LEADING ARTICLE

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Newer Immunosuppressive Drugs
Their Potential Role in Rheumatoid Arthritis Therapy

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Abstract

Rheumatoid arthritis (RA) is a chronic immune-mediated disease characterised by chronic synovitis, which leads to cartilage damage and joint destruction. It is generally a progressive disease with radiographic evidence of joint damage, functional status decline and premature mortality. Proinflammatory cytokines, such as interleukin 1 and tumour necrosis factor α, play an important role in maintaining the chronicity of RA and mediating tissue damage. New approaches in the therapy of RA with anticytokine biological agents, which neutralise or block cytokines or their receptors, are now the first generation antirheumatic drugs in clinical practice.

A better understanding of the signal transduction systems and gene regulation by transcription factors involved in cytokine production has opened the way for the discovery of novel therapeutic compounds useful in treating patients with RA. Overactivation of selective kinases or aberrant function of downstream transcription factors could help convert a normal immune response to a chronic disease state. This provides a unique opportunity for novel therapeutic interventions, since specific signal transduction or transcription factor targets might interrupt the perpetuation mechanisms in RA. The availability of potent and selective p38 mitogen activated protein kinase inhibitors provide a means in further dissecting the pathways implicated in cytokine production, which in turn maintain the chronicity of RA. Many studies conclude that these compounds are very useful in the treatment of chronic synovitis and therefore are very promising for RA treatment.

Rheumatoid arthritis (RA) is a chronic inflammatory disease, affecting primarily the synovial membrane. The presentation of an appropriate antigen to an immunogenetically susceptible host is believed to be the event that initiates a complex series of steps, which ultimately results in chronic inflammatory synovitis. Activated autoreactive T cells and macrophages, as well as an increased number and activity of synoviocytes, have been implicated in the pathogenesis of bone and joint destruction in RA.[1-3] Aberrant overproduction of proinflammatory cytokines such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α by inflammatory cells leads to persistent up-regulation of various molecules responsible for the inflammatory and destructive processes in the joints of patients with RA [4,5] (figure 1). The overproduction of IL-1, TNFα and other molecules is regulated via specific transcription factors. A number of transcription factor families including activator protein

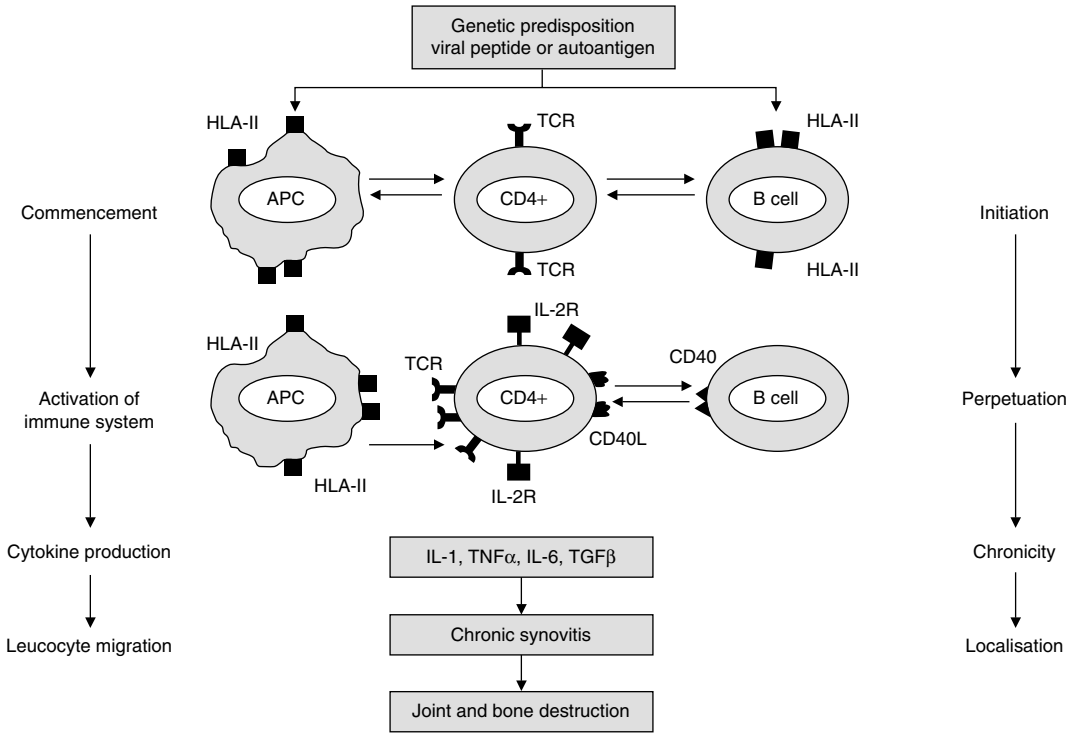

Fig. 1. In a genetically predisposed individual a viral peptide or an autoantigen is recognised by the antigen-presenting cell. This leads to initiation of a series of events, immune system activation and disease perpetuation. As a consequence of this process proinflammatory cytokine production occurs which leads to disease chronicity. This inflammatory event causes leucocyte migration across the epithelium to the synovial membrane, localisation and establishment of the disease. **APC** = antigen presenting cell; **CD40L** = CD40 ligand; **HLA-II** = human leucocyte antigen class II; **IL** = interleukin; **IL-2R** = IL-2 receptor; **TCR** = T-cell receptor; **TGF**β = transforming growth factor β; **TNF**α = tumour necrosis factor α.

(AP)-1, activating transcription factor (ATF)-2, nuclear factor κB (NF-κB), nuclear factor of activated T cells (NF-AT), signal transducer and activator of transcription (STAT), p53 and nuclear hormonal receptors have been implicated as critical regulators of gene expression in the setting of inflammation in rheumatoid joints.[6-8] These factors usually increase the rate of transcription of the gene and therefore increase the formation of messenger RNA (mRNA) and protein (figure 2).

Current therapies in RA are designed to reduce the availability or inhibit the activity of TNFα and IL-1 by administration of various biological agents like monoclonal antibodies (mAbs) against TNFα, soluble TNFα receptors and IL-1 receptor (IL-1R) antagonists.[5,9] It is therefore important to study the signalling pathways involved in the regulation of transcription factors responsible for gene activation and cytokine production. In this review we discuss the signal transduction systems and the regulation of gene expression and the possible sites of therapeutic intervention, with a focus on selective p38 mitogen-activated protein kinase inhibitors.

1. Signal Transduction Cascades

Cell growth and cell activation are initiated by the binding of a signalling agent, most commonly a growth factor, an antigen, or cytokine to a specific receptor. Signal transduction is the process by

which extracellular signals are detected and converted into intracellular signals, which in turn generate a specific cellular response. Signal transduction systems are typically arranged as networks of sequential protein kinases. The most important ones are the mitogen activated protein kinase (MAPK), phosphoinositide (PI)-3 kinase, inositol lipid (IP3), cyclic adenosine monophosphate (cAMP), jenus kinases (JAKs) and STATs signalling system (figure 3).

