evidence of RA in Europe first appeared in early 17th century art, especially by the Dutch Masters (Fig. 2), and Sydenham published the first case report in 1676.

Although intermittent case series were subsequently reported, the disease was not fully recognized until it was defined by Garrod in 1859. He named it ‘rheumatoid’ arthritis to distinguish it from the two well-known forms arthritis, rheumatic fever and gout. By the early 20th century, RA was viewed as separate from osteoarthritis (‘arthritis deformans’). In 1957, Charles Short described RA definitively and clearly set it apart as a defined clinical entity distinct from the seronegative spondyloarthropathies, crystal-induced disease, osteoarthritis, systemic lupus erythematosus, and many other conditions.

2.3. Classification of Rheumatoid arthritis:
The currently accepted classification scheme for rheumatoid arthritis (RA) is the 1987 American Rheumatism Association (ARA) criteria (Table 1). The 1987 criteria were developed to replace existing classification scheme that had not been revised since 1960s. The newer criteria are simpler to apply and with more sensitivity than previous ones. A patient fulfilling four of seven criteria is said to have RA.

Table 1

<table>
<thead>
<tr>
<th>The 1987 ARA criteria for rheumatoid arthritis classification</th>
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<td>1. Morning stiffness</td>
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2. Arthritis in three or more joints
Soft tissue swelling or fluid observed by a physician, present simultaneously for at least 6 weeks

3. Arthritis of hands
Swelling of wrist, MCP or PIP joints at least 6 weeks

4. Symmetric arthritis
Simultaneous improvement of the same joint area on both sides of the body for at least 6 weeks

5. Rheumatoid nodules
Subcutaneous nodule over bony prominences, extensor surfaces or in juxta-articular regions

6. Rheumatoid factor
Detected by a method positive in fewer than 5% of normal controls

7. Radiographic changes
Typical of RA on postanterior hand and wrist radiographs

2.4. Prevalence
Rheumatoid arthritis has a worldwide prevalence of approximately 1% (Akhter et al 2011). The prevalence of RA in adults has been reported to vary from 0.5 to 3.8% in women and from 0.15 to 1.37% in men, with peak incidence in the fourth decade of life (Malaviya et al 1993) and is consistently observed to affect women 2-3 times more frequently than men. The occurrence of RA is not, however, the same throughout the world (Fig. 3). There are some interesting exceptions and its prevalence and incidence vary from one population to another (Del Puente et al 1989; Mijiyawa 1995) and from time to time (Silman 1992; Spector 1993). In individual patients, it takes a variable course with remissions and exacerbations, and has a variable outcome, from a remitting disease leaving no damage to a severe disease bringing disability and even death (Rasker & Cosh 1992).

Prevalence rates are low in the less developed and rural parts of the world and it have been suggested that RA is a modern disease, its appearance seeming to coincide
with industrialisation or urbanisation. A study in South Africa found a low frequency of RA among Bantu-speaking people in their traditional rural environment but higher rates in the same ethnic group living in the modern urban townships of Soweto, similar in fact to Caucasians living in nearby Johannesburg (Silman & Pearson 2002). This apparent influence of urbanisation was not however observed in a study comparing rural Chinese with those living in the highly industrialised society of Hong Kong. The frequency of RA was low in both the Chinese populations studied. Other factors such as diet and a lower or different genetic susceptibility, may explain these apparently contradictory findings.

2.5. Factors Affecting Arthritis Risk

A greater risk of arthritis is associated with certain factors. Non-modifiable risk factors (those that cannot be changed) include female gender, advanced age and genetic predisposition. Modifiable risk factors (those that may be changed/prevented) include physical activity levels, obesity, joint injuries, infections and certain occupations such as those with repetitive knee bending or other repetitive joint movements (Worthington et al 2006). Awareness of these risks is important in order to identify groups at higher risk and effectively target intervention efforts.

Non-Modifiable Factors:

- Female Gender: Women aged 15 years and older account for 60% of all arthritis cases. In 1995, it was reported that at least 26.4 million U.S. women have arthritis, listing it as the leading chronic condition among women. An estimated 36 million women will be affected by arthritis by 2020.
- Advanced Age: Older age is associated with an increased risk of arthritis as half of all adults 65 and older are affected by arthritis.
- Genetic Predisposition: Genetic predisposition is known to affect risk for certain types of arthritis. The exact role of these genetic factors is still unclear, but there is evidence that certain genes are known to be associated with a higher risk of some types of arthritis. Rheumatoid arthritis, ankylosing spondylitis, and lupus erythematosus are most associated with a genetic influence.

Modifiable Factors:

- Activity Levels: Physical inactivity can complicate the problems associated with arthritis. Pain from arthritis can lead to a failure to use the affected joints, which in turn can lead to muscle atrophy, as well as joint capsule and tendon contracture. The result is decreased flexibility and ultimately a loss of independence. Regular, moderate physical activity helps maintain joint health and can improve aerobic capacity and alleviate
depression. Studies have also shown that an appropriate exercise program will help reduce pain and improve functional capacity in people with arthritis.

- Overweight and Obesity: Obesity and overweight increases the risk of arthritis, especially osteoarthritis of the knee and hip. Conversely, weight loss has been shown to be effective in the management of arthritis. A weight loss of 5 kg (approximately 11 pounds) is associated with a 50% decrease in the risk of developing symptomatic knee osteoarthritis.

- Other: Infection, injuries and occupational injuries are other risk factors for arthritis. Primary prevention aimed at preventing Lyme disease, carpal tunnel syndrome and injuries to the joints can reduce the prevalence of arthritis.

2.6. Clinical course of the disease

There are three forms of clinical presentation (Fig. 4) into which most cases of RA can broadly be recognized.

- A chronic progressive form in which the disease begins with minimal joint involvement and then progresses slowly over a period of years to multiple joint disease with severe functional limitation. This is the most common pattern of arthritis seen.

Figure 4. Three patterns of the clinical progress of rheumatoid arthritis are demonstrated. The most common pattern is of chronic progressive disease.
An intermittent course punctuated by acute episodes of arthritis with periods of remission in between.

An explosive onset with multiple joint involvement and acute synovitis which may go into partial remission after 3 years or so. This pattern of RA is more commonly seen when RA begins in the elderly patient.

2.7. Pathogenesis of Rheumatoid arthritis

The synovial membrane in patients with rheumatoid arthritis is characterized by hyperplasia, increased vascularity, and an infiltrate of inflammatory cells, primarily CD4+ T cells, which are the main orchestrator of cell-mediated immune responses (Choy & Panayi 2001). In genetic studies, rheumatoid arthritis is strongly linked to the major histocompatibility complex class II antigens HLADRB1*0404 and DRB1*0401 (Lanchbury 1992). The main function of HLA class II molecules is to present antigenic peptides to CD4+ T cells, which strongly suggests that rheumatoid arthritis is caused by an unidentified arthritogenic antigen (Gregersen et al 1987). The antigen could be either an exogenous antigen, such as a viral protein, or an endogenous protein. Recently, a number of possible endogenous antigens, including citrullinated protein, human cartilage glycoprotein 39, and heavy-chain-binding protein, have been identified (Blass et al 1999).

2.7.1. Cellular Mediators of Inflammation and Joint Damage

Antigen-activated CD4+ T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce the cytokines interleukin-1, interleukin-6, and TNFα and to secrete matrix metalloproteinases (Fig. 5) through cell-surface signaling by means of CD69 and CD11 (Isler et al 1993) as well as through the release of soluble mediators such as interferon and interleukin-17. Interleukin-1, interleukin-6, and TNFα are the key cytokines that drive inflammation in rheumatoid arthritis. Activated CD4+ T cells also stimulate B cells (Fig. 5), through cell-surface contact and through the binding of integrin, CD154 (CD40 ligand), and CD28, to produce immunoglobulins, including rheumatoid factor. The precise pathogenic role of rheumatoid factor is unknown, but it may involve the activation of complement through the formation of immune complexes. Activated CD4+ T cells express osteoprotegerin ligands that stimulate osteoclastogenesis (Fig. 5). Such activated T cells caused joint damage in an animal model of rheumatoid arthritis (Kong et al 1999). These activated macrophages, lymphocytes, and fibroblasts, as well as their products, can also stimulate angiogenesis, which may explain the increased vascularity found in the synovium of patients with rheumatoid arthritis. Endothelial cells
in the synovium are activated and express adhesion molecules that promote the recruitment of inflammatory cells into the joint. This process is enhanced by the release of chemokines, such as interleukin-8, by inflammatory cells in the joint. The detailed mechanisms of these complex cellular interactions remain elusive.

![Diagram of cytokine signaling pathways](image)

**Figure 5. Cytokine Signaling Pathways Involved in Inflammatory Arthritis.** The major cell types and cytokine pathways believed to be involved in joint destruction mediated by TNFα and interleukin-1 are shown. Th2 denotes type 2 helper T cell, Th0 precursor of type 1 and type 2 helper T cells, and OPGL osteoprotegerin ligand.

### 2.7.2. Soluble Mediators of Inflammation and Joint Damage

Monocytes, macrophages, fibroblasts, and T cells release numerous cytokines on stimulation. Most of these cytokines, including TNFα and interleukin-1, can be detected in synovial fluid from patients with rheumatoid arthritis (Houssiau 1995). Both TNFα and interleukin-1 are likely to have primary roles in the pathogenesis of rheumatoid arthritis. The serum and synovial concentrations of both cytokines are high in patients with active rheumatoid arthritis (Saxne et al 1988; Chikanza et al 1995). Furthermore, TNFα and
interleukin-1 are potent stimulators of mesenchymal cells, such as synovial fibroblasts, osteoclasts, and chondrocytes, that release tissue-destroying matrix metalloproteinases (Shingu et al 1993). Interleukin-1 and TNFα also inhibit the production of tissue inhibitors of metalloproteinases by synovial fibroblasts (Shingu et al 1993). These dual actions are thought to lead to joint damage. Perhaps by inducing the production of interleukin-11, TNFα stimulates the development of osteoclasts, which are responsible for bone degradation (Girasole et al 1994).

2.7.2.1. Macrophages in RA

Monocytes are derived from myeloid progenitor cells in the bone marrow. Macrophages are usually the first immune cells to encounter pathogens, and act primarily as phagocytic cells that engulf microorganisms to destroy them in intracellular vesicles. Macrophages recognize pathogens with receptors that display conserved structures on microorganisms, present antigens to T-cells and also activate them.

![Activated macrophages and cytokines targets in RA](image)

Macrophages play a central role in the inflammation of the synovial membrane and the cartilage-pannus junction in patients with RA. The abundance and activation of macrophages at the inflamed synovial membrane correlates significantly with the severity of the disease (Mulherin et al 1996). Activated macrophages overexpress MHC class II molecules and produce pro-inflammatory or regulatory cytokines (Fig. 6) and growth factors (IL-1, IL-6, IL-10, IL-13, IL-15, IL-18, TNF-α and granulocyte macrophage...
colony stimulating factor (GM-CSF)), chemokines (IL-8, macrophage inflammatory protein (MIP)-1, monocyte chemoattractant protein (MCP)-1), metalloproteinases and neopterin, that are detected in the inflamed joint (Kinne et al 2000). The activation of the monocyte-lineage cells is not restricted to synovial macrophages as it extends also to circulating monocytes and polymorphonuclear cells (Kinne et al 2000).

