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Current Opinion in Rheumatology was launched in 1989. It is one of a successful series of review journals whose unique format is designed to provide a systematic and critical assessment of the literature as presented in the many primary journals. The field of rheumatology is divided into 15 sections that are reviewed once a year. Each section is assigned a Section Editor, a leading authority in the area, who identifies the most important topics at that time. Here we are pleased to introduce the Journal’s Section Editors for this issue.

Section Editors

Iain B. McInnes, FRCP, PhD

Professor McInnes studied medicine in the University of Glasgow graduating with honours in 1989 before training in internal medicine and rheumatology, completing MRCP in 1992 and FRCP in 2003. Thereafter he trained to PhD and post doctoral studies via fellowships from The Wellcome Trust, ARC (UK) and Fogarty Program in NIH in both Glasgow and in the National Institutes of Health in Bethesda, Maryland. He is now Professor of Experimental Medicine/Honorary Consultant Rheumatologist in the Center for Rheumatic Diseases University of Glasgow. His research interests include understanding the role of cytokines in inflammatory synovitis, both from the basic perspective of functional activity and using a translational medicine approach whereby such molecules also offer therapeutic utility. He leads a trials unit specialising in the use of biologic agents in early clinical trials in inflammatory arthritis. Recently these studies have extended to include the role of inflammation in promoting vascular disease, particularly atherogenesis. He serves on editorial boards for Rheumatology and Arthritis Research and Therapy, and this year will Chair the Abstract Committee for the European Rheumatology Congress in Vienna.

Larry W. Moreland, MD

Dr Moreland received his undergraduate degree from West Liberty State College, West Liberty, West Virginia. He received his medical degree from West Virginia University in Morgantown, and performed his internship and residency in Internal Medicine at the West Virginia University Medical Center. He completed a fellowship in Immunology and Rheumatology at the University of Alabama at Birmingham (UAB) where he continues to work today.

Dr Moreland is a Professor of Medicine in the Division of Clinical Immunology and Rheumatology at the University of Alabama at Birmingham and was named the Anna Lois Waters Chair of Medicine in Rheumatology in 2001. He is Director of the Pittman General Clinical Research Center and the Arthritis Clinical Intervention Program. Dr. Moreland is also the Associate Dean for Clinical Research at the School of Medicine.

He is a member of the American Society for Clinical Investigation and serves on editorial boards of the American Journal of Medicine and Journal of Rheumatology. Dr. Moreland served on the Veterans Administration Merit Review Subcommittee for Immunology (1997–2000) and the Food and Drug Administration (FDA) Arthritis Advisory Committee (1998–2000). He has served on the National institute of Allergy and Infectious Diseases (NIAID) Allergy, Immunology, and Transplantation Research Committee (1998–present), and the Osteoarthritis Research Society International Board of Directors.

Dr Moreland is also heavily involved in conducting innovative research. He is internationally recognized for his clinical research initiatives in rheumatoid arthritis (RA), especially with biologic therapies. His primary research interest over the past 15 years has been the evaluation of biologic response modifiers and their mechanisms that are targeted at the disease process in RA. Several pivotal phase I, II, and III studies have been performed at UAB evaluating such biologic response modifiers as anti-CD4 monoclonal antibodies, tumor necrosis factor (TNF) and interleukin-1 (IL-1) inhibitors, and co-stimulatory blockers. The first biologic agent approved by the FDA was etanercept, a TNF inhibitor first evaluated in arthritis patients by Dr. Moreland at UAB. In collaboration with other UAB scientists he established the multi-institutional Consortium for the Longitudinal Evaluation of African-Americans with Early Rheumatoid Arthritis Registry (CLEAR) that is identifying and enrolling approximately 600 subjects. He was also recently awarded funding (with Dr. Howard as Co-PI) as a coordinating center for ‘Treatment of Early Aggressive RA,’ a multicenter clinical trial.
Geraldine M. McCarthy, MD