The signal transduction systems transfer information to the nucleus were specific changes occur in the regulation of gene expression.[10-12] This regulation is frequently achieved at the level of the transcription of genes, and the latter is controlled by regulatory factors known as transcription factors, which then have a vital role in controlling cell growth and cell activation. Transcription factors exhibit a modular structure composed of specific types of domains, including domains for DNA binding and for transcriptional regulation (regulatory domain). The regulatory domain allows the protein either to increase (activation domain) or to decrease (suppressive domain) transcription. Transcription factors are phosphorylated by specific proximal kinases and such phosphorylation can change the subcellular localisation of the transcription factor or its affinity for DNA, which in turn alters gene expression.[13,14]

The activity of at least three transcription factors relevant to the inflammatory process in RA such as AP-1, NF-κB and STATs, is regulated directly or indirectly by MAPK pathways.[14,15] These phosphorylation cascades are activated by diverse extracellular stimuli and act to modulate gene transcription in the inflammatory process. Three groups of MAPKs have been identified in mammalian cells. There are the extracellular signal regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38 kinases.[15-17] MAPK cascades consist of three or four tiered signalling modules in which the MAPK or ERK is activated by a MAPK kinase (MAPKK or MEK), which in turn is activated by a MAPKK kinase (MAPKKK or MEKK). The MAPKKK is itself activated by a small G protein, such as Ras, either directly or via another upstream kinase (figure 4). ERKs have been activated by mitogens and growth factors, while the JNKs and p38 kinases are activated in response to the inflammatory cytokines TNFα, IL-1 and by cellular stress (heat shock, osmotic shock, reactive oxygen metabolites, ultraviolet irradia-

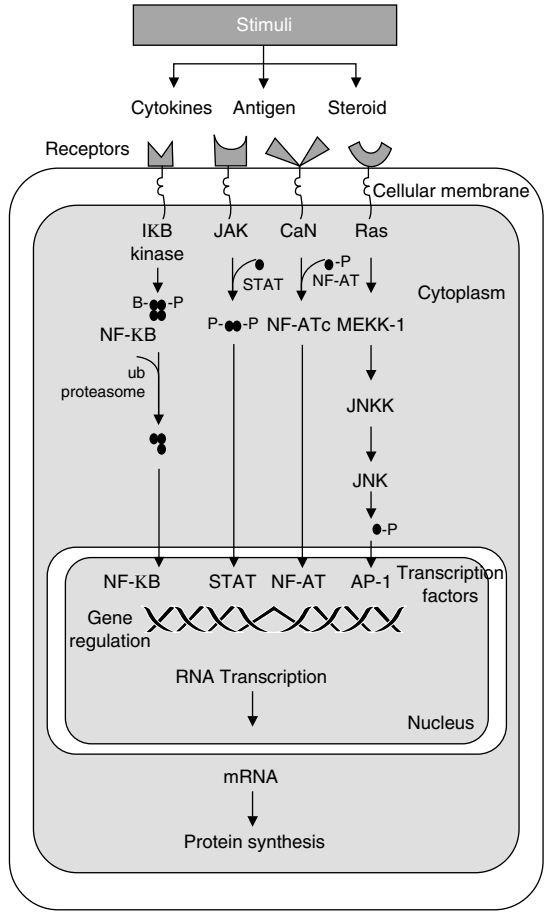

Fig. 2. The up-regulation of proinflammatory cytokines in rheumatoid arthritis is regulated by transcription factors, which are activated through the signal transduction systems. **AP-1** = activator protein 1; **CaN** = calcineurin; **IκB** = Inhibitor of nuclear factor κB; **JAK** = jenus activated kinase; **JNK** = jun-N terminal kinase; **JNKK** = JNK kinase; **MEKK** = mitogen-activated protein kinase kinase kinase or MAPKKK; **NF-AT** = nuclear factor of activated T cells; **Ras** = oncoprotein ras; **NF-κB** = nuclear factor κB; **STAT** = signal transducers and activator of transcription; **ub** = ubiquitin.

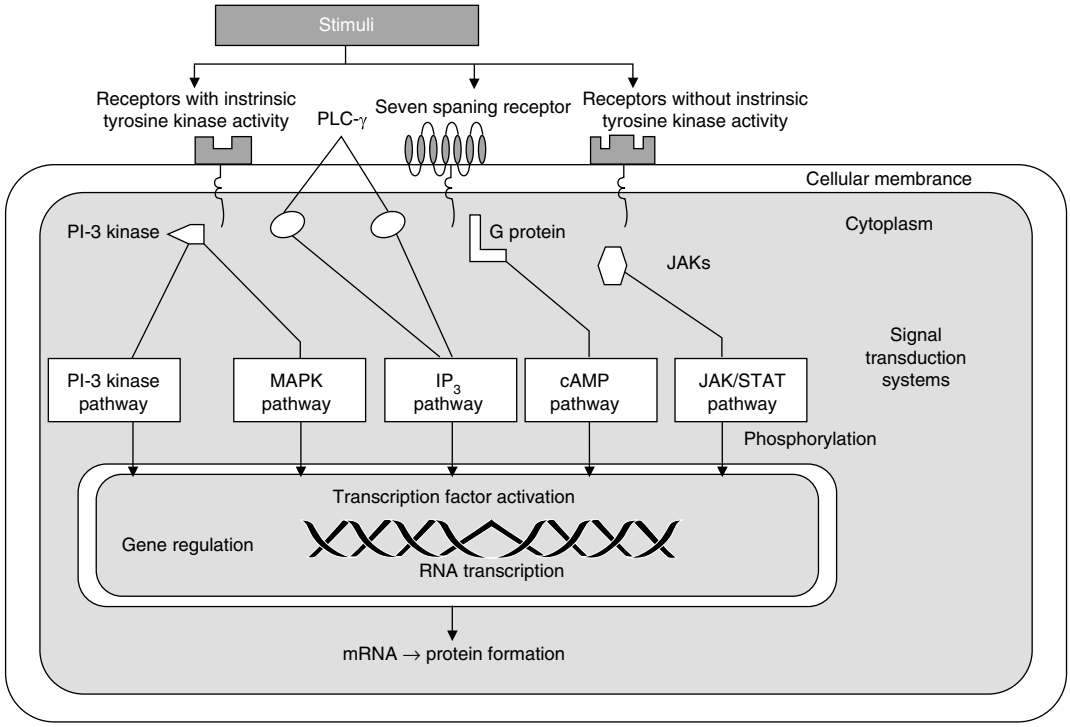

Fig. 3. Simplification of the three major classes of cell surface receptors. On ligand binding, they deliver signals to the nucleus by using a variety of signal transduction pathways. **cAMP** = cyclic adenosine monophosphate; **IP_3** = inositol lipid 3; **JAK** = jenus activated kinase; **MAPK** = mitogen-activated protein kinase; **PI-3** = phosphoinosidil-3 kinase; **PLC**γ = phospholipase C-γ; **STAT** = signal transducers and activation of transcription.