![Diagram of Biological actions of TNF-α, which is produced mainly by activated macrophages in the inflamed synovial membrane tissue.](image)

Macrophages activated through the alternative pathway have been exposed to IL-4, IL-10, IL-13, tumor growth factor (TGF)-β or glucocorticoids. These macrophages produce anti-inflammatory cytokines and are resistant to re-activation. Alternatively activated macrophages are also involved in matrix synthesis and stabilization, enhancement of cell survival and proliferation, angiogenesis and antigen presentation (Fig.7). Macrophages activated by either pathway induce the phagocytosis of debris and apoptotic cells (Opferman & Korsmeyer 2003).

2.7.2.2. T-cells

T-cells develop from bone marrow derived common lymphoid progenitors. The progenitor cells migrate to the thymus where the actual maturation of T-cells occurs. T-cells are divided into Type 1, Type 2 and Type 3 effector cells. Type 1 T-cells are characterized by the expression of chemokine receptors CCR5 and CXCR3 and by the secretion of IFN-γ and IL-2. These cells help cytotoxic T-cells in the clearance of
intracellular pathogens, they activate macrophages and are considered to be effector cells in several cell-mediated autoimmune diseases. Type 2 T-cells express CCR3, CCR4 and CCR8 and secrete IL-4, IL-5, IL-6, IL-10 and IL-13 cytokines. Type 2 cells activate B-cells to produce antibodies, which bind to and help destroy extracellular pathogens. Type 2 T-cells also have a central role in allergic reactions (Figure 5). Type 3 CD4 T-cells originate from the mucosa, are activated by mucosal antigens and secrete TGF-β. These cells provide assistance for IgA production and can enhance their own differentiation by secreting TGF-β. The chemokine receptor expression of type 3 T-cells is not known. Type 2 and 3 cytokines play anti-inflammatory roles in suppressing type 1 responses. An additional type of regulatory T-cell, which is driven by IL-10 and secretes both IL-10 and TGF-β, has been termed as Tr1 cell (Groux et al 1997).

Autoimmune diseases are believed to arise when cells of specific tissues become targets of T-lymphocytes and/or autoantibodies. T-cells are important in promoting and initiating the tissue damage seen in these disorders. The mechanisms by which T-cells cause the destruction of tissues include direct damage through cell-mediated cytotoxicity processes or indirect damage mediated by non-lymphocyte mechanisms (Opferman & Korsmeyer 2003).

2.7.2.3. T-cells in RA

The role of T-cells in the pathogenesis of RA is still not completely understood. In favour of the assumption that T-cells play a role in RA is the association of disease susceptibility and outcome with HLA-DR antigens (van Zeben et al 1991). Also, a high number of CD4 T-cells has been found in the synovial membranes of patients with RA (Iannone et al 1994). Further evidence in favour of T-cells in the pathogenesis of RA has been described from animal models in which a single T-cell clone was shown to transfer the disease (Klareskog et al 1983; Taurog et al 1983). In addition, the depletion of T-cells in non-obese diabetic-severe combined immunodeficiency (NOD-Scid) mice, that are engrafted with synovial tissue isolated from patients with active disease, show a decreased production of macrophage derived cytokines (IL-1β, TNF-α and IL-15). This suggests that the decrease in cytokine production is due to the disappearance of synovial CD68+ macrophages, which depend on T-cells for their survival (Weyand & Goronzy 2000). Also the beneficial effects of CD4 blocking agent on disease symptoms in patients further support the role of T-cells in RA (Choy et al 2002).

However, since the most striking feature of RA T-cells is hyporesponsiveness, the primacy of T-cells in early and chronic RA pathogenesis has been questioned (Smeets et
al 1998). The hyporesponsiveness of RA T-cells is reflected by a reduced response to mitogenic stimulation and decreased calcium-influxes (Kingsley et al 1987; Allen et al 1995), defective TCR mediated signalling (Maurice et al 1997), lowered T-cell proliferation rate (Cush & Lipsky 1991) and reduced expression of T-cell derived cytokines, such as IFN-\(\gamma\) and IL-2 (Firestein et al 1988; Dolhain et al 1996).

Most of the T-cells infiltrating the rheumatoid synovium express CD45RO and CD4, indicating that they are of helper and of memory subset T-cells. Instead only ten to fifteen percent of T-cells in the RA synovium contain granzyme A and perforin, the molecules specific for cytotoxic T-cells. This suggests that T-cells expressing CD8 are infrequent in the RA synovium, whereas in RA SF CD4 and CD8 T-cells are equally represented (Forre et al 1982). TCR \(\alpha/\beta\) is expressed on most of the T-cells in RA synovium and only a minority show TCR \(\gamma/\delta\) expression, although it has been found that expression of TCR \(\gamma/\delta\) is increased in the synovium and PB of patients with active RA (Jacobs & Haynes 1992).

2.7.2.4. B-cells in RA

B-cells develop from the same bone marrow derived common lymphoid progenitors as T-cells and mature in the bone marrow before entering the circulation. RA was originally considered as an antibody-driven disease (Johnson et al 1973; Vaughan 1973), but the precise role of B-cells in RA is not well understood. Nevertheless, the role of B-cells appears to be significant; B-cells act as potential links between the cells of adaptive and innate immune systems, and direct the cellular components, such as cytokines and chemokines, in inflammation. The indicators proposing an enhanced B-cell activation in patients with RA include formation of T-cell and B-cell aggregates in the synovium, expression of co-stimulatory molecules, such as CD40 ligand, enhanced production of autocrine cytokines for B-cells (IL-6 and IL-10) and abnormalities in the negative selection of B-cells in RA (Schroder et al 1996; Weyand et al 2001). In Peripheral blood, an overall high activation of B-cells and enhanced frequency of memory B-cells has been reported in patients with RA (Bohnhorst et al 2001; Lindenau et al 2003). In the synovium a reduced proliferative capacity and an enhanced receptor revision of the B-cell receptor have been found to occur (Itoh et al 2000; Reparon-Schuijt et al 2001).

2.7.3. Cytokines

Since chronic inflammation of the joints occurs in patients with RA, many inflammatory mediators have been shown to play roles in prolonging the inflammatory
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Removal of literature

process. Cytokines and chemokine attract lymphocytes to the inflammed tissue and thereby contribute to chronic inflammation present in the RA joint.

Cytokines are locally acting protein mediators that are involved in almost all biological processes, including cell growth and activation, inflammation, immunity and differentiation. These proteins form a network of synergistic, complementary, antagonistic and inhibitory factors in a biological response in a tissue that depends on the balance between such molecules present (Fig. 8). Cytokines induce the activation of a signaling cascade inside the cell after binding specific receptors. They are mostly

![Figure 8 Cytokine cascade in rheumatoid arthritis](image)

produced by activated inflammatory cells, although epithelial cells, chondrocytes and hepatocytes are also known to secrete cytokines. The role of cytokines in the pathogenesis of autoimmune diseases has been the object of intensive study in recent years.

Analysis of the expression of cytokines at mRNA and protein levels in patients with RA has revealed that many proinflammatory cytokines are abundant in synovial tissue (Vervoordeldonk et al., 2002). These proinflammatory cytokines such as TNF-α, IL-1, IL-6 and GM-CSF, and chemokines such as IL-8, are mainly secreted by cells of the monocyte/macrophage lineage. It has been shown that the absolute number of cytokine producing T-cells in rheumatoid SF is low, yet the spontaneous production of cytokines at the level of a single T-cell is increased in the arthritic joint when compared to PB T-cells (Ronnelid et al 1998). The increased expression of inflammatory mediators in rheumatoid joints is counteracted to some degree by the production of anti-inflammatory cytokines such as IL-10 and TGF-β and by cytokine inhibitors such as the IL-1 receptor antagonist (IL-1ra) and the soluble TNF-α receptor (TNF-R) (Lotz et al 1990; Cope et al 1992; Firestein et al 1994; van Roon et al 2001). Despite the anti-inflammatory
mediators, a pronounced chronic inflammation is characteristic of the RA joint. Cytokines found in increased concentrations in RA synovium are listed in table 2.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Main producing cell in RA</th>
<th>synovial membrane</th>
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<tr>
<td><strong>Proinflammatory Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>macrophages</td>
<td>+</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>T-cell</td>
<td>+</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>macrophages</td>
<td>+</td>
</tr>
<tr>
<td>LT</td>
<td>T-cell</td>
<td>+</td>
</tr>
<tr>
<td>IL-1α &amp; β</td>
<td>macrophages</td>
<td>+</td>
</tr>
<tr>
<td>IL-2</td>
<td>T-cell</td>
<td>+/-</td>
</tr>
<tr>
<td>IL-6</td>
<td>FLS, macrophages</td>
<td>+</td>
</tr>
<tr>
<td>IL-12</td>
<td>macrophages, dendritic cell</td>
<td>+</td>
</tr>
<tr>
<td>IL-15</td>
<td>FLS, macrophages</td>
<td>+</td>
</tr>
<tr>
<td>IL-17</td>
<td>activated memory CD4+ T cells</td>
<td>+</td>
</tr>
<tr>
<td>IL-18</td>
<td>macrophages</td>
<td>+</td>
</tr>
<tr>
<td><strong>Anti-inflammatory Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-β</td>
<td>FLS</td>
<td>?</td>
</tr>
<tr>
<td>IL-4</td>
<td>Th2 cells</td>
<td>-</td>
</tr>
<tr>
<td>IL-11</td>
<td>FLS</td>
<td>+</td>
</tr>
<tr>
<td>IL-13</td>
<td>Th2 cells</td>
<td>+</td>
</tr>
<tr>
<td><strong>Regulatory Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>Th2 cells, macrophages, B-cells</td>
<td>+</td>
</tr>
<tr>
<td>TGF-β</td>
<td>macrophages</td>
<td>+</td>
</tr>
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</table>

Table 2. Cytokines expressed in RA synovium. The classification of cytokines is made based on the standard practice used in the study of RA (modified from Vervoordeldonk et al., 2002 and Feldmann et al, 1996). TNF-tumor necrosis factor, IFN-interferon, IL-interleukin, LT-lymphotoxin, TGF-transforming growth factor, Th-Th helper cell, FLS-fibroblast-like synoviocyte

2.7.3.1. Pro-inflammatory cytokines

TNF-α

TNF-α is a 17 kD protein composed of three identical subunits. It is produced mainly by monocytes and macrophages, but also by activated helper T-, B- and NK-cells, mast cells polymorphonuclear leukocytes, astrocytes and smooth muscle cells. Its main function appears to promote inflammation by inducing fever, shock, tissue injury, energy substrate mobilization, cachexia, bone resorption, differentiation and proliferation of hematopoietic cells and production of acute-phase proteins. It is the most rapidly released cytokine under stress and has the ability to stimulate the production of other
pro-inflammatory cytokines, such as IL-1, IL-6, and chemokines such as IL-8 (Tracey et al 1987; Butler et al 1995).