Dr McCarthy grew up in Ireland and graduated in Medicine from University College Dublin. Trained in General Internal Medicine in Ireland, she completed her Fellowship in Rheumatology at the Medical College of Wisconsin in 1991 where she was mentored by Drs Daniel McCarty, Lawrence Ryan, Robert Wortmann and Herman Cheung. During her Fellowship training, she developed her interest in calcium crystal deposition diseases and received a post-doctoral fellowship from the Arthritis Foundation to pursue her research in the area. Her research remains focused on the biologic effects of calcium-containing crystals in degenerative joint disease as well as in atherosclerosis and breast cancer. Her research funding includes the National Institutes of Health, American Federation for Aging Research, US Department of Defense and the Wellcome Trust. She was promoted to Associate Professor of Medicine at the Medical College of Wisconsin in 1996 where she remained until her return to Dublin in 1998. She transferred her laboratory to the Royal College of Surgeons in Ireland where her bench research program continues and where she teaches. She was appointed Consultant in Rheumatology at the Mater Misericordiae University Hospital, Dublin in 1999. She is the author of over 60 publications and has spoken at many national and international meetings. She has been winner of several research and teaching awards and has mentored medicine and science graduates in clinical practice and in research.
This past decade has been marked by rapid advances in our understanding of the pathogenesis of many musculoskeletal disorders. Parallel to these events, there have been remarkable advances in our therapeutic options for many patients. Now, with a decade of experience with many of these novel agents, we can now reflect on which of these approaches have been successful, which have been unsuccessful, and, more importantly, better understand the reasons for the successes and failures. In this section of Clinical Therapeutics, there is a comprehensive review of the state of the art of our new therapies as they apply to rheumatoid arthritis (RA), spondyloarthropathies, vasculitis associated with anti-neutrophil cytoplasmic antibodies (ANCA), and pediatric clinical research.

There is no doubt that the treatment of adults with RA has dramatically been altered by the introduction of inhibitors of tumor necrosis factor (TNF). Three TNF inhibitors (adalimumab, etanercept, and infliximab) have now been approved for the treatment of RA. The most important recent data suggest that many patients who receive the combination of an anti-TNF agent plus methotrexate (MTX) have a superior response as measured by improvement in signs and symptoms and radiographic damage. The challenge now remains to identify which patients need to be treated early with the combination and which patients should be receiving monotherapy with either the anti-TNF agents or MTX. In addition, it still remains to be shown that the combination of MTX plus anti-TNF therapy is superior to more traditional combination of other disease-modifying antirheumatic drugs (DMARDs) such as MTX, hydroxychloroquine, and sulfasalazine. The studies addressing this important question are currently in progress.

In addition, it is now becoming more obvious with data that are emerging from long-term registries a large percentage of patients who initially receive anti-TNF therapy no longer continue that therapy after 2 to 5 years. There are numerous reasons, but some include the possibility of immunogenicity of the administered protein, other pro-inflammatory mechanisms of tissue inflammation, and mechanisms of tissue damage are operative independent of TNF. Thus, there are significant unmet needs and opportunities to continue our search for better understanding of the pathogenesis of RA as well as understanding the mechanisms by which patients respond to anti-TNF therapies and, equally important, understand why those who initially respond and then subsequently lose that response.

Although there are numerous potential molecular targets at this time, the targets that have received the most attention, and from which we have clinical trial data, include those that block interleukin (IL)-6, costimulatory pathway, and B cells. These three new targets are reviewed in detail by Choy. Multiple other cytokines (IL-12, IL-13, IL-17, IL-18, etc.) have been shown to potentially attractive targets with biologic therapies. IL-6 is a pleotropic cytokine that has multiple pro-inflammatory activities. Several clinical trials have shown that MRA (atilizumab) can demonstrate significant improvements in signs and symptoms in patients with RA [1–3]. Pivotal phase III studies are now under way to better define the efficacy and also the long-term safety profile of this novel biologic agent. Some dose-related adverse events have been reported in studies to date, including elevated liver enzymes, decreased white blood cell counts, and a rise in serum cholesterol.

Abatacept is a costimulatory blocker that has now been evaluated in several placebo-controlled trials [4–6]. Abatacept selectively blocks the engagement of cytotoxic T-lymphocyte-associated protein 4 with CD80/CD86, which provide the essential secondary costimulatory signals for T cell activation. It selectively inhibits the function of T cells but does not deplete the circulating T cells.
Abatacept has been studied in patients who have been MTX partial responders and in patients who have responded partially or not at all to anti-TNF therapy.

Another exciting area in the field of RA has been the role of rituximab, a specific inhibitor of CD20 [7**]. Pivotal phase III studies are in progress to determine the long-term safety and efficacy of this agent. In addition, other inhibitors of B cell function are in early development at this stage.