tion, etc.). All three MAPK pathways are involved in the transcriptional regulation of *Fos* and *Jun* family genes (figure 4). The ERKs, JNKs and p38 MAPKs each contributes to up-regulation of *c-fos* gene transcription by phosphorylating and activating the Ets family transcription factor ELK-1 and stress-activated protein (SAP)-1. ELK-1 is phosphorylated by ERK and JNK, whereas SAP-1 is a target of p38 MAPK (figure 4).[8,17,18]

2. Role of p38 Mitogen-Activated Protein Kinase (MAPK) in Cytokine Production

Since the discovery of p38 MAPK in 1994, our understanding of its biology has progressed dramatically.[13] It is now known that there are four members of the p38 MAPK family (p38 α, β, γ, δ). They differ in their tissue distribution, regulation of kinase activation and subsequent phosphorylation of downstream substrates. The best-studied isoform is p38α the activation of which has been observed in many hematopoietic and non-hematopoietic cell types upon treatment with appropriate stimuli.

Significant progress has been made in defining the exact molecular pathways of p38 MAPK activation and subsequent signalling. Members of the p38 MAPK family are phosphorylated on Thr and Tyr residents in a Thr-Gly-Tyr motif by a dual specificity MAPK kinase (MKK). Initially, it was thought that among upstream MKKs, MKK3, 4 and 6 all activated p38 MAPK. However, recent studies suggest that MKK6 and under certain conditions,

MKK3 activate p38 MAPK. MKK6 is a dual specific kinase that phosphorylates p38 on Thr180 and Tyr182. MKK6 can be activated by phosphorylation on Ser151 and Thr155 residues by an upstream enzyme termed MAPKKK (figure 4).[18,19]

Selective activation of the p38 MAPK pathway by MKK6 leads to mRNA stabilisation possibly through MAPKAPK-2 (APK = activated protein kinase) activation, the downstream target of p38 kinase. Thus, it seems that the p38 MAPK pathway contributes to inducible gene expression by stabilising mRNA through a MAPKAPK-2 and 3′ untranslated region (3′-UTR) adenosine-uridine (AU) rich motif targeted mechanism. This hypothesis has been confirmed by the phenotypic changes observed in transgenic mice expressing TNF mRNA lacking the AU rich elements. Interestingly, TNF 3′-UTR AU element-deficient mice spontaneously express TNF in targeted tissues leading to pathological conditions similar to RA and inflammatory bowel disease. Moreover, TNF production by macrophages from these mice was insensitive to p38 MAPK inhibitors despite normal activation of the p38 MAPK pathway. In addition, mice deficient in MAPKAPK-2 have been shown to be resistant to lipopolysaccharide (LPS)-induced endotoxic shock. This resistance appeared to be due primarily to a marked reduction in TNF translation, and not at the level or stability of TNF mRNA or secretion. This result further confirms that p38 MAPK through its activation of MAPKAPK-2 is essential for the regulation of TNF biosynthesis at a post-transcriptional level.[8,18,19]

The p38 MAPK pathway is important also for T-cell cytokine production as well. Mice deficient in MKK3, an upstream kinase p38, are defective in the production of IL-12 and interferon (INF)-γ by antigen-presenting cells and CD4+ cells. p38 MAPK may be involved in mediating IL-10 induced effects, by suppression of TNFα production, in monocyte-derived dendritic cells. These effects include an increase in the T cell stimulatory capacity and expression of cell surface molecules, as well as the suppressive effect of endocytosis and chemotactic migration.[8,11,18]

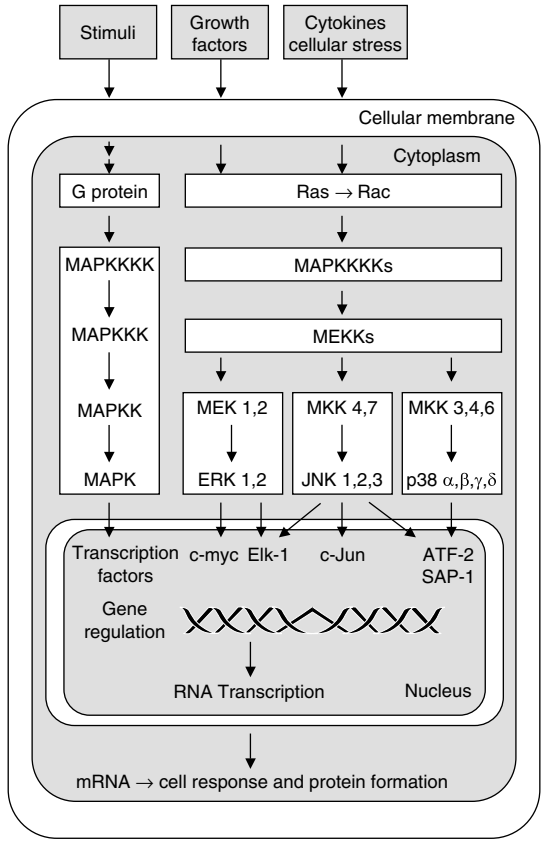

Fig. 4. Simplified scheme of mitogen-activated protein (MAP) kinase signal transduction pathway. **ATF-2** = activating transcription factor 2; **ERK** = extracellular signal-regulated kinase; **JNK** = jenus N-terminal kinase; **MAPK** = MAP kinase; **MAPKK** = MAPK kinase or MEK; **MAPKKK** = MAPKK kinase or MEKK; **MAPKKKK** = MAPKKK kinase; **MKK** = MAP kinase kinase; **p38** = p38 kinase; **Ras** = oncoprotein ras; **SAP-1** = stress activated protein 1.

3. Rationale for the Use of Immunosuppressive Agents in Rheumatoid Arthritis

The better understanding of the signal transduction pathways involved in inflammatory cytokine production and signalling has provided a number of intracellular molecular targets that can be exploited for the development of novel therapeutic agents for the treatment of RA.[20] Several agents

commonly used to treat RA affect the transcription, synthesis, release and/or activity of cytokines, especially those cytokines that contribute to the pathogenesis and the destructive process of the disease.