The primary sources of TNF-α, monocytes and macrophages, are also one of its main targets. TNF-α causes the activation and differentiation of monocytes and macrophages, acts as a chemotactic agent for monocytes and induces the expression of adhesion molecules on endothelial cells, which further induces the migration of leukocytes to the site of TNF-α release. TNF-α also promotes inflammation by upregulating the production of matrix metalloprotein-1 (MMP-1) and PGE₂ (Dayer et al, 1985). TNF-α reduces the expression of MHC class II proteins on macrophages and acts as a growth factor for monocytes by preventing programmed cell death (Mangan et al 1991; Watanabe & Jacob 1991).

Resting T-cells do not respond to TNF-α as they do not express the receptor, TNF-R. Instead, activated T-cells increase the expression of the IL-2 receptor (IL-2R) and thus the proliferative response to IL-2 as well as the production of IL-2 in response to TNF-α. IL-2R expression is also increased on NK-cells upon exposure to TNF-α (Ostensen et al., 1987). Endothelial cells, which are also major targets of TNF-α, increase their expression of adhesion molecules, such as intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, when responding to TNF-α (Osborn, 1990). In addition to this, TNF-α recruits leukocytes to the site of inflammation by inducing angiogenesis and the release of chemokines produced by a variety of cells. TNF-α appears to inhibit collagen synthesis by fibroblasts and to stimulate the resorption of proteoglycans in cartilage and inhibit their synthesis (Osborn 1990). TNF-α has also the ability to kill cells infected with viruses and to inhibit viral replication (Feduchi & Carrasco 1991).

On the other hand, TNF-α has been shown to have an anti-inflammatory role by stimulating the release of corticotrophin, a hormone that stimulates the release of cortisol from the adrenal cortex, which indirectly downregulates inflammation (Tilders et al 1994). Practically all cells have TNF-R and therefore respond to TNF-α. Two different forms of TNF-R, p55 and p75, have been described (Tartaglia et al., 1992). TNF-Rp55 is ubiquitously expressed on all cells, whereas TNF-Rp75 is mainly found on hemopoietic and endothelial cells (Tartaglia et al, 1992). Signaling through TNF-Rp55 has an effect on antiviral activity, fibroblast proliferation and induction of superoxide dismutase, whereas signaling through TNF-Rp75 affects the proliferation of thymocytes and cytotoxic T-cells (Tartaglia et al, 1992). The receptors are also produced naturally as soluble
molecules, which inhibit the action of TNF-α by competition of binding with cell surface receptors (Aderka et al 1992; Koch et al 1995).

IL-1

Interleukin-1 is a 17-kd protein that is mostly produced by monocytes and macrophages (Fig. 9) but is also produced by endothelial cells, B cells, and activated T cells (Koch et al 1995). The interleukin-1 signaling system is more complex than the TNF α system. Interleukin-1 binds to two types of cell-surface receptors (Sims et al 1988; McMahan et al 1991; Sims et al 1993). Only type I receptors have a cytoplasmic tail and are capable of intracellular signalling (Sims et al 1993). Type II receptors are decoy receptors: they bind circulating interleukin-1 but do not deliver any intracellular signals (Colotta et al 1993).

The type I receptor whereas the type II receptor is expressed primarily on neutrophils, monocytes, and B cells. Soluble forms of both types of interleukin-1 receptor compete with cell surface receptors, thereby decreasing interleukin-1- mediated activation of cells. In addition, a naturally occurring antagonist, interleukin-1-receptor antagonist, binds the type I receptor with high affinity without triggering a signal, thus providing another mechanism for the inhibition of interleukin-1 activity (Svenson et al 1995). The biologic activity of interleukin-1 is dependent on the precise quantities of many interacting molecules. Studies of arthritis in animals have strongly implicated interleukin-1 in joint damage. Injection of interleukin-1 into the knee joints of rabbits results in the degradation of cartilage (Pettipher et al 1986) whereas the injection of antibodies against interleukin-1 ameliorates collagen induced arthritis in mice and decreases the damage to cartilage (Joosten et al 2008). Macrophages in the synovial
tissue of patients with rheumatoid arthritis appear to be an important source of interleukin-1 (Arend & Dayer 1995). Like TNFα, interleukin-1 may cause damage by stimulating the release of matrix metalloproteinases from fibroblasts and chondrocytes (Fig. 10) (MacNaul et al. 1990). The concentrations of interleukin-1 receptor antagonist are high in the synovial fluid of patients with rheumatoid arthritis (Chomarat et al. 1993).

Figure 10. Role of IL-1 in activation of inflammation, pannus formation, cartilage breakdown and bone resorption.

IFN-γ

Biologically active IFN-γ is a 34 kD homodimer glycoprotein and a Type 1 cytokine that has receptors on virtually all cells of the body. Activated T-cells and NK-cells are the main producers of IFN-γ, but macrophages also secrete IFN-γ. IFN-γ is the major activator of macrophages and it stimulates several macrophage functions including tumor cell cytotoxicity (Pace et al. 1983), antimicrobial activity (Nathan 1992), increased killing of intracellular pathogens (Torrico et al. 1991) and induction of MHC class II proteins (Basham & Merigan 1983). IFN-γ stimulates the production of IFN-β, IL-1α, IL-1β, TNF-α, interferon-γ inducible protein (IP-10) and IL-12 in human and murine systems and inhibit the expression of some cytokines and chemokines, such as IL-8, IL-10 and MCP-1 in human monocytes (Ohmori & Hamilton 1994). It also upregulates the Fcγ receptors on phagocytes (Fertsch & Vogel 1984) and adhesion molecules, such as ICAM-1, on endothelial cells (Dustin et al. 1986). IFN-γ suppresses Type 2 responses by inhibiting IL-4, which mediates the antibody isotype switch (favoring IgE) and by inhibiting macrophage derived production of IL-10 (Chomarat et al. 1993). IFN-γ promotes T-cell proliferation in low concentrations, T-cell apoptosis in high concentrations and can augment the development of cytotoxic lymphocyte activity in vitro (Siegel 1988). IFN-γ is mainly induced by IL-12 and IL-18, which are secreted by monocyte lineage cells. The pro-inflammatory actions of IFN-γ synergise with TNF-α in many in vitro test systems. However, this might critically depend on the state of
differentiation of the cells, as seen when TNF-α was found to enhance IFN-γ-triggered MHC class II expression in undifferentiated macrophages, yet it inhibited such enhancement in mature macrophages (Watanabe & Jacob 1991).

IL-2

IL-2, originally known as "T-cell growth factor", has both immunostimulatory and immunosuppressive effects on the immune system. IL-2 promotes the proliferation of naïve T-cells and their maturation into Type 1 deviated lymphocytes, it enhances the cytotoxicity of T-cells and promotes the production of pro-inflammatory cytokines. Furthermore, IL-2 promotes the proliferation of NK-cells and of γ/δ subsets of T-cells (Robertson & Ritz 1990). IL-2 can also synergize with IL-12 to facilitate NK-cells to produce IFN-γ and TNF-α (Carson et al 1995). However, IL-2 suppresses the immune response by promoting programmed cell death, differentiation of Th2 cells and IL-10 and TGF-β secreting immunoregulatory T-cells.

IFN-γ and IL-2 in RA

Pro-inflammatory cytokines produced by T-cells, such as IFN-γ and IL-2, are found in the joints of patients with RA. In RA, SF-derived T-cells have been shown to express increased levels of IFN-γ when compared to PB (Kusaba et al 1998), and the expression of IFN-γ mRNA in RA synovial membrane has been shown to be increased when compared to the synovial membrane of patients with spondyloarthritis (Canete et al 2000).

Locally administered IFN-γ was found to promote development of CIA in mice, whereas systemically administered IFN-γ exerted a protective effect (Nakajima et al 1990). On the contrary, in patients with RA, the production of IL-1 by synovial macrophages and further the IL-1 and TNF-α induced matrix metalloproteinase production and glycosaminoglycan release by cultured cartilage fragments were found to be inhibited by IFN-γ (Ruschen et al 1989). In cultured human articular chondrocytes, IFN-γ synergizes with TNF-α in prostaglandin E2 production, but decreases TNF-α-induced production of cascinase, an indicator of the proteoglycan degradation enzyme, stromelosin. IFN-γ also inhibits bone resorption in an in vitro system (Gowen et al 1986). In CIA, IFN-γ production declines with time (Mussener et al 1997), whereas in man no difference in the expression of IFN-γ can be found in synovial membranes between patients with recent-onset RA and RA of long duration (Smeets et al 1998).
2.7.3.2. Anti-inflammatory cytokines

IL-4

IL-4 is a 20 kD anti-inflammatory cytokine produced by activated T-cells, mast cells, NK-cells, basophils and eosinophils. IL-4 is characteristic of Type 2 T-cells and its main function is to direct T-cell differentiation towards Type 2, while suppressing the Type 1 immune response. IL-4 promotes the growth and differentiation of cytotoxic T-cells and mast cells, but acts as a growth inhibitor for immature thymocytes and as a maturation promoting factor for CD4+/CD8 cells in the thymus (Trenn et al 1988). On B-cells, IL-4 acts as an isotype switch factor in favour of IgE and IgG4 (IgG1 in mouse). Inflammatory functions, such as H2O2 production, intracellular antimicrobial activity and induction of IFN-γ responsive genes, are downregulated by IL-4 in monocytes and macrophages. Furthermore, IL-4 increases the expression of MHC class II molecules, IL-1ra and TNF-R, while downregulating the production of pro-inflammatory cytokines IL-1, TNF-α, IL-6, IL-8 and IL-12. In vivo the overproduction of IL-4 has been associated with elevated IgE and allergic diseases, where IL-4 induces the rolling on and adhesion of circulating eosinophils to endothelial cells (Bochner & Schleimer 1994).

IL-5

IL-5 is a homodimeric glycoprotein secreted mostly by Type 2 cells. In the mouse IL-5 induces the differentiation of activated conventional B cells into Ig-secreting cells, as well as the growth of progenitors of CD5+ B-cells and the production of IgM by B-cells. In man, the biologic effects of IL-5 are best characterized in eosinophils. IL-5 induces terminal maturation of eosinophils, prolongs eosinophil survival by delaying apoptotic death, possesses chemotactic activity for eosinophils, increases eosinophil adhesion to endothelial cells and enhances eosinophil effector functions (Foster et al 2002).

IL-13

IL-13 has a low degree of sequence homology with IL-4, despite sharing most of its biological functions. This includes induction of IgG4 and IgE production by B cells and the upregulation of CD23, CD71 and MHC class II expression. It also induces the downregulation of NK and monocyte function, yet distinct from IL-4 synergism only IL-13 is required for worm removal. While being produced at high levels by Type 2 T-cells, IL-13 can also be expressed by both Type 1 and naive T-cells. IL-13 has a critical role as an effector molecule during established allergic inflammation, whereas it is responsible for the hypersecretion of mucus by mucus cells (Foster et al 2002).
IL-4, IL-13 and IL-5 in RA

IL-4 is believed to play a protective role in arthritis, although its virtual absence in synovial samples from patients with RA points to its lack of protective mechanisms rather than to its active regulation (Miossec et al. 1992; Cohen et al. 1995). In the CIA mouse, IL-4 delays the onset and diminishes the clinical symptoms of CIA, as well as preventing joint damage and bone erosion (Horsfall et al. 1997). In patients with RA, IL-4 has been shown to inhibit the production of pro-inflammatory cytokines and to induce the production of the natural anti-inflammatory cytokine IL-1ra in ex vivo synovial tissue specimens (Chomarat et al. 1995). In vitro, IL-4 inhibits the activation of Type 1 helper T-cells and in this way decreases the production of IL-1, TNF-α and TNF-R, and it inhibits cartilage damage (van Roon et al. 1997). Furthermore, recombinant IL-4 has been shown to inhibit the spontaneous production of total IgG and IgM and IgM RF in non-stimulated T plus B cell cultures from patients with RA (Fidaka et al. 1992). IL-4 also inhibits bone resorption through an effect on osteoclast activity and survival in patients with RA (Miossec et al. 1994).