Although the major impact seen to date in the use of biologic response modifiers has been with adults, there have been an equal number of improvements or breakthroughs in our therapeutic opportunities in children with musculoskeletal disorders. Drs. Ruth and Lovell review the new information on juvenile systemic lupus erythematosus, juvenile idiopathic arthritis, and juvenile dermatomyositis. There is a special emphasis on strategies to improve the health-related quality of life in children with these musculoskeletal disorders. Perhaps one of the most important findings has been the data demonstrating that in children who do not respond to oral methotrexate, either because of inefficacy or toxicity, the use of subcutaneous methotrexate has a very high likelihood of success without any significant increased toxicity [8]. Understanding compliance and administering adequate and proper doses to children is a critically important issue. We have the ability to use methotrexate at its maximum effective dose and combine that with other agents such as anti-TNF agents. For now, we have the opportunity to significantly affect the quality of life and functioning of our children who have juvenile RA.

A major finding over the past several years has been the consistent data that patients with RA have a shortened lifespan. Further research has determined that a major cause of this is an increase in cardiovascular disease in patients with this chronic inflammatory condition. Drs. Snow and Mikuls review the most recent information regarding this area of rapidly developing news with regard to the mechanisms as well as potential therapeutic ways to prevent adverse cardiovascular outcomes in RA patients. The increased cardiovascular morbidity and mortality in RA are most likely multifactorial. The most likely explanation is that the ongoing systemic inflammation in the joints leads to dysfunction in endothelial cells and premature arthrosclerosis. Understanding the pathogenesis of arthrosclerosis is obviously important to understanding the role of the immune system in this process. In addition, many patients with RA have significant comorbid conditions that may predispose them to cardiovascular risks. Finally, several medications used to treat RA may contribute to the cardiovascular risks. The role of methotrexate, glucocorticoids, and NSAIDs in the premature cardiovascular events in RA patients remains an area of investigation.

One key unanswered question at this time is whether our new treatment paradigm of early aggressive treatment of RA with disease-modifying drugs alters the cardiovascular outcome. Indeed, in a recent study by Krishnan et al. [9**], there seems to be evidence that the incidence in birth cohorts of RA patients, investigators noted temporal improvements in myocardial infarction–related mortality. Additional attention is needed in this area to better define the cost effectiveness of long-term therapies with these new aggressive approaches.

Finally, data have emerged about the role of statins and their ability to be anti-inflammatory agents. Thus, in addition to lowering cholesterol levels, statins have the potential to be anti-inflammatory agents and could provide a unique opportunity to further our potential to improve the cardiovascular risks in patients with RA. The most compelling and comprehensive study has been a trial of atorvastatin agent in which RA patients were treated with atorvastatin compared with placebo for 6 months [10]. There were significant improvements in inflammatory markers as well as the expected declines in cholesterol low-density lipoprotein and triglycerides.

The therapeutic approach to spondyloarthopathies has been markedly altered in the past few years with the availability of TNF inhibitors. Drs. Anandarajah and Ritchlin review the rapidly advancing field of new therapies for ankylosing spondylitis and psoriatic arthritis from some of the recent published data and the recent approval of these agents by the US Food and Drug Administration and European authorities. All three anti-TNF therapies (etanercept, adalimumab, and infliximab) are approved, or are being studied in clinical trials for approval, for both ankylosing spondylitis and psoriatic arthritis [11–13].

Although it has not yet been demonstrated by studies, some of the same remarkable clinical outcomes can be expected to occur with the spondyloarthopathies that have been seen with RA. Thus, it will be important that we better study the factors that predict good response and what combination of therapies, whether additional DMARDs plus anti-TNF agents or some other combination, would be most useful to treat these seronegative spondyloarthopathies. In this same context, several other targets are attractive for therapy of both ankylosing spondylitis and psoriatic arthritis; they include IL-1, costimulatory pathway, and adhesion molecules, to name a few.