3.1 Cyclosporin

Cyclosporin, widely used to prevent transplant rejection, is a fungal cyclic peptide very effective in the treatment of RA. Its mechanism of action is through the reduction in the synthesis of lymphocyte activating cytokines, particularly by reducing the transcription of IL-2. After entering into the T cell, cyclosporin binds to cyclophilin and this dimeric complex inhibits calcineurin activity (CaN). Normally CaN removes a phosphate group from NF-AT and AP-1 thus leaving NF-AT and AP-1 to move into the nucleus and stimulate IL-2, INFγ and most T cell growth factors.[21,22] Cyclosporin also interferes with the inducible degradation of NF-κB inhibitors in the nucleus and causes a modest direct decrease of NF-κB, leading to a net decrease in NF-κB and increases transcription and synthesis by T cells and macrophages of the transforming growth factor (TGF)-β1, which down-regulates the T cell response.[21,22] Cyclosporin has been used for many years to treat RA and other autoimmune diseases.

Before 1990, cyclosporin was used in patients with refractory long standing RA, with investigators using high doses of cyclosporin (5 to 10 mg/kg/day). This was associated with many adverse effects, especially hypertension and nephrotoxicity.[23] The renal adverse effects have been overcome by the use of lower doses of cyclosporin (<5 mg/kg/day) without loss of therapeutic efficacy, following the international consensus recommendations.[24] Thus, in recent years a number of prospective, randomised double-blind clinical trials have been reported showing the efficacy of cyclosporin at doses of 2.5 to 5 mg/kg/day.[25,26]

In addition, long-term comparative studies have been performed to compare the efficacy of cyclosporin with other disease modifying antirheumatic drugs (DMARDs). In these studies, cyclosporin was found to have equal efficacy to chloroquine, azathioprine and penicillamine (D-penicillamine). Retardation of radiological progression in patients with active RA has been found in recent controlled studies.[24-26] Studies in patients with early RA showed that cyclosporin has an equal value compared with methotrexate, without serious adverse effects and no radiological progression.[27-30] Furthermore, in another long-term observational study with patients with RA treated early, it appears that cyclosporin is associated with the longest survival time, more that 6 years, and that it was better than hydroxychloroquine, penicillamine and gold salts.[31]

3.2 Tacrolimus and Sirolimus

Tacrolimus (FK-506) and sirolimus (rapamycin) are purified from fungi. Despite the variable antifungal properties they have been developed primarily because of their potent immunosuppressive properties. Their immunosuppressive effects are similar to those of cyclosporin inhibiting the activation of T cells.[22] Although tacrolimus and sirolimus share structural similarities, they suppress T-cell activation at different levels. Tacrolimus inhibits T-cell activation through a series of calcium-associated signal events involved in cytokine gene transcription. In contrast, sirolimus appears to inhibit T-cell activation post-transcriptionally.[22]

3.2.1 Tacrolimus

Tacrolimus has been approved for the prevention of rejection of allogenic organ transplants. In comparison to cyclosporin, tacrolimus is 10 to 100 times more potent in suppressing arthritis.[32] Thus, in rodents, tacrolimus has been demonstrated to prevent the development of collagen-induced arthritis, antigen-induced arthritis and transferred arthritis.[33] Tacrolimus significantly suppressed the activity of arthritis once it was present in both the collagen-induced model and the transferred antigen-induced model. A recent study showed that tacrolimus is effective in suppressing staphylococcal enterotoxin B potentiated collagen-induced arthritis in mice. In addition to suppressing the clinical symptoms, changes in immunological pa-

rameters, especially the expression of CD25 (IL-2 receptor) on T cells, were show with the drug.[34] Therapeutic administration of tacrolimus was demonstrated to have a profound anti-inflammatory effect on the development of the chronic, erosive arthritis induced by peptidoglycan polysaccharide in rats. The decrease in joint inflammation was associated with suppression of IL-6 and nitric oxide (NO) production.[35,36]

In a clinical trial, 12 patients with RA refractory to many DMARDs received tacrolimus 2 to 6 mg/day. Seven of 12 patients completed 6 months of treatment. These seven patients showed significant clinical improvement as evaluated by the reduction of the number of swollen and tender joints. All seven patients achieved the 20% American College of Rheumatology (ACR) response criteria, while five out of the seven patients satisfied the 50% ACR response criteria. Serum creatinine levels were unchanged in all patients during the study and no hypertension was seen. However, five patients withdrew from the study in the first 3 months of treatment. Three because of gastrointestinal symptoms, one because of chest pain and one because of neuropathic pain.[37]

These data suggest that tacrolimus may be effective in the treatment of RA, however, a large randomised, double-blind, placebo-controlled study is needed to demonstrate the efficacy, tolerability and safety of tacrolimus in this setting.[37,38]

3.2.2 Sirolimus

Sirolimus has been shown to be effective in the treatment and prevention of allogenic organ transplantation in animal studies.[39,40] Oral administration of sirolimus was demonstrated to prevent the development of adjuvant arthritis in rats. However, equivalent doses did not affect established arthritis.[41] Using a rat adjuvant arthritis model, oral sirolimus significantly reduced both periarticular inflammation and bony erosions compared with controls. This beneficial effect of sirolimus was seen in rats that had already developed arthritis and extended beyond the time of drug discontinuation.[38]

In preliminary studies, sirolimus was effective at inhibiting streptococcal cell wall-induced arthritis, but only if started at the time of antigen immunisation. Initiation of sirolimus therapy during established arthritis had no effect on clinical or histological inflammation. To date, no studies of sirolimus have been conducted in patients with RA. However, sirolimus is a promising drug and may be effective in these patients.[38]

3.3 Mycophenolate Mofetil

Mycophenolate mofetil is a morpholinoethyl ester of mycophenolic acid (MPA), which is rapidly absorbed and hydrolysed to form free MPA. In 1898, Dr Gosio first isolated MPA from a penicillium culture.[42] He noted its antibacterial properties but was unable to isolate enough material for further analysis. The chemical structure was discovered by Dr Birkinshaw and his colleagues.[43] Although it was initially investigated as an antifungal and antibacterial agent, its antiviral, antitumour and immunosuppressive properties were soon discovered.[44,45]

MPA is the active metabolite of mycophenolate mofetil inhibiting the *de novo* pathway of purine synthesis in T and B cells. In the *de novo* pathway synthesis, inosinate (inosine monophosphate, IMP) is formed from amino acid precursor and 5′-phosphoribosyl-1-pyrophosphate (PRPP). IMP is then converted to xanthylate by enzymatic action of inosine dehydrogenase (IMPDHase). Xanthylate is then converted to guanylate by the enzymatic action of guanylate synthetase (GMPSase). Subsequently, guanylate is converted to guanosine triphosphate (GTP) to be used for RNA, DNA and protein synthesis (figure 5). On the other hand, IMP is converted to adenylate by a different set of enzymes and intermediates. Guanylate and adenylate can also be formed, in most cells, directly from guanine and adenine via the salvage pathways catalysed by hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and adenine phosphoribosyl transferase, respectively. In addition, inosinate can also be formed from hypoxanthine through the salvage pathway catalysed by HGPRT.[46,47]