Reports exist stating the absence of IL-5 expression in SF and IL-5 mRNA in the rheumatoid nodule (Bakakos et al. 2002; Hessian et al. 2003), however the role of IL-5 in RA remains virtually unknown. IL-13 has been detected in the SF of patients with RA and recombinant IL-13 has been shown to reduce the expression of pro-inflammatory cytokines produced by SF macrophages (Isomaki et al. 1996). In a recent study, Relic et al. showed that IL-13 and IL-4 have a protective role on human synoviocytes against apoptosis (Relic et al. 2001).

2.7.3.3. Regulatory cytokines

IL-10

IL-10 has a dual role in the inflammatory process since it has both anti-inflammatory and proinflammatory potential. IL-10 is a Type 2 cytokine in mice, yet both Type 1 and Type 2 cells can produce it in humans (Yssel et al. 1992). It is also produced by B-lymphocytes, mast cells, eosinophils, monocytes, keratinocytes and a variety of tumor cells. IL-10 inhibits the production of pro-inflammatory cytokines secreted by macrophages and upregulates the production of anti-inflammatory molecules, such as IL-1ra and soluble p55 and p75 TNF-receptors. IL-10 also downregulates the production of activator and co-stimulatory molecules on monocytes and dendritic cells, such as ICAM-1, CD80 and CD86, and it downregulates macrophage production of toxic oxygen radicals, nitric oxide synthase and prostaglandin synthesis 2 (Mertz et al. 1994). In T-cells, IL-10 inhibits T-cell...
proliferation by inhibiting the production of IL-2 and IFN-γ (de Waal Malefyt et al 1993). IL-10 may also contribute to the induction of T-cell anergy by downregulating the ligand-receptor co-stimulatory interaction between antigen presenting cells and T-cells (Schwartz 1996). On the other hand, IL-10 enhances immune activity by stimulating the proliferation and activation of NK-cells, B-cells and IL-2-activated cytotoxic T-cells (Rousset et al 1992; Carson et al 1995) and by upregulating Fc receptors on monocytes which enhances antibody mediated cytotoxicity (te Velde et al 1992).

IL-10 in RA

In patients with RA no difference has been seen in the number of cells spontaneously producing IL-6 or IL-10 in PB when compared to controls (Berg et al 2001). Instead, high IL-10 concentrations have been detected in the serum and SF of patients with RA (Moller et al 2002). IL-10 levels have also been shown to correlate with serum RF titers and in vitro levels of spontaneous IgM RF production (Cash & Lipsky 1991), thus suggesting that in RA activation of IL-10 secretion is linked to inflammatory activity. In patients with RA, IL-10 also plays a part in cartilage degradation that is mediated by antigen-stimulated mononuclear cells (van Roon et al 1997). In CIA, systematically administered IL-10 reduces joint swelling, cellular infiltration, pro-inflammatory cytokine production and cartilage degradation (Persson et al 1996).

2.7.4. Inflammation and biochemical mediator in arthritic inflammatory reaction

Inflammation is a physiological response of the organism to different stimuli such as trauma, infection or immune reactions (Henrotin et al 2001). A variety of biochemical mediators (Fig. 11) act in concert to initiate and perpetuate the inflammatory reaction. The major biochemical mediators include phospholipase A2 (PLA2), cyclooxygenase (COX), lipoxigenase (LOX), matrix metalloproteases (MMPs), nitric oxide synthases (NOS), indoleamine 2,3-dioxygenase (IDO), tissue inhibitors of metalloproteases (TIMPs), prostaglandins (PG), leukotrienes (LT) and nitric oxide (NO). These mediators act via different interconnected pathways resulting in arthritic inflammation (Fig. 11). The functions of PLA2, COX, LOX, MMPs, NOS and IDO are summarized in Table 3.

2.7.4.1. Phospholipase A2 (PLA2)

PLA2 hydrolyzes the fatty acid from the sn-2 position of membrane phospholipids. Free fatty acids thus released can be metabolized to various lipid mediators of biological importance (Funk 2001). The remaining lysophospholipids also serve important roles in biological processes (Rivera & Chun 2008; Xu et al 2010). There are more than 14 distinct groups of PLA2 enzymes (Schaloske & Dennis 2006; Burke & Dennis 2009).
Among the four main types of PLA2 are the secreted PLA2 (sPLA2), cytosolic PLA2 (cPLA2), calcium-independent PLA2 (iPLA2) and platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein-associated PLA2 (LpPLA2). cPLA2 is the predominant type synthesized at the site of inflammation (Raichel et al. 2008) and it is the only PLA2 with a preference for arachidonic acid in the sn-2 position of phospholipids (Clark et al. 1991; Ghosh et al. 2006). As arachidonic acid is the precursor of eicosanoids, cPLA2 represents the central enzyme involved in the generation of eicosanoids and hence, is the mediator of many inflammatory processes, including RA (Bonventre et al. 1997; Uozumi et al. 1997; Uozumi & Shimizu 2002; Niknami et al. 2009). In addition, cPLA2 upregulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in neutrophils and monocytes, releasing superoxides during the inflammatory process (Riesenber et al. 1997; Raichel et al. 2008). sPLA2 can hydrolyze different fatty acids at the sn-2 position of the substrate phospholipid (Singer et al. 2002). Further, the role of the mammalian sPLA2 in eicosanoid generation is not clear. Different studies on this subject have yielded inconclusive results, and clinical trials of the efficacy of sPLA2...
against arthritis and allergies revealed no significant therapeutic effects (Schevitz et al 1995; Lambeau & Gelb 2008).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Isoforms</th>
<th>Substrate/target</th>
<th>Product released</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA₂</td>
<td>cPLA₂, iPLA₂, LpPLA₂, sPLA₂</td>
<td>Phospholipids containing arachidonic acid at sn-2 position</td>
<td>Arachidonic acid</td>
<td>Generation of precursor (arachidonic acid) for eicosanoid synthesis, release of free radicals</td>
</tr>
<tr>
<td>COX</td>
<td>COX-1 and COX-2</td>
<td>Arachidonic acid</td>
<td>PGE₂, TXA₂</td>
<td>Vasodilation, neutrophils infiltration, extracellular matrix degradation, induction of pain and edema, endothelial cell migration</td>
</tr>
<tr>
<td>LOX</td>
<td>5-LOX, 12-LOX and 15-LOX</td>
<td>Arachidonic acid</td>
<td>LTB₄</td>
<td>Leukocyte infiltration, expression of pathogenic TNF-α and IL-1β, activation of neutrophils to release superoxides and MMPs</td>
</tr>
<tr>
<td>MMPs</td>
<td>MMP-1, MMP-2, MMP-3, MMP-9, MMP-12, MMP-13</td>
<td>Collagen and proteoglycan</td>
<td>-</td>
<td>Degradation of cartilage and bone, osteoclast resorption and angiogenesis</td>
</tr>
<tr>
<td>NOS</td>
<td>bNOS, ecNOS, iNOS</td>
<td>L-arginine</td>
<td>NO</td>
<td>Production of TNF-α, IL-1β, IFN-γ and MMPs</td>
</tr>
<tr>
<td>IDO</td>
<td>-</td>
<td>Tryptophan</td>
<td>Kynurenine</td>
<td>Reduction in autoreactive T cells, development of immune tolerance, induction of regulatory T cells</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of the biochemical mediators of immune pathology in RA. The isoforms shown in bold font are the key enzymes in that group. bNOS—brain nitric oxide synthase, COX—cyclooxygenase, cPLA₂—cytosolic phospholipase A₂, ecNOS—endothelial cell nitric oxide synthase, IDO—indoleamine 2,3-dioxygenase, IL—interleukin, iNOS—inducible nitric oxide synthase, iPLA₂—calcium independent phospholipase A₂, LOX—lipooxigenase, LpPLA₂—platelet activating factor acetyl hydrolase/oxidized lipid lipoprotein-associated phospholipase A₂, LTB₄—leukotriene B₄, MMPs—matrix metalloproteases, NO—nitric oxide, PGE₂—prostaglandin E₂, sPLA₂—secreted phospholipase A₂, TNF—tumor necrosis factor and TXA₂—thromboxane A₂.
2.7.4.2. Cyclooxygenase (COX) and prostaglandins (PG)

COX converts arachidonic acid into prostaglandin H2 (PGH2), which is further catalyzed by distinct synthases to five major bioactive prostaglandins (PGE2, PGI2, PGF2, PGD2, (Fig. 12) and thromboxane A2 (TXA2))(Narumiya et al 1999). There are two isoforms of COX that are designated as COX-1 and COX-2. COX-1 is constitutively expressed in most tissues, whereas COX-2 is induced by a range of mitogenic and inflammatory stimuli. Prostaglandin synthesis in inflammatory conditions is attributable largely to COX-2. However, COX-1 also is associated with the generation of pro-inflammatory prostaglandins (Chen et al 1999). PGE2 and TXA2 are potent inflammatory mediators that contribute to the pathogenesis of RA (Weissmann & Korchak 1984; Trebino et al 2003; Honda et al 2006; Lemos et al 2009). PGE2 causes vasodilatation and recruits neutrophils to the affected joints in RA. The latter effect is attributable both to the production of IL-23-induced IL-17 and the impaired production of IL-12 and IFN-c production (Lemos et al 2009). Moreover, PGE2 mediates matrix degradation and cartilage destruction(Miwa et al 2000). PGE2 also plays a role in angiogenesis evoked by inflammation by stimulating the production of vascular endothelial growth factor (VEGF)(Alcaogi et al 2006). Moreover, PGE2 contributes to

![Diagram](Figure 12. Arachidonic acid and lipooxygenase pathway)
inflammatory pain by sensitizing to bradykinin as well as histamine induced nociceptive stimuli, and to edema via plasma extravasation. In addition, the effects of IL-1, IL-6 and TNF-a on bone resorption have been shown to be PGE2 dependent (Yano et al 2001). TXA2, the other product of COX, induces rapid irreversible aggregation of human platelets and it is a potent inducer of smooth muscle contraction (Dogne et al 2000). TXA2 also is a mediator of endothelial cell migration as well as angiogenesis (Daniel et al 1999).

2.7.4.3. Lipoxgenase (LOX) and leukotrienes (LT)

LOX constitutes a group of non-heme iron-containing dioxygenases. So far, 5-LOX, 12-LOX, and 15-LOX have been identified, which stereospecifically integrate oxygen at carbon atom 5, 12 or 15, respectively of the substrate fatty acid (Hagmann 1997). 5-LO catalyzes the synthesis of leukotriene B4 (LTB4) from arachidonic acid, and it is known to play an important role in the pathogenesis of RA (Mathis et al 2007). In contrast, 12- and 15-LOX represent major anti-inflammatory enzymes operative during the course of inflammatory joint disease (Kronke et al 2009). LTB4 is a chemoattractant and mediates the infiltration of leukocytes into the RA joint (Mathis et al 2007). There, these cells proliferate and form an invasive pannus, which leads to cartilage and bone destruction (Chen & Lu 2006).