The spectrum of vasculitides has also been an area of major advances in our understanding of pathogenesis and thus the ability to provide better therapies. Dr. Stone outlines the recent pivotal studies of three randomized
controlled trials that have been performed and reviews the results of these important studies that highlight the lessons learned from targeting patients with ANCA-associated vasculitis [14,15**,16]. The major conclusions from these three trials are as follows:

1. Azathioprine plus maintenance-dose steroids is as effective as cyclophosphamide plus maintenance steroids in sustaining disease remission in ANCA-associated vasculitis.
2. Methotrexate is as effective as cyclophosphamide for the induction of remission within 6 months of treatment; however, there were more relapses in the methotrexate-treated group than in the cyclophosphamide-treated group after initial induction.
3. Etanercept was not superior to placebo for the maintenance of disease remission in Wegener’s granulomatosis.

The lessons learned from these extremely important, well designed, multicenter, international collaborative studies will be fruitful for a better understanding of the pathogenesis of these diseases and in developing new targets.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:• of special interest ** of outstanding interest


This phase III trial evaluated rituximab in rheumatoid arthritis patients.


This observational cohort study demonstrated a decrease in cardiovascular outcomes in patients with rheumatoid arthritis.


This placebo-controlled trial evaluating the efficacy of etanercept in patients with Wegner’s granulomatosis.


Rheumatoid arthritis and cardiovascular disease: the role of systemic inflammation and evolving strategies of prevention

Marcus H. Snow and Ted R. Mikuls

Purpose of review
The incidence and mortality of cardiovascular disease are increased in the context of rheumatoid arthritis. The purpose of this review is to examine our evolving understanding of the pathogenesis of cardiovascular disease in rheumatoid arthritis and to underscore the importance of tailored prevention of cardiovascular disease in this select population.

Recent findings
Recent reports have highlighted the shared pathobiology of cardiovascular disease and rheumatoid arthritis, both of which represent inflammatory disorders. Several reports have also provided much-needed insight into the deleterious impact that select therapies (including cyclo-oxygenase-2-specific inhibitors) may have in terms of the risk of cardiovascular disease in rheumatoid arthritis. Although further study is warranted, preliminary investigations also suggest that aggressive anti-inflammatory therapy, including the adjutant use of statins, may play important cardioprotective roles in rheumatoid arthritis.

Summary
The pathogenesis of cardiovascular disease in rheumatoid arthritis is complex and involves several intermediate factors, including dyslipidemia, elevations in serum homocysteine, impaired insulin sensitivity, and endothelial dysfunction. Given the burden of cardiovascular disease in this population, it is important that health care providers caring for rheumatoid arthritis patients adopt a treatment course that is both comprehensive and individualized to address specific risk factors for cardiovascular disease.

Keywords
3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, cardiovascular disease, congestive heart failure, endothelial dysfunction, glucocorticoid, homocysteine, myocardial infarction, nonsteroidal anti-inflammatory drugs, rheumatoid arthritis, statin

Abbreviations
BMI body mass index
CHF congestive heart failure
COX-2 cyclo-oxygenase-2
CRP C-reactive protein
CVD cardiovascular disease
DMARD disease-modifying antirheumatic drug
HDL high-density lipoprotein
HRT hormone replacement therapy
LDL high-density lipoprotein
MI myocardial infarction
NSAID nonsteroidal anti-inflammatory drug
RA rheumatoid arthritis
TNF-α tumor necrosis factor-α

Introduction
Rheumatoid arthritis (RA) leads to progressive joint deformity and disability, and cardiovascular disease (CVD) is arguably its most lethal manifestation. Recent studies have shown that the risk of myocardial infarction (MI) or congestive heart failure (CHF) in patients with RA is significantly higher than in age-matched and sex-matched control individuals, leading some to suggest that CVD is an extra-articular manifestation of RA [1,2]. Others have reported that approximately one third to one half of RA-related deaths are directly attributable to CVD [3]. The reasons for increased CVD morbidity and mortality in RA are likely multifactorial, with burgeoning data supporting a predominant role for systemic inflammation in promoting endothelial dysfunction and premature atherosclerosis (Fig. 1). In this review, we shall examine the role of systemic inflammation in CVD pathogenesis in the context of RA. Furthermore, we shall discuss the need for a more comprehensive approach to RA care, one that incorporates both primary and secondary CVD prevention as a central treatment strategy.