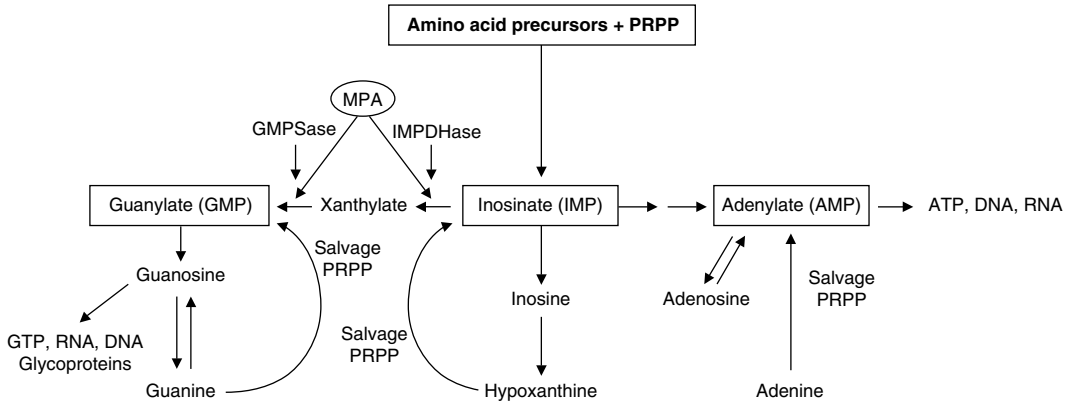

Fig. 5. Schematic representation of mycophenolate acid (MPA) action. It inhibits inosine monophosphate dehydrogenase (IMPDHase) and guanosine monophosphate synthetase (GMPSase), blocking the production of guanylate from inosinate. **AMP** = adenosine monophosphate; **ATP** = adenosine triphosphate; **GMP** = guanosine monophosphate; **GTP** = guanosine triphosphate; **IMP** = inosine monophosphate; **PRPP** = 5′ phosphoribosyl-1′ pyrophosphate.

MPA is a reversible, non-competitive inhibitor of IMPDHase and GMPSase. This inhibition causes intracellular depletion of GTP and deoxyguanosine triphosphate without affecting ATP and deoxy-ATP.[48] Thus, DNA synthesis is affected to a much greater degree than is RNA or protein synthesis. This leads to inhibition in the proliferation of T and B cells. The addition of guanosine or deoxy-guanosine can reverse the antiproliferative effects of this agent, but not xanthine or adenine. MPA does not inhibit the formation of IL-2 or the expression of IL-2 receptor on mitogen-activated T cells. It also inhibits antibody formation by B cells. Finally, MPA inhibits the proliferation of the monocyte cell lines.[46-48]

Mycophenolate mofetil is an effective immunosuppressant agent in animal transplantation models, as well as in early human transplantation trials.[49] Although effective in animal models as a single agent, this drug appears to be most effective when it is used in combination with cyclosporin or tacrolimus. Mycophenolate mofetil also appears effective in animal models of immunologically mediated type 1 diabetes mellitus, adjuvant-induced arthritis (AIA) and experimental autoimmune uveoretinitis with very minimal adverse effects. Mycophenolate mofetil is more effective than azathioprine in preventing acute rejection of renal allografts. The results from studies in animals and anecdotal clinical reports suggest that this agent might have a role in the treatment of lupus nephritis.[50] Recently, a study from China suggests that the combination of mycophenolate mofetil and prednisone is as effective as a regimen of cyclophosphamide and prednisone followed by azathioprine and prednisone.[51]

Mycophenolate mofetil is also being evaluated in patients with RA. In a 1-year, nonblind study, 29 patients with RA refractory to many DMARDs were treated with mycophenolate mofetil. The results showed significant reduction of swollen and tender joints, as well as improvement of the investigator global score and the global score of the patients. In addition, a decrease of immunoglobulin levels and total CD3+ T cells were also found.[52,53]

This agent appears promising in the treatment of patients with RA, and the broad range of its effects suggests that it may be effective in early and established disease.

3.4 Leflunomide

Leflunomide is an isoxazole derivative that is structurally unrelated to other known immunosup-

pressive drugs. Following oral administration it is rapidly and almost completely metabolised within the liver to its active metabolite teriflunomide (A-771726). Leflunomide acts by inhibiting dihydroorotate dehydrogenase (DHOOH) in the intramitochondrial pyrimidine biosynthesis pathway, resulting in decreased levels of pyrimidine nucleosides, such as ribonucleotide uridine monophosphate (rUMP) (figure 6). Dividing cells need to increase the pool of pyrimidine precursors 8-fold to move from the G1 phase to the S phase of cell growth. In the presence of leflunomide, this pool can increase only 2-fold through cellular salvage pathways.[54,55] It is thus thought that clonal expansion of CD4+ T cells would be inhibited in patients with RA who receive leflunomide, contributing to its mechanism of action. Recently, it has been demonstrated that leflunomide may also inhibit NF-κB activation, as well as tyrosine kinase activity.[56]

Pharmacokinetic studies demonstrate that absorption of leflunomide is unaffected by food and that plasma concentrations of teriflunomide are linearly related to the dose. Teriflunomide is highly bound to plasma proteins and has an elimination half-life of approximately 16 days. In order to reach steady-state concentrations more quickly, a 100mg loading dose of leflunomide is given daily for the first 3 days of therapy. On day 4, patients are switched to a 20 mg/day maintenance dose.

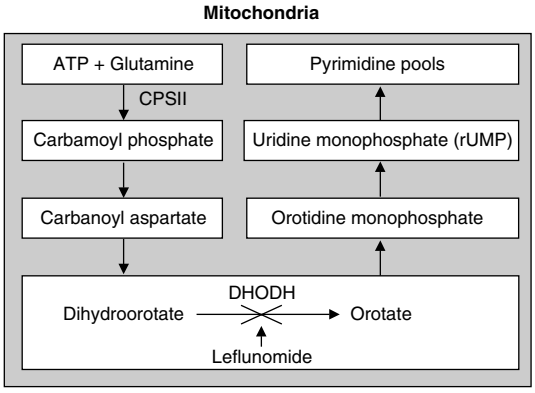

Fig. 6. *De novo* synthesis of pyrimidines and leflunomide action. Starting with adenosine triphosphate (ATP) and glutamine, uridine synthesis is initiated by the enzyme carbamoyl phosphate synthetase (CPSII). A key step occurs in the mitochondria, where dehydrooratate is converted to orotate by the enzyme dehydroorate dehydrogenase (DHODH). Leflunomide inhibits the enzyme DHODH, leading to diminished levels of orotate and, thus, to diminished synthesis of ribonucleotide uridine monophosphate (rUMP).