Recent reports suggest that LTB4 increases the production of pathogenic TNF-a and IL-1b at both the mRNA and the protein level (Xu et al 2010) LTB4 not only serves as a chemoattractant, but also activates neutrophils to release superoxides and proteolytic enzymes, which in turn cause matrix destruction (Wipke & Allen 2001). The release of inflammatory lipid mediators, particularly PGE2, TXA2 and LTB4 is regulated by a cascade of reactions starting from PLA2. Table 3 depicts the functions of PLA2, COX and LOX. Selective inhibitors of LOX or COX display suppressive effect against inflammation in the joint (Anderson et al 1996; Cortes-Burgos et al 2009). However, dual inhibitors of LOX and COX are more effective than selective single-enzyme inhibitors in preventing arthritis in experimental models (Martel-Pelletier et al 2003; Araico et al 2007). In comparison, the inhibition of over-expressed cPLA2 should simultaneously diminish the activity of multiple lipid mediators that facilitate the recruitment of neutrophils to the site of inflammation and the release of superoxides (Magrioti & Kokotos 2010) products described above involves inhibition of NF-kB activity, which in turn suppresses the activity of COX and other inflammation-related biomolecules.
Matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteases (TIMPs)

In arthritic conditions, inflammatory cytokines such as IL-1β and TNF-α stimulate the production of MMPs, enzymes that can irreversibly degrade components of extracellular matrix (ECM), including the articular cartilage and bone (Poole et al 2003; Kevorkian et al 2004; Burrage et al 2006). Cartilage is made up of proteoglycans and type II collagen, while bone is composed primarily of type I collagen. The degradation of collagen by MMPs is the rate-limiting step in cartilage and bone damage. MMP-1 is produced primarily by the synovial cells that line the joints, while MMP-13 is a product of the chondrocytes that reside in the cartilage. MMP-13 degrades collagen as well as the proteoglycan molecule, aggrecan. The expression of other MMPs such as MMP-2, MMP-3, MMP-9, MMP-12, and MMP-14 is also elevated in arthritis (Andersen et al 2004). These enzymes degrade non-collagenous protein components of the matrix resulting in complete joint destruction. In addition, they play a critical role in angiogenesis (Moses 1997; Raza & Cornelius 2000) which is one of the vital components of the pathogenic process in inflammatory arthritis. Inhibiting the activities of pathogenic MMPs can prevent or significantly reduce joint destruction, thereby benefiting arthritis patients with an improved quality of life. TIMPS 1–4 are the natural inhibitors of MMPs, and they also inhibit pro-inflammatory cytokines and tissue damage in the joint (Carmichael et al 1989; Mohammed et al 2003). Significant effort has been invested in designing effective inhibitors of MMP activity and/or synthesis (van der Laan et al 2003) that display anti-arthritic activity in experimental animal models (Shaw et al 2000). Moreover, a number of MMP inhibitors derived from herbal products have been shown to suppress arthritis (Table 3). For example, the anti-arthritis activity of total glucosides of paeony (TGP), a TCM product, in rats is attributable in part to the inhibition of the production of IL-1β and TNF-α by macrophage-like synoviocytes, and that of MMP-1 and MMP-3 by the fibroblast-like synoviocytes. Furthermore, this concurrent inhibition of different mediators of inflammation can be explained by the fact that IL-1β and TNF-α regulate the expression of MMP-1 and MMP-3. Similarly, in another study, Triphala guggulu, an Ayurvedic medicine, is shown to inhibit certain key enzymes involved in tissue damage in arthritis, including hyaluronidase, collagenase and MMPs. Ursolic acid suppresses the expression of MMP-9 (Shishodia et al 2003) one of the NF-κB-regulated genes.
2.7.5. Free radicals

Free radicals are continually generated within metabolically active cells of aerobic organisms and they utilize molecular oxygen (dioxygen or $O_2$). The major reactive oxygen species (ROS) generated are the superoxide anion radical (dioxygen or $O_2^-$), the hydroxyl radical (OH) and the peroxynitrite anion (ONOO⁻) (Fig. 13). Free radicals are highly reactive (de Groot 1994) and they can be quite toxic and cause cellular dysfunction and even cell death (Kehrer 1993). The harmful effects of free radicals are owing to their tendency to interact with and to damage macromolecules such as DNA, proteins, carbohydrates and lipids (Kehrer 1993). Oxygen radical generation is relatively high in the RA joint (Woodruff et al. 1986; Merry et al. 1989). In regard to RA pathogenesis, the effects of free radicals on connective tissue macromolecules (collagen, hyaluronic acid (HA), proteoglycans), intact tissues and immunoglobulins are of high relevance (Cuzzocrea 2006). The free radicals generated by polymorphonuclear cells (PMNs) alter IgG, which could in turn activate PMNs to generate additional superoxides (Zlabinger et al. 1993). Free radicals themselves also activate PMNs (Weiss et al. 1985). ROS might also...
perpetuate inflammation by facilitating the generation of chemotactic factors at the local site. Superoxide dismutase (SOD) is a ubiquitously distributed anti-oxidative enzyme that affords protection against free radical damage. In addition, anti-oxidants can scavenge the free radicals and limit damage.

NO is a free radical that serves as an important messenger molecule in inflammatory conditions (Kerwin & Heller 1994). NO is synthesized from the guanidino group of L-arginine by a family of enzymes termed NO synthases (NOS), and this process involves the incorporation of molecular oxygen into L-arginine. Inducible macrophage type NOS (iNOS), endothelial cell NOS (e-NOS) and brain NOS (bNOS), represent different isoforms of NOS (Marletta 1993; Stuehr 1997; Geller & Billiar 1998). A variety of immunological stimuli including pro-inflammatory cytokines induce the expression of iNOS in a number of non-hematopoietic cells, including fibroblasts (Nathan 1992). The induction of iNOS may have either a toxic or a protective effect (Palmer et al 1992; Szabo & Thiemermann 1994; Salzman 1995; Kim et al 1997). In arthritis, NO induces the production of pathogenic cytokines such as TNF-a, IL-1b and IFN-c, as well as collagenase (Brenner et al 1997; Ajuebor et al 1998; Diefenbach et al 1998; Hierholzer et al 1998; Mclnnes et al 1998). NO also induces certain chemokines that contribute to the disease progression in arthritis. The functions of NOS are summarized in Table 3. Decreased production of NO via suppressing or inhibiting NOS reduces arthritic symptoms and affords protection against the loss of body weight (McCartney-Francis et al 1993). Anti-oxidants that are present in a number of plant extracts scavenge NO and other free radicals. Plant-derived compounds also can suppress iNOS and increase SOD activity. For example, oral feeding to rats of Quercetin, a flavonoid, ameliorates adjuvant arthritis (AA), and this effect is associated with reduced production of various mediators of inflammation, including NO by macrophages.

2.7.5. Indoleamine 2,3-dioxygenase (IDO)
Tryptophan is an essential amino acid that is critical for cell survival and proliferation (Munn et al 1999; Mellor et al 2002). It can be catabolized by IDO yielding kynurenine, which can induce apoptosis of T cells. Furthermore, IDO-mediated deprivation of tryptophan inhibits T cell proliferation. IDO is expressed in dendritic cells (DC) and activated macrophages but not in T cells. IDO-positive DC play an important role in the induction and maintenance of peripheral tolerance via the depletion of self-reactive T cells (Szanto et al 2007) and the generation/activation of regulatory T cells (Pallarino et al...
2003; Hayashi et al 2004). It has been shown in the CIA model that the induction of IDO significantly reduces both the accumulation of pathogenic Th1 and Th17 cells in the arthritic joints (Criado et al 2009) and the severity of the disease (Bianco et al 2009). However, it has also been reported that inhibiting IDO activity might attenuate rather than aggravate arthritis (Scott et al 2009). The activity of IDO can be modulated by IFN-c (Hassanain et al 1993) as well as CD4+CD25+ regulatory T cells (Treg) (Fallarino et al 2003). Furthermore, the cytoplasmic enzyme tryptophanyl-tRNA-synthetase (TTS) mediates the association of tryptophan with its specific tRNA (Fleckner et al 1995) and this accumulation of tryptophan can antagonize the IDO-mediated deprivation of tryptophan (Murray 2003; Boasso et al 2005). It has been reported that autoreactive T cells in the rheumatoid joints resist IDO-mediated inhibition and persist during disease progression (Zhu et al 2006). This effect might be because of the enhanced expression of TTS in T cells by inflammatory cytokines such as IFN-c and TNF-a (Zhu et al 2006).

2.7.7. Molecular mediators of inflammation and arthritis

The initiation and progression of arthritic inflammation requires transduction of signals from the arthritogenic stimuli. Defined ligands bind to the appropriate receptors on the target cells, initiating a chain of reactions, including the activation of transcription factors. The generation of a variety of mediators (e.g., cytokines, chemokines, MMPs and other enzymes) of inflammation and tissue damage in RA are controlled at the transcriptional level (Okamoto et al 2008). Hence, cell signaling pathways and

<table>
<thead>
<tr>
<th>Molecular mediator</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERK</td>
<td>Production of pro-inflammatory cytokines and MMPs, lymphocyte activation and differentiation</td>
</tr>
<tr>
<td>P38</td>
<td>Release of pro-inflammatory cytokines, Cox-2 and MMPs</td>
</tr>
<tr>
<td>JNK</td>
<td>Expression of cytokines, growth factors, cell surface receptors, cell adhesion molecules and MMPs</td>
</tr>
<tr>
<td>Nuclear factor</td>
<td>Expression of cytokines (TNF-a, IL-1β, IL-6, IL-17, IFN-γ, etc), chemokines (MCP-1, MCP-4, CCL18, etc.), adhesion molecules (E-selectin, ICMA-1, VCAM-1, etc.), MMPs, VEGF, NOS and COX</td>
</tr>
<tr>
<td>AP-1</td>
<td>Activation of cytokines production, T-cell differentiation, interaction with and trans-repression of the glucocorticoid receptor, and MMP expression</td>
</tr>
</tbody>
</table>

Table 4. Function of the key molecular mediators associated with the pathogenesis of RA. COX—cyclooxygenase, ICAM—intracellular adhesion molecule, IFN—interferon, IL—interleukin, MCP—monocyte chemottractant protein, MMPs—matrix metalloproteases, NOS—inducible nitric oxide synthase, TNF—tumor necrosis factor, VEGM—vascular cell adhesion molecule and VEGF—vascular endothelial growth factor.
transcription factors are important components of the effector pathways leading to arthritis. The roles of major signaling molecules and transcription factors are summarized in Table 4.

2.7.7.1. Cell signaling pathways
Mitogen-activated protein (MAP) kinases are central components of signal transduction pathways leading to the enhanced expression of mediators of inflammation that play a key role in the pathophysiology of RA (Fig. 14) and other inflammatory diseases (Sweeney & Firestein 2004). Consequently, members of the MAP kinase pathways are potential therapeutic targets in RA. MAP kinases are proline-directed serine/threonine protein kinases. Nuclear translocation of activated MAP kinases facilitates the modulation of gene transcription via the induction and/or transactivation of transcription factors (Robinson & Cobb 1997; Chang & Karin 2001). The 3 major mammalian MAP kinase pathways include the ERK pathway, the JNK/SAPK pathway, and the p38 pathway. The kinases in each pathway have multiple isoforms that may be differentially expressed in various tissues and play different roles.