Cardiovascular disease burden in rheumatoid arthritis
In their seminal study, Wolfe et al. [4] reported a standardized mortality ratio (the ratio of observed to expected deaths) for CVD-related mortality of 2.2 among RA patients. RA has been associated with a significant two-fold increase in the risk for development of CHF and a 40% increase in the risk for incident MI [5,6]. In a large population-based study using the UK General Practice Research Database, patients with RA were 30 to 60% more...
likely than those with osteoarthritis or no arthritis to experience a major vascular event (i.e., stroke or MI) over a mean follow-up period of approximately 5 years [7]. Corroborating the findings of several previous studies, investigators from the Nurses’ Health Study recently observed an adjusted relative risk for CVD in patients with RA that was twice that in control individuals (RR = 2.0; 95% CI 1.23–3.29) [8]. In the same study, the risk of CVD was more than threefold (RR = 3.10; 95% CI 1.64–6.87) for RA patients with more than 10 years of disease duration, even after adjustment for known CVD risk factors. In a particularly ominous report, investigators recently found that RA patients are substantially more likely than non-RA controls to experience unrecognized MIs (OR = 2.1; 95% CI 1.1–4.0) and sudden deaths (OR = 1.9; 95% CI 1.1–3.6) even after adjusting for multiple CVD risk factors [9]. Moreover, the risk of CVD in affected patients appears to precede the formal diagnosis of RA.

Cardiovascular disease risk factors in rheumatoid arthritis

Hypertension, diabetes mellitus, male sex, advanced age, cigarette smoking, obesity, and hyperlipidemia have long been recognized as important determinants of higher CVD susceptibility [10]. The increased CVD burden in RA, however, does not seem to be completely explained by an increased prevalence of traditional risk factors. In fact, findings from risk assessments in RA are sometimes counter to what has been observed among the general population. For instance, RA patients with low body mass index (BMI) (<20 kg/m²) have a substantially higher risk of cardiovascular death (RR = 3.34; 95% CI 2.23–4.99, compared with normal BMI) than do individuals with normal or even increased BMI [11*]. It has been speculated that low BMI, resulting from so-called rheumatoid cachexia, may simply serve as a marker for more severe RA and a higher systemic inflammatory burden [12].

With the exception of cigarette smoking, which is associated with both RA disease onset and severity, there does not seem to be an increase in the frequency of other CVD risk factors in RA patients compared with the general population [13–17]. Solomon et al. [18*] recently compared the prevalence of traditional CVD risk factors among women with and without RA. They found no differences between these groups in terms of BMI, prophylactic aspirin use, diabetes, hypertension, physical activity, and family history of CVD. In addition to the presence of systemic inflammation and the possible iatrogenic role of medications, disease-specific factors that may predispose RA patients to increased CVD risk include elevations in serum homocysteine, dyslipidemia, and endothelial dysfunction [19–26].

Cardiovascular risk associated with medications

The iatrogenic effects of RA treatments, including disease-modifying antirheumatic drugs (DMARDs), may contribute to CVD risk. For instance, methotrexate use leads to increased serum homocysteine, a factor that has been independently associated with a higher incidence of cardiovascular morbidity in non-RA patients [19,20]. Glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs) may adversely affect blood pressure control, hyperglycemia, and serum lipids, thus contributing to a heightened CVD susceptibility [27–33]. In a recent study involving 92 RA patients, previous exposure to oral prednisone therapy was associated with significant declines in insulin sensitivity, an independent risk factor for CVD [34]. Although this was not supported by the results of a recent observational study, tumor necrosis factor-α (TNF-α) inhibition has been associated with increased mortality among RA patients with severe CHF, leading manufacturers to caution against the use of these agents in high-risk patients [35,36]. Recent studies have shown that TNF-α blockade may lead to declines in serum levels of high-density lipoprotein (HDL), an effect that could potentiate CVD risk [37].

The potential for adverse cardiovascular outcomes with RA treatments was recently underscored with the voluntary recall of rofecoxib (a cyclo-oxygenase [COX]-2 specific inhibitor) in the wake of investigations demonstrating enhanced cardiovascular morbidity and mortality with the use of this agent [32,38*,39,40••]. The negative impact of rofecoxib on CVD risk may be mediated in part through its detrimental effects on blood pressure. In a large retrospective cohort study, patients taking rofecoxib were at a substantially higher risk for the development of new-onset hypertension than were those taking
either another COX-2 specific inhibitor (RR = 1.6; 95% CI 1.2–2.1 compared with celecoxib) or a nonspecific NSAID (RR = 1.4; 95% CI 1.1–1.9) [41••]. Although controversial, recent reports have highlighted potential problems associated with other COX-2 inhibitors including