The clinical efficacy and safety of leflunomide were established in controlled, randomised trials in patients with active RA. The drug was compared to placebo, methotrexate and sulfasalazine in two phase III, placebo-controlled, double-blind multicentre trials. Leflunomide was also studied versus methotrexate in a comparative phase III, double-blind, parallel group study. In all phase III clinical studies, leflunomide demonstrated a rapid onset of action with significant clinical response evident at 1 month, that was sustained for up to 2 years of treatment.[57,58] Leflunomide demonstrated: (i) significant improvement in the signs and symptoms of RA; (ii) equal efficacy for early and established RA; (iii) significant reduction of radiographically assessed disease progression; and (iv) significant improvement of the functional ability and health-related quality of life of the patient. Leflunomide was, in general, well tolerated. The most common adverse events included gastrointestinal disturbances, rash, hair loss, and liver dysfunction.[57,58] Thus, a patient with a history of viral hepatitis or alcohol consumption should probably not receive leflunomide. Similarly, combination treatment with methotrexate should be used with caution.

3.5 Anticytokine Therapy

Cytokines can be divided into cytokines that enhance inflammation (proinflammatory cytokines such as IL-1 and TNFα) and cytokines that decrease inflammation (anti-inflammatory cytokines such as IL-4 and IL-10). In RA there is an imbalance at the site of inflammation between pro- and anti-inflammatory cytokines in favour of the proinflammatory cytokines. This imbalance plays an important role in disease perpetuation and the induction of tissue destruction in patients with RA (figure 1). This has resulted in the investigation of therapies with compounds that either block the ef-

fects of proinflammatory cytokines or enhance the effect of anti-inflammatory cytokines.[5]

Therapeutic strategies aimed at enhancing the effect of anti-inflammatory cytokines are still controversial with regard to the rheumatoid disease process. Therefore, this review deals only with compounds that block the effects of proinflammatory cytokines.

3.5.1 Blockade of Tumour Necrosis Factor (TNF)-α

TNFα is a proinflammatory cytokine that was originally described as a monocyte product that induced tumour lysis. It exerts powerful effects including the induction of inflammatory mediators such as NO and prostaglandins, as well as metalloproteinases and adhesion molecules.[59] An important role of TNFα in RA was further supported by the observation that mice transgenic for TNFα developed destructive arthritis spontaneously.[60] The administration of mAbs prevented this form of arthritis. Other animal studies further support the view that TNFα plays a key role in the pathogenesis of RA. Intramuscular administration of TNFα induces synovitis. Similarly in collagen-induced arthritis, TNFα administered during the development of arthritis leads to a more severe form of joint inflammation, whereas mice receiving anti-TNFα mAbs in the same time period showed significant amelioration of the disease process.[61]

With tools from molecular biology, two types of TNFα inhibitors have been developed: a chimeric antibody cA2, now called infliximab, and a soluble receptor, etanercept. Infliximab has the variable region of a murine antibody grafted to the constant region of a human antibody. Etanercept is a true designer molecule of a dimer consisting of a TNFα receptor joined to the Fc domain of a human immunoglobulin (Ig)G1 molecule. Both compounds potently bind TNFα and block inflammation by inhibiting the downstream effects of this cytokine. However, these agents differ in several ways. Etanercept can bind the cytokine lymphotoxin-α, as well as TNFα. The two TNFα antagonists also differ in their method of administration. Etanercept is given subcutaneously twice a week, whereas infliximab is given intravenously every two months.[5]

The precise mechanisms of action remain to be defined. These include: (i) binding and inactivation of TNFα in the fluid phase; (ii) binding to transmembrane TNFα; (iii) down-regulation of the expression of proinflammatory cytokines [suggested by the decrease of C reactive protein (CRP)]; and (iv) blockage of cell trafficking (suggested by reduction and expression of adhesion molecules).[5] There are also some differences concerning the biological effects of infliximab and etanercept. The mAb might have the advantage of causing cytotoxicity to cells that express membrane-bound TNFα, whereas the high affinity of the TNF-Fc fusion proteins and the possibility of binding TNFβ in addition to TNFα are relevant to the efficacy and remain to be demonstrated.

3.5.2 Therapy with Anti-TNFα Antibodies

At the beginning of the 1990s, clinical trials using chimeric human/mouse or humanised anti-TNFα mAbs in patients with RA provided the first direct evidence that inhibitors of TNFα might be useful therapeutic agents.

Thus, a nonblind trial of the TNFα mAb infliximab showed significant improvement in swollen joints and a reduction of CRP levels in all RA patients.[62] In a subsequent double-blind, multicentre European trial, 73 patients were randomly assigned to single infusions of either placebo, low doses of infliximab (1 mg/kg) or high-dose infliximab (10 mg/kg). Seventy nine percent of patients treated with the high-dose infliximab showed a 20% Paulus response after 4 weeks of treatment as did 44% of the patients treated with the low dose, which both clearly contrasted with only the 8% of placebo responders.[63]

In another trial in a small number of patients with active RA, infliximab in combination with small doses of methotrexate 7.5 mg/week showed an enhanced degree and duration of efficacy.[64] Recent, short- and long-term studies of infliximab in combination with methotrexate showed that infliximab is well-tolerated and can be used safely, and may reduce the rate of joint damage in patients

with RA.[9,65,66] However, the safety aspects of infliximab therapy need further evaluation in long-term regimens for the following reasons: (i) human antichimeric antibody responses occur in a considerable number of patients which may lead to shortening of clinical response and the development of adverse effects; (ii) >10% of the infliximab recipients developed anti-double stranded DNA antibodies and one of these patents showed symptoms of a drug-induced lupus syndrome; (iii) major chronic infections such as chest tuberculosis may develop;[67] and (iv) some of the treated patients developed malignancy. Seeking causal relationships between infliximab therapy and malignancy is complicated by the higher risk of malignancy in patients with RA compared with the general population. Thus, to assess the risk of major infections or malignancy following infliximab therapy, a long-term, world-wide registry might be required.

3.5.3 Therapy with Soluble TNFα Receptors

Recently, investigators have studied the effects of etanercept, a soluble TNFα receptor (p75) fusion protein (sTNFR-Fc). This molecule is a dimer composed of two molecules of the recombinant form of the human p75 sTNFR fused to a Fc fragment of human IgG1 (see section 3.5.1).[5]

The safety, pharmacokinetics and potential clinical efficacy of etanercept were first evaluated in a double-blind, placebo-controlled, dose-escalation study in patients with active refractory RA. In this study, etanercept was administrated in doses of 2 to 16 mg/m^2 twice weekly by subcutaneous injection for 4 weeks after a single intravenous loading dose. An over 50% reduction in individual response variables such as swollen and tender joint count, and CRP was observed in patients receiving the etanercept.[68] Pharmacokinetic data showed that the twice-weekly dosage schedule, in all four dosage groups, resulted in an elevation of receptor concentration compared with baseline in all patients through day 35 (i.e. up to 6 days after the last injection).