2.7.7.2. Extracellular-signal-regulated kinase (ERK) pathway
The ERK pathway is activated by the MAP kinase kinases (also known as MAP kinase/ERK kinases (MEKs)). MEKs phosphorylate critical tyrosine and threonine residues of ERK (Cobb 1999). MEK/ERK pathway plays an important role in lymphocyte activation and differentiation (DeSilva et al 1998; Chen et al 1999; Pages et al 1999) in the production of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6 (Scherle et al 1998; Tuyt et al 1999; Dumitru et al 2000; Schett et al 2000) in the production of MMPs (Brogley et al 1999; Brauchle et al 2000) and in the development of synovitis, pain, and tissue destruction in RA. Accordingly, MEK inhibitors are being exploited to inhibit diverse inflammatory pathways. For example, a selective MEK inhibitor demonstrates anti-arthritic activity (Thiel et al 2007). In this regard, medicinal plants being used in CAM might be an invaluable resource for novel MEK/ERK inhibitors.

2.7.7.3. p38 MAP kinase pathway
The p38 MAP kinase has many isoforms (p38a, b, c and d), and p38a is believed to be a critical regulator of the inflammatory response, including the release of cytokines by immune competent cells and the functional response of neutrophils to inflammatory stimuli (Herlaar & Brown 1999; Ono & Han 2000). p38 MAP kinase phosphorylates several transcription factors, including signal transducer and activator of transcription
2.7.7.3. Nuclear factor of activated T cells (NFAT) pathway

NFAT regulates a variety of genes involved in inflammation, such as TNF-α, IL-1β, IL-6, IL-8, COX-2, and MMPs (Ono & Han 2000). The p38 pathway also mediates cellular functions, including cell migration, cell survival, and cell death (Hannigan et al. 2001; Kontoyiannis et al. 2002; Kodyarov et al. 2002). Inhibition of p38 MAPK suppresses paw swelling, joint damage, and the production of inflammatory cytokines (Badger et al. 1996; Adams et al. 2001).

2.7.7.4. c-Jun N-terminal kinase (JNK) pathway

JNKs phosphorylate and activate transcription factors and other cellular factors which regulate the expression of many genes encoding cytokines (TNF-α, IL-2), growth factors, cell surface receptors, cell adhesion molecules (E-selectin) and degradative enzymes (MMPs). Activated JNK can be detected in synovial fibroblasts and chondrocytes from the joints of arthritic patients but not from normal controls, and it has been implicated in chondrocytes injury and cartilage degeneration (Clancy et al. 2001; Han et al. 2001). Furthermore, the disease-suppressive effect of a JNK inhibitor in an animal model of arthritis has been reported (Gaillard et al. 2005). Inhibitors of JNK can be found in certain Chinese herbs that are used in CAM for the treatment of several inflammatory
disorders including RA (Ehrman et al. 2010). Ikarisoside, a purified compound from Epimedium koreanum, has inhibitory effects on JNK and Akt (besides NF-κB) when tested for its effects on osteoclastogenesis using monocyte/macrophage RAW 267.7 cells. The molecules targeted here are involved in abnormal bone lysis in RA. In another study, 6-dehydrogingerdione, a compound purified from ginger, was shown to enhance the activity of JNK without much effect on ERK and p38, resulting in the induction of apoptosis in the target cells (Hsu et al. 2010).

2.7.8. Transcription factors

2.7.8.1. Nuclear factor-κB (NF-κB)

The transcription factor NF-κB regulates the expression of a wide variety of genes. RelA, RelB, c-Rel, NF-κB1 and NF-κB2 are members of NF-κB family. These members activate characteristic sets of genes in a cell-type and stimulus-type manner, thus regulating the transcription of genes (Silverman & Maniatis 2001; Dejardin et al. 2002; Ghosh & Karin 2002; Li & Verma 2002; Udalova et al. 2002). NF-κB remains in an inactive form by binding to the inhibitor of NF-κB proteins (IκB), but cellular stimuli including cytokines, mitogens and stress activate IκB via activating NF-κB kinase (IκB kinase (IKK) complex) and subsequent degradation of IκB (Uhlar & Whitehead 1999; Mullan et al. 2006). The activated NF-κB translocates to the nucleus and stimulates the transcription of genes containing the consensus κB sequence 50-GGGPuNNPyPyCG-3' (where Pu denotes a purine and Py denotes a pyrimidine). Such genes include those encoding certain cytokines and chemokines, adhesion molecules, MMPs, VEGF, iNOS, COX-2, etc. Most of these genes have been reported to have important role in the pathogenesis of RA (Li & Verma 2002). VEGF as well as a few other molecules involved in angiogenesis are attractive targets for therapeutic agents against RA (Lainer-Carr & Braun 2007).

2.7.8.2. Activator protein-1 (AP-1)

AP-1 is another transcription factor that transduces extracellular signals in immune cells. AP-1 gets activated in response to a variety of inflammatory stimuli. Activated AP-1 interacts with the binding site(s) in their promoter/enhancer regions resulting in the expression of specific target genes encoding MMPs and pro-inflammatory cytokines (Benbow & Brinckerhoff 1997; Foster et al. 2000; Harrison et al. 2004). AP-1-mediated cytokine production is in cooperation with transcription factors of the nuclear factor of activated T cells (NFAT) family (Rooney et al. 1995) wherein AP-1 and NFAT form stable ternary complexes on DNA-binding sites. AP-1-mediated activation of NFAT and
integration of the signals via the receptor activator for nuclear factor-kB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) are required for osteoclast differentiation (Takayama 2005). AP-1 also regulates the differentiation of naïve T cells into T helper 1 (Th1) or T helper 2 cells (Th2), and it interacts with and trans-represses the glucocorticoid receptor (Rincon et al 1997; Brogan et al 1999).

2.7.9. Treatment, medications and therapy for Rheumatoid Arthritis

The conventional approaches to treatment of RA are either produce symptomatic relief (NSAIDs) or modify the disease process (DMARDs). Some medications are used only for pain relief; others are used to reduce inflammation. Still others, often called disease modifying antirheumatic drugs (DMARDs), are used to try to slow the course of the disease. Though effective, their use is also limited by their side effects including gastrointestinal ulcers and perforation, cardiovascular complications and emergence of opportunistic infections due to immunosuppressant. Owing to the chronic nature of disease and side effects associated with long-term use of these agents, patients with rheumatoid arthritis rely on other option like use of complementary and alternative medicine (CAM) and according to reports CAM therapy is on rise as 60-90% dissatisfied patients are likely to seek option of CAM therapy (Salahuddin et al 2005).

Special diets, vitamin supplements, and other alternative approaches have been suggested for treating rheumatoid arthritis. Although many of these approaches may not be harmful in and of themselves, controlled scientific studies either have not been conducted on them or have found no definite benefit to these therapies. Some alternative or complementary approaches may help the patient cope or reduce some of the stress associated with living with a chronic illness.

2.7.9.1. Analgesics and Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Analgesics relieve pain; NSAIDs are a large class of medications useful against pain and inflammation. A number of NSAIDs are available over the counter. More than a dozen others—including a subclass called COX-2 inhibitors—are available only with a prescription. NSAIDs can cause stomach irritation or, less often, can affect kidney function. The longer a person uses NSAIDs, the more likely he or she is to have side effects, ranging from mild to serious. Many other drugs cannot be taken when a patient is being treated with NSAIDs because they alter the way the body uses or eliminates these other drugs. NSAIDs sometimes are associated with serious gastrointestinal problems, including ulcers, bleeding, and perforation of the stomach or intestine.
Aspirin reduces the production of prostaglandin E2 through acetylation of the isoforms 1 and 2 of the cyclooxygenase. Although its use is limited by the gastric side effects (mostly dependent on cyclo-oxygenase-1 inhibition), there is a renewed interest in aspirin derivatives that potently and selectively inactivate the inducible cyclo-oxygenase-2 isoform in isolated macrophages and in local inflammation (Kalgiutkar et al 1998; Crofford et al 2000). Cyclo-oxygenase-2-dependent mechanisms selectively induce the production of IL-6 (Ross et al 1997), the cytokine that is most highly increased in the RA synovial fluid. Aspirin may affect macrophages also by decreasing the TNF-a production via nuclear factor-kB (NF-kB) mechanisms (Shackelford et al 1997).

2.7.9.2. Corticosteroids

These are steroids given by mouth or injection. They are used to relieve inflammation and reduce swelling, redness, itching, and allergic reactions. These steroids are available in pill form or as an injection into a joint. Improvements are seen in several hours up to 24 hours after administration. The potent anti-inflammatory effects of corticosteroids in RA can be at least partly explained by transcriptional downregulation of the inflammatory cytokines IL-1 and IL-6, or, as recently reported, transcriptional and post-transcriptional downregulation of TNF-a in monocytes (Amano et al 1993). Corticosteroids may also affect the balance of the functionally distinct membrane-bound and soluble TNF-a (Grell et al 1995). Interestingly, in vitro studies suggest that addition of low doses of IL-4 and IL-10 decreases the dose of corticosteroids that is necessary to downregulate TNF-a, possibly via a coordinated attack on activated macrophages. Corticosteroid also decreases the production of IL-8 and MCP-1 (Loetscher et al 1994; Seitz et al 1997); this, once optimally exploited, may limit the self-perpetuating ingress of monocytes into the inflamed joint. There is potential for serious side effects, especially at high doses. They are used for severe flares and when the disease does not respond to NSAIDs and DMARDs.

2.7.9.3. Disease-modifying antirheumatic drugs (DMARDs)

These are common arthritis medications. They relieve painful, swollen joints and slow joint damage, and several DMARDs may be used over the disease course. They take a few weeks or months to have an effect, and may produce significant improvements for many patients. Exactly how they work is still unknown. Side effects vary with each medicine. DMARDs may increase risk of infection, hair loss, and kidney or liver damage. Few of these DMARDs are discussed below.
2.7.9.4. **Gold sodium thiomalate**

This was one of the first DMARDs used to treat rheumatoid arthritis. Administration of gold compounds to RA patients results in gold accumulation in the lysosomes of synovial macrophages, especially in lysosome-rich sublining macrophages (Nakamura & Igarashi 1977). In monocytes, gold compounds inhibit Fc and C3 receptor expression, oxygen radical generation and IL-1 production (Burmester et al 1997). Through their effects on macrophages as accessory cells, gold compounds also inhibit T-cell proliferation in response to antigen or mitogen (Burmester et al 1997). Gold compounds inhibit the production of IL-1, IL-8, and MCP-1 (Seitz et al 1997) and decrease monocyte chemotaxis in vitro [2]. In the synovial lining, this is paralleled by a significant decrease in macrophage numbers and IL-1, IL-6 and TNF-a production (Yanni et al 1994; Bondeson 1997). In vitro, gold salts also inhibit angiogenic properties of macrophages, probably through their thiol moiety (Koch et al 1991), which colocalizes in macrophage lysosomes together with gold (Nakamura & Igarashi 1977). In experimental arthritis, gold salts seem much more effective as long-term disease-modifying drugs than as anti-inflammatory agents (Lewis et al 1998).

2.7.9.5. **Methotrexate**

One of the most effective DMARDs, methotrexate also impairs chemotaxis of blood monocytes (Burmester et al 1997) and monokine production, while increasing the production of cytokine inhibitors, including soluble TNF-a receptor R75. Because methotrexate shifts the IL-1/IL-1 receptor antagonist balance in favour of IL-1 receptor antagonist, this drug may pharmacologically correct the imbalance between these two mediators (Seitz et al 1997). A change in the monokine balance, including that of TNF-a, may indirectly cause a selective decrease in collagenase production in the synovial tissue. A surprising caveat of methotrexate therapy, however, is the accelerated formation of rheumatoid nodules in some patients (Williams et al 1998).

2.7.10. **Biologic Response Modifiers**

These drugs selectively block parts of the immune system called cytokines. Cytokines play a role in inflammation. Long-term efficacy and safety are uncertain. Increased risk of infection, especially tuberculosis. Increased risk of pneumonia and listeriosis are some of the reckoned side effects of these diseases though specific biologic response modifiers have specific side effects.
2.7.10.1. Tumor Necrosis Factor Inhibitors
These medications are highly effective for treating patients with an inadequate response to DMARDs. They may be prescribed in combination with some DMARDs, particularly methotrexate.

2.7.10.2. Etanercept
Etanercept is a human soluble TNF receptor fusion protein, which interferes with binding of TNF-α to its cell bound receptor, by mimicking the actions of naturally occurring soluble TNF receptors. Etanercept is self-administered subcutaneously twice a week and can be used alone or with methotrexate. Although it is not necessary to co-prescribe methotrexate, good evidence supports the possibility that the two together may be more efficacious than an individual agent. Can be side effects: Pain or burning in throat; redness, itching, pain, and/or swelling at injection site; runny or stuffy nose.

2.7.10.3. Infliximab
Infliximab is a monoclonal antibody that binds to both soluble and membrane-bound TNF-α preventing it from binding with its cell surface receptor, thus blocking its activity and reducing intra-articular TNF-α. Infliximab is administered as an intravenous infusion at commencement, after two and six weeks and then every 6–8 weeks. It is given concurrently with methotrexate to reduce the risk of allergic reaction to the murine protein. Dosage of the drug is worked out according to the patient’s weight at 3 mg per kg. It is made up on the day of administration and is mixed in 250 ml of normal saline and infused over a period of 2 h. Side effects range from abdominal pain, cough, dizziness, fainting, headache, muscle pain, runny nose, shortness of breath, sore throat, vomiting, wheezing.

2.7.10.4. Interleukin1 Inhibitor
Anakinra
This medication requires daily injections. Long-term efficacy and safety are uncertain. Side effects are redness, swelling, bruising, or pain at the site of injection; headache; upset stomach; diarrhea; runny nose; and stomach pain.

2.7.11. Complementary and alternative medicine (CAM) in treatment of rheumatoid arthritis
Agents derived from plants that can modulate the expression of pro-inflammatory signals clearly have potential against arthritis (Table 3). These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins, all
of which are known to have anti-inflammatory effects. Some of these polyphenols, which have been tested for the treatment of arthritis, are as follows.

2.7.11.1. Curcumin (Curcuma longa)

Curcumin ( diferuloylmethane) is a yellow colouring agent present in turmeric (Curcuma longa) that has been used for centuries as a spice on the Indian subcontinent (Aggarwal et al. 2006). Curcumin has been well documented in Ayurveda, an Indian system of medicine, as an anti-inflammatory agent. Several lines of evidence, both in vitro and in vivo, suggest that curcumin may have potential against arthritis. The work from our laboratory and others have shown that curcumin can downregulate activation of the transcription factor NF-κB (Singh & Aggarwal 1995), thus leading to downregulation of the expression of TNF-α (Shishodia et al. 2005), adhesion molecules (Kumar et al. 1998), MMPs, COX-2 (Aggarwal et al. 2006), 5-LOX (Sla-zyiDczak-Jankun et al. 2003) and other

![Diagram of inflammatory arthritis](image)

Figure 15. Pathophysiology of inflammatory arthritis. The figure shows current therapeutic targets and their sites of action.

inflammatory intermediates (Aggarwal et al. 2003), all of which are associated with arthritis. That curcumin indeed has potential against arthritis was first reported in 1980 (Deodhar et al. 1980). Neutral matrix MMPs are responsible for the pathological features of RA such as degradation of cartilage; however, the upregulation of MMP mRNA associated with arthritis was inhibited by curcumin (Onodera et al. 2000). This polyphenol has also been shown to suppress the expression of TNF-α-induced MMP-13 in primary
chondrocytes (Liacini et al. 2003). Jackson et al. (Jackson et al. 2006) found that curcumin inhibited neutrophil activation, synoviocyte proliferation, angiogenesis, and collagenase and stromelysin expression, thus suggesting that curcumin has therapeutic potential in arthritis. It has also been reported to potentiate the growth-inhibitory and pro-apoptotic effects of the COX-2 inhibitor celecoxib in osteoarthritis synovial adherent cells. Indeed, a recent study showed that the suppression of NF-κB activation by curcumin leads to inhibition of the expression of COX-2 and MMP-9 in human articular chondrocytes (Shaldbaei et al. 2007). Besides in vitro studies, in vivo studies also suggest that curcumin might have potential against arthritis. For example, oral administration of curcumin has been shown to decrease elevated levels of the glycoprotein Gp A72, with concomitant lowering of paw inflammation in arthritic rats (Joe et al. 1997).

2.7.11.2. Resveratrol (Vitis vinifera)

Resveratrol (or trans-3,5,40-trihydroxystibene) was first isolated in 1940 as a constituent of the roots of white hellebore (Veratrum grandiflorum O. Loes), but has since been found in various plants including grapes, berries and peanuts. The work from our laboratory has shown that resveratrol can suppress the activation of NF-κB (Manna et al. 2000) and downregulate inflammatory gene products such as COX-2, 5-LOX, IL-1β, and IL-6 (Aggarwal et al. 2004), all of which play crucial role in arthritis. Recent studies indicate that this stilbene might play a role in the prevention and treatment of arthritis; for example, Tang et al. showed that resveratrol can suppress the proliferation, and induce caspase-3-mediated apoptosis, of synoviocytes in vitro (Tang et al. 2006). Elmali and colleagues determined the in vivo effects of intra-articular injections of resveratrol on cartilage and synovium in an experimental OA model in rabbits (Elmali et al. 2005; EknaK et al. 2007). They found that resveratrol significantly reduced cartilage tissue destruction, and hence concluded that resveratrol could protect cartilage against the development of experimentally induced OA. Well-designed clinical trials are now needed to establish the efficacy of resveratrol in the prevention and treatment of arthritis.

2.7.11.3. Guggulsterone (Commiphora mukul)

Guggulsterone [4, 17(20)-pregnadiene-3,16-dione] is a plant sterol derived from the gum resin (guggulu) of the tree Commiphora mukul. This sterol can inhibit NF-κB activation and downregulate the expression of inflammatory gene products such as COX-2 and MMP-9, which are major players in the development of arthritis (Shishodia & Aggarwal 2004). Recent work showed that guggulsterone can suppress osteoclastogenesis induced by RANKL (receptor activator of NF-κB ligand), a bone-resorbing cytokine (Ichikawa &
Aggarwal 2006). The anti-arthritic and anti-inflammatory activities of gum guggul were first demonstrated by Gujral et al. in 1960 (Gujral et al. 1960). Subsequently, the anti-inflammatory activity of C. mukul (guggul) has been compared with that of NSAIDs, namely phenylbutazone and ibuprofen (Sharma 1977). In another study, Singh et al. (Singh et al. 2003) conducted both preclinical and clinical investigations of guggul for the reduction of pain, stiffness, improved function and tolerability in older patients with a diagnosis of OA of the knee. They demonstrated significant improvement in patients during the trial, in both Western Ontario and McMaster Universities (WOMAC) and visual analogue scales and objective measures used for assessment purposes. There were no side effects reported during the trial. Therefore, guggul appears to be a relatively safe and effective supplement to reduce symptoms of OA.

2.7.11.4. Withanolide (Withania somnifera)

Withanolides, which are extracted from Withania somnifera, are employed in the treatment of arthritis and are known to be potent inhibitors of angiogenesis, inflammation and oxidative stress. Recent studies showed that withanolides can indeed inhibit the activation of NF-kB and NF-kB-regulated gene expression (Ichikawa et al. 2006), which could explain their anti-arthritic actions. Begum and Sadique (Begum & Sadique 1988) showed for the first time the long-term effects of W. somnifera on adjuvant-induced arthritis in rats. More recently, Rasool and Varalakshmi (Rasool & Varalakshmi 2006) investigated the effect of W. somnifera root powder on paw volume and serum lysosomal enzyme activities in rats in which arthritis was induced with MSU crystal.

<table>
<thead>
<tr>
<th>Herbs targeting PLA₂, COX-2, LOX, PGE₃ and/or LTB₄</th>
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<th></th>
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<tbody>
<tr>
<td>Allium cepa</td>
<td>Multiple</td>
<td>Quercetin</td>
</tr>
<tr>
<td>Aralia cordata</td>
<td>Korea</td>
<td>7-Oxisandaracopimaric acid</td>
</tr>
<tr>
<td>Boswellia serata</td>
<td>India</td>
<td>Boswellic acid</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>China</td>
<td>Epigallocatechin-3-gallate</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>China/India</td>
<td>Curcumin</td>
</tr>
<tr>
<td>Ocimum sanctum</td>
<td>India</td>
<td>Ursolic acid</td>
</tr>
<tr>
<td>Sinomenium acutum</td>
<td>China</td>
<td>Sinomenine</td>
</tr>
<tr>
<td>Tripterygium wilfordii</td>
<td>China</td>
<td>Triptolide, teiptonide and celastrol</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>Multiple</td>
<td>Reserverol</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>China/India</td>
<td>Gingerol and zingerone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Herbs targeting MMPs and/or TIMPs</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Achyranthes bidentata</td>
<td>China</td>
<td>Oleanolic acid</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>China</td>
<td>Epigallocatechin-3-gallate</td>
</tr>
</tbody>
</table>
Table 3 Herbs mentioned in bold font were studied in the adjuvant arthritis (AA) model. Active compound identified in each herbal extract is listed. Some of these compounds have been tested for their specific inhibitory activity.