These initial encouraging clinical results were confirmed in a multicentre, randomised, double-blind, placebo-controlled study in 180 patients with RA.[69] In another 24-week, double-blind trial, 89 patients with persistently active RA despite at least 6 months of methotrexate therapy at a stable dose of 15 to 25 mg/week were randomly assigned to receive either etanercept 25mg or placebo subcutaneously twice weekly while continuing to receive methotrexate. After 6 months of therapy, the combination of etanercept and methotrexate was safely used and well tolerated, and provided greater clinical benefit than methotrexate alone.[70] Etanercept was also evaluated in 632 patients with early RA for 1 year. It was shown that etanercept, compared with oral methotrexate, acted more rapidly to decrease symptoms and slow joint damage in patient with early RA.[71]

Safety aspects of therapy with soluble TNFα receptors needs further evaluation in long-term studies for the following reasons: (i) >40 patients developed serious infections, including sepsis, and some of these died within 2 to 16 weeks of starting treatment; (ii) some patients developed blood dyscrasias; and (iii) others developed demyelinating central nervous system disorders. Thus to assess the risk of the above adverse effects after etanercept therapy, a long-term, world-wide registry needs to be established.

3.5.4 Interleukin-1 Blockade

IL-1 is a potent proinflammatory cytokine that activates its receptor and stimulates the production of prostaglandins, NO, and other substances that mediate inflammation and tissue remodelling. IL-1Rα is a naturally occurring cytokine and a member of the IL-1 family whose only function is to prevent the biological response to IL-1. When IL-1 occupies its receptor various proinflammatory events are initiated, but when IL-1Rα occupies the receptor, no such events occur because IL-1 cannot activate the cells.[72]

The finding that mice which lacked the ability to produce IL-1Rα developed an inflammatory disease spontaneously was of considerable importance. In mice in which the gene for IL-1Rα had been knocked out, two distinct diseases developed spontaneously which were similar to RA and vasculitis in humans.[73,74] These studies concluded

that IL-1Rα produced by healthy mice protects them from the effects of IL-1, which is also produced in healthy animals under normal conditions. These findings are consistent with the concept that the increased production of IL-1 during an inflammatory process in humans contributes to the pathologic event by binding to and triggering its receptor. On the other hand, normally enough IL-1Rα is produced to control IL-1 mediated inflammation. However, in case of runaway inflammation, there is an insufficient amount of IL-1Rα to control the activity of IL-1. Thus, IL-1 is a potent target of therapeutic intervention in a variety of inflammatory and autoimmune disorders.

The administration of exogenous IL-1Rα or other agents that reduce the effects of IL-1 should ameliorate the inflammatory process. The first therapeutic approach in patients with RA has been the intra-articular and subcutaneous administration of recombinant human IL-1R. This treatment achieved a limited therapeutic efficacy. More recently, IL-1Rα proved to be successful in the collagen-induced arthritis model and in the antigen-induced arthritis model.[75] Subsequent short-term, double-blind, placebo-controlled studies in patients with refractory RA, as well in early RA, demonstrated a significant clinical improvement ranging from 20 to 35%.[76,77] No long-term studies have been reported so far in large numbers of patients to evaluate the efficacy and safety of this compound. It seems that the IL-1Rα is a promising agent for the treatment of RA, but its efficacy and safety should be further demonstrated.

4. p38 MAPK Inhibitors in Development

All three families of MAPKs are expressed and can be activated under appropriate conditions in both normal and rheumatoid synovial tissues.[78,79] A recent study showed that activation of ERK, JNK and p38 MAPKs were observed exclusively in synovial tissues from patients with RA but not in tissue from patients with osteoarthritis.[78-80] In addition, significant differences were observed in the exact localisation of those molecules in RA tissue. The activation of ERK was localised to microvessels, JNK was activated around and within mononuclear cell infiltrates, and p38 MAPK was activated in the synovial lining and in endothelial cells. In all cells, TNFα and IL-1 were the major inducers of all three MAPKs.[81]

A lot of pharmacological evidence indicates that p38 MAPK is one of the most validated targets for the treatment of RA.[20] This has been made possible by the discovery of specific inhibitors of p38 MAPK activity. These were first described by Lee et al.[13] as a target of the pyridinyl imidazole class of compounds that inhibit the production of inflammatory cytokines such as IL-1 and TNFα. It is worth noting that they are highly specific and reversible inhibitors of p38 MAPK activity but do not affect its activation by upstream MAPKKs.[82,83] Several reports indicate that inhibition of p38 MAPK prevents the translation of TNFα and IL-1 in LPS-stimulated monocytes. Decreased MAPKAPK-2 activity is a direct indicator of the inhibition of p38 MAPK within the cell.[20,82,83]

A bicyclic imidazole, SKF-86002 was found to have a noticeable inhibitory effect on TNFα and IL-1 production in LPS-stimulated human monocytes [50% inhibitory concentration (IC_{50}) = 1 μmol/L], and therefore the term cytokine-suppressive anti-inflammatory drugs (CSAID) was proposed to indicate this class of cytokine inhibitors.[84]

SB-203580 is a member of a new series of pyridinyl imidazole compounds that inhibit IL-1 and TNFα production from LPS-stimulated human monocytes and the human monocyte cell line THP-1 with IC_{50} values of 50 to 100 nmol/L.[85] The molecular target of SB-203580 has been identified as a pair of closely related MAPK homologues, termed CSAID binding proteins (CSBPs) p38 or RK. Binding of the pyridinyl imidazole compounds to CSBP in THP-1 cytosol correlates with their ability to inhibit cytokine synthesis in response to various stimuli. In addition to blocking TNFα and IL-1 production, SB-203580 blocked IL-1 stimulated NO production in chondrocytes, IL-1 induced cyclooxygenase 2 (COX-2) and ma-

trix metalloproteinase (MMP)-1 and -3 mRNA in fibroblasts, and decreased the stability of COX-2 mRNA in activated monocytes.[86-90] Studies in several transgenic and knockout mouse models, as well as *in vitro* studies, using SB-203580 strongly implicate p38 in the regulation of IL-12 and INFγ gene transcription, and hence in the regulation of T helper cell 1 (Th1) type immune response.[87,89] Studies have shown that SB-203580 binds to the ATP-binding pocket suggesting that the compound competes with ATP for binding to the enzyme.[91]

SB-203580 has been shown to have many interesting activities in both antigen-induced arthritis in the rat and type II collagen-induced arthritis in mice. These experimental inflammatory arthritides are characterised by T cells involving the synovial tissue, similar to that seen in RA. Since SB-203580 also inhibits JNK and other kinases, its activity may not be specific. Despite these interesting activities *in vivo*, toxicological studies on rats have shown liver weight to be increased and a significant elevation of hepatic cytochrome P450 (CYP) enzymes while *in vitro* studies have demonstrated inhibitory effects on human CYP enzymes.[85] However, more recent analogues, such as SB-226882, which incorporate an aminopyrimidine moiety, display a markedly reduced CYP inhibition profile.