2.7.11.5. Green tea extracts

The constituents of green tea are polyphenolic compounds termed catechins. The most abundant catechin in green tea is (−)-epigallocatechin 3-gallate (EGCG), but (−)-epigallocatechin, (−)-epicatechin 3-gallate (ECG) and (−)-epicatechin are also present. The most widely recognized properties of the green tea catechins are their antioxidant activities. Benefits of green tea have been recognized in cardiovascular disease and cancer (Yang & Wang 1993; Singh et al 2010). More recently, the benefits of the catechins extracted from green tea have been recognized in models of arthritic disease. Studies by Haqqi et al (Haqqi et al 1999) reported prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. More recently (Adcocks et al 2002), in vitro models of cartilage degradation were used to study the effects of the individual catechins extracted from green tea. In this study, using a bovine in vitro model of cartilage degradation, it was shown that EGCG and ECG inhibit IL-1-induced proteoglycan release and type II collagen degradation in cartilage explants (Adcocks et al 2002). Similarly, in a human in vitro model of cartilage degradation, EGCG suppressed IL-1β-induced iNOS (inducible nitric oxide synthase) mRNA and protein expression and production of nitric oxide, concomitant with attenuated activation of the transcription factor NF-κB (Singh et al 2002). A recent study (Vankemmelbeke et al 2003) has shown that the catechin gallate esters found in green tea potently inhibit the aggrecan-degrading activity of the aggrecanases ADAMTS-1, -4 and -5. Interestingly, the concentrations needed for aggrecanase inhibition were two orders of magnitude lower than those needed to inhibit either collagenase or another cell-surface enzyme involved in cytokine release, ADAM-10. Thus, these extracts of green tea appear to show a preferential inhibition of certain members of the ADAMTS group of proteolytic enzymes, the
aggrecanases. It is not known whether or not these components of green tea are inhibiting these matrix proteases at the protein and/or gene expression level. From the above studies, molecular evidence appears to be emerging explaining why catechins extracted from green tea that exhibit both anti-inflammatory and chondroprotective effects might be beneficial to arthritis sufferers. However, further studies are required to determine whether or not oral consumption of green tea can lead to sufficiently high concentrations of catechins within the joint to mimic the effects that were observed in the in vitro studies.

2.7.12. Animal models of rheumatoid arthritis

Animal models have contributed to the understanding of basic mechanisms of joint disease. Arthritis has been induced by various stimuli and there is marked diversity among the numerous models. These include the generation of autoimmunity to cartilage components, nonspecific skewing of autoimmunity with adjuvants, and triggering with exogenous agents such as bacteria and viruses (van den Berg 2009). Rodent models of rheumatoid arthritis (RA) have been developed in both rats and mice. Other species have also been used over the years, however rodent models are most common, due to cost, homogeneity of the genetic background, the capacity to use genetically modified strains. Most RA in animals is produced by treatment with an inducing agent, and even "spontaneous" models can be considered induced since they develop by the introduction or deletion of specific genes in animals with the proper immunologic milieu for susceptibility(Kannan et al 2005).

2.7.12.1. Rat models of rheumatoid arthritis

The first model of polyarthritis was developed in rats when Stoerk et al. (Stoerk et al. 1954) and Pearson and Wood (Pearson and Wood, 1959) found that injection of rats with complete adjuvant induced polyarthritis, possibly by a mechanism involving heat shock proteins (HSP). This model is termed the adjuvant arthritis (AA) model, and has been used to test new drugs for inflammatory arthritis. Interestingly HSPs have been implicated in the pathogenesis of human RA as well (Res et al 1988). Many early studies of this model addressed the role of lymphocytes, suppressor cells, and the type of antigen (or lack thereof) administered with the adjuvant for induction of arthritis. In fact it was recently found that AA could be induced by incomplete Freund's adjuvant (IFA) alone in the DA strain of rats. More recently this model has also been reproduced in mice (Knight et al 1992). Adjuvant arthritis has been used in the evaluation of nonsteroidal inflammatory drugs (NSAIDs) such as phenylbutazone and aspirin during the early
1960s (Wooley 1991, 2004), and later COX-2 inhibitors such as celecoxib (Geis 1999). AA in rats shares many features with human RA including genetic linkage, synovial CD4+ cells and T cell dependence (Goodson et al 2003). One of the major differences between the AA model and human RA is simply that the inciting agent is known in the model, though the need for any specific antigen is controversial.

2.7.12.2. Collagen induced arthritis (CIA)

Collagen-induced arthritis is an extensively studied animal model of RA because it shares both immunological and pathological features of human RA. CIA is primarily an autoimmune disease of joints, requiring both T and B cell immunity to autologous type II collagen (CII) for disease manifestation. This model is reproducible in genetically susceptible strains of mice with MHC haplotypes H-2q or H-2r by immunization with heterologous type II collagen in complete Freund’s adjuvant (CFA). Collagen from a variety of sources has been used including bovine, porcine, chick, and human, and response varies with strain and injection conditions. Not surprisingly, mouse collagen gives a poor response. Highly purified collagen prepared under a defined protocol should be used, for the presence of minor contaminants or deglycosylated protein preparation yield either false positive results or may be less arthritogenic (Andersson & Holmdahl 1990). Rats injected with native type II collagen also develop polyarthritis, but the mouse model is most commonly used for its applicability in genetically modified strains.

The requirement for T cells in the development of CIA is clear, whereas the underlying mechanisms are not. Contrary to expectations, passive transfer of collagen-II specific T cells induces only minor changes in the synovium, while the transfer of collagen-II specific antibody results in severe inflammation, and co-transfer of antibody and T cells induce chronic disease. Although T cells play a prominent role in the regulation and development of CIA, autoantibody to murine CII appears to be the primary mechanism of immunopathogenesis in this model. During the early stages of disease development, anticollagen antibodies bind to the joint cartilage and activate the complement cascade (Joe et al 1999). Consistent with autoantibody being a major pathogenic factor is the fact that passive transfer of anti-collagen II sera produces an inflammatory arthritis not only in strains considered genetically susceptible to CIA, but also in CIA-non-susceptible strains.

2.7.13. Drugs used in our studies

2.7.13.1. Boswellic acid (Boswellia serrata)

Boswellic acid (BA) is an active component of Boswellia serrata (also known as Salai guggul). Extensive research in the past 30 years identified the active component of this
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Resin as BA (a pentacyclic triterpenic acid) and its derivatives (acetyl-β-boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid). BA, a mixture comprised of four major pentacyclic triterpene acids: β-boswellic acid, 3-aceteyl β-boswellic acid, 11-keto-β-boswellic acid and 3-acetyl-11-keto-β-boswellic acid, isolated from the oleo gum resin of Boswellia serrata is reported to be effective as anti-inflammatory (Singh et al., 1996), immunomodulatory (Sharma et al., 1996), anti-tumor (Huang et al., 2000), anti-asthmatic (Gupta et al., 1998) and in chronic colitis (Gupta et al., 2001). In animal models of inflammation, BA has been shown to be an effective adjuvant mitigating bovine serum albumin-induced arthritis (Reddy & Dhar 1987; Sharma et al 1989) and OA (Kimmatkar et al 2003). The anti-arthritic potential of BA is a result of its anti-inflammatory activity, mediated through inhibition of NF-κB, COX-2 and 5-LOX (Safayhi et al 1995; Takada et al 2006). (Kimmatkar et al 2003) conducted a randomized double blind placebo-controlled crossover study to assess the efficacy, safety and tolerability of B. serrata extract in 30 patients with OA of the knee. Fan et al. (Fan et al 2005) examined the effects of an acetone extract of Boswellia carterii gum resin on adjuvant-induced arthritis in Lewis rats. The results show that B. carterii extract had significant anti-arthritic and anti-inflammatory properties, and suggest that these effects may be mediated via the suppression of pro-inflammatory cytokines.

2.7.13.2. Thymoquinone (TQ)

Nigella sativa L., commonly known as black seed or black cumin, is used in folk medicine as a natural remedy for a number of disease and condition such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and gastrointestinal disturbances (Ali and Blunden, 2003). Nigella sativa seeds and oil have been used traditionally in the Middle East, Northern Africa and India to treat RA, diabetes and cancer patients [Ali and Blunden, 2003; Salem, 2005].
Thymoquinone (TQ) is an abundant component of black seed oil extract. Its beneficial effects are related to anti-oxidant, anti-infective, anti-tumour and anti-inflammatory properties [Ragheb et al., 2009]. In an experimental model of RA, Tekeoglu et al. [2007] reported that TQ suppressed adjuvant-induced arthritis (AIA) in rats, an action similar to that of methotrexate.

Thymoquinone (TQ) has been shown to exert antioxidant, antineoplastic and anti-inflammatory effects (Houghton et al., 1995; Abdel-Fattah et al., 2000) blocked pancreatic cancer cell growth and killed the cells by enhancing the process of programmed cell death (apoptosis). In Islam, it is regarded as one of the greatest forms of healing medicine available. Prophet Muhammad (SAW) once stated that the black seed can heal every disease—except death.

TQ, active constituent of N. sativa seeds, is a pharmacologically active quinone, which possesses several properties including protects organs against oxidative damage induced by a variety of free radical generating agents including doxorubicin induced cardiotoxicity (Nagi and Mansour, 2000), carbon tetrachloride evoked hepatotoxicity (Nagi et al., 1999), nephropathy produced by cisplatin (Badary et al., 1997), autoimmune as well as allergic encephalomyelitis (Mohamed et al., 2003) and gastric mucosal injury induced by ischemia reperfusion (El-Abhar et al., 2003). The volatile oil of N. sativa was shown to contain about 24% thymoquinone (El-Dalkakhny, 1963). Thymoquinone acts as a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen (Badary et al., 2003). Thymoquinone was found to inhibit arachidonic acid metabolism (Houghton et al., 1995; Marsilc et al., 2005) demonstrated that thymoquinone acts as an inhibitor of cyclooxygenase-1 and -2. TQ is able to inhibit lipid peroxidation (Houghton et al., 1995).

2.7.13.3. Piperine (PIP)

Piperine is a phenolic component of black pepper (Piper nigrum) and long pepper (Piper longum). Black pepper and long pepper are important medicinal plants which are used in traditional medicine by many people in Asia and the Pacific islands, especially in Indian medicine (Singh, 1992). It stimulates the digestive enzymes of pancreas, protects against oxidative damage, lowers lipid peroxidation, and enhances the bioavailability of a
number of therapeutic drugs. Piperine is shown to be an effective antioxidant and offers protection against the oxidation of human low density lipoprotein (LDL) as evaluated by copper ion-induced lipid peroxidation of human LDL by measuring the formation of thiobarbituric acid reactive substance and relative electrophoretic mobility of LDL on agarose gel (Naidu and Thippeswamy, 2002). Studies have also indicated that various spice principles form an important group as antioxidants. Pipeline has been demonstrated in in vitro experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species and inhibit lipid peroxidation (Mittal and Gupta, 2000). Pipeline was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the Fenton reaction resulting in increased generation of hydroxyl radicals. Rauscher et al. (2000) investigated that piperine treatment (10 mg/kg/day, i.p. for 14 days) would protect against diabetes-induced oxidative stress. Khajuria et al. (1998) have investigated that piperine is able to inhibit or reduce the oxidative changes induced by chemical carcinogens in a rat intestinal model. Selvendiran et al. (2004) have recently investigated the impact of piperine on alterations of mitochondrial antioxidant system and lipid peroxidation in benzo(a)pyrene (B(a)p) induced experimental lung carcinogenesis. Vijayakumar et al. (2004) have recently examined the effect of supplementation of black pepper or piperine on tissue lipid peroxidation, enzymic, and non-enzymic antioxidants in rats fed a high-fat diet and observed that these spices can reduce high-fat diet induced oxidative stress. The anti-inflammatory activity of piperine has been reported in rats employing different experimental models like carrageenan-induced rat paw edema, cotton pellet granuloma, and croton oil-induced granuloma pouch (Mujumdar et al., 1990).