SB-220025 is a new compound belonging to the NSAID class of cytokine biosynthesis inhibitors, which inhibits human p38 MAPK with an IC_{50} value and 50- to 100-fold selectivity versus other kinases tested.[92] *In vivo* SB-220025 reduced the LPS-induced production of TNFα at a 50% effective dose (ED_{50}) value of 7.5 mg/kg. In the inflammatory angiogenesis model, over the course of granuloma development Jackson and collaborators[92] observed elevated levels of TNFα and IL-1 during the chronic inflammatory phase when intense angiogenesis occurred. Oral SB-220025 30 mg/kg twice daily was able to reduce the expression of these proinflammatory cytokines and inhibit angiogenesis by approximately 40%. To further study the effects of p38 MAPK inhibition in angiogenesis-dependent chronic inflammatory disease, SB-220025 was tested in murine collagen-induced arthritis. In this model, the compound was found to prevent the progression of established arthritis.[92]

Thus, SB-220025 can reduce inflammatory cytokine production and inhibit angiogenesis, and may be an effective treatment for chronic inflammatory diseases like RA in which angiogenesis and panus formation induced by IL-1 and TNFα are the major histological findings.

SB-242235 is a new member of the pyridinyl imidazole class of compounds that has exhibited potent anti-inflammatory activity. SB-242235, when tested against a panel of representative protein kinases, showed a superior kinase selectivity profile compared to SB-203580. As with SB-203580, SB-242235 inhibits CSBP/p38a MAPK (IC_{50} = 0.1μmol/L) and p38β2 MAPK (IC_{50} = 1μmol/L), but not p38γ or p38δ MAPKs. In contrast to SB-203580, SB-242235 does not inhibit JNK2β1 or ERK2.

In recent studies, SB-242235 has been demonstrated to be a potent inhibitor of LPS-induced TNFα production in the plasma of normal rats. The optimal pre-treatment time was 1 to 2 hours, although highly significant activity was seen 4 hours post-dosing. SB-242235 demonstrated excellent inhibitory activity with regard to TNFα production after oral administration of a range of doses. The ED_{50} for inhibition of LPS-stimulated TNFα was 3.99 mg/kg given orally. Anti-inflammatory activity was observed in antigen-induced arthritis in Lewis rats when SB-242235 was administered orally at 10, 30 and 60 mg/kg either prophylactically or therapeutically. There was a 73% inhibition of paw oedema and a 53% normalisation of bone mineral density at the 30 mg/kg dose. This is significant activity for a small molecular cytokine inhibitor in such an aggressive arthritis model. Additional evidence for disease modifying activity for the compound was provided by the obvious improvement observed in the magnetic resonance images, in which 80% of the joints were significantly protected by treatment at the 60 mg/kg dose. In addition, micro computed tomography technology

showed clear protection with SB-242235.[93] Protection of bone, cartilage and soft tissues was also shown histologically. Finally, serum IL-6 levels were decreased in rats with antigen-induced arthritis receiving the 60 mg/kg dose.

All these data indicate that SB-242235 exerts a protective effect on joint integrity and appears to have disease-modifying properties, and may be a useful tool for treating patients with RA.

A series of diphenylimidazoles, typified by RPR-66425, have been developed. RPR-132331, a 2-(2-diaxanyl) imidazole, was identified as an inhibitor of TNFα release from LPS-stimulated human monocytes. A novel series of inhibitors, derived from RPR-132331 has led to identification of RPR-200765A as a development candidate for the treatment of RA.

RPR-200765A is a potent and selective inhibitor of p38 MAPK (IC_{50} = 50 nmol/L). It inhibits LPS-stimulated TNFα release both *in vitro*, from human monocytes (EC_{50} = 160 nmol/L), and *in vivo* in Balb/c mice (ED_{50} = 6 mg/kg). At oral dosages between 10 and 30 mg/kg/day, it reduces incidence and progression in the rat streptococcal cell wall (SCW) arthritis model when administered in either prophylactic or therapeutic dosing regimens. The compound showed very good bioavailability (F = 50% in the rat) and also excellent chemical stability. Thus, the drug could be considered as having modifying activity in patients with RA.[94]

The pharmacological profile that we have described for p38 MAPK inhibitors would appear to be one that would be desirable for an antirheumatic therapeutic agent. However, the discovery of new pyrimidinylimidazole inhibitors of p38 MAPK with decreased inhibition for hepatic CYP enzymes will be soon available.[95-97]

5. Conclusions

Current treatments for RA with first- and second-line drugs are inadequate in that they only partially control established RA. They also have many adverse effects that limit their use during the disease process and interfere with prolonged administration. Thus, despite optimal use of current DMARDs, the outcome for many patients with RA is a severe functional decline, work disability and premature death.

In the last few years significant advances have been made in our understanding of the molecular mechanisms underlying RA pathogenesis. Proinflammatory cytokines, TNFα and IL-1 play an important role in maintaining the chronicity of RA and mediating tissue damage. Biological anti-TNFα agents and IL-1 receptor antagonists that neutralise their activity are now the first generation antirheumatic drugs in clinical practice. Since several factors are involved in the pathogenesis of RA, neutralising one or some of these factors may be of only limited benefit. A better outcome for patients with RA stresses the need for new therapeutic regimens in terms of controlling the production and the activity of the factors involved in disease pathogenesis.

Our understanding of the signal transduction systems and gene regulation by transcription factors involved in cytokine production has opened the way for the discovery of novel therapeutic compounds useful in treating RA. The availability of potent and selective p38 MAPK inhibitors provides a means to further dissect the pathways implicated in cytokine production which maintain the chronicity of RA. It seems that p38 MAPK inhibitors are very promising drugs for the treatment of patients with RA. The advantages that we expect in using p38 MAPK inhibitors may be multiple. Firstly, instead of blocking or neutralising cytokines or their receptors, these compounds reduce the proinflammatory cytokine production. Secondly, p38 MAPK inhibitors should be easy to prescribe and would be administrated orally without the need for admission of the patient to the clinic. Thirdly, their adverse effects are mild and include liver, heart and blood dyscrasias. Finally, p38 MAPK inhibitors could be used in combination with other immunosuppressive drugs, such as cyclosporin or mycophenolate mofetil, or even in combination with biological agents. However, in order to confirm these predicted advantages, further studies with a large number of patients are

needed. These include the discovery of new compounds with minimal adverse effects, and comparative or combination studies with multiple therapeutic agents.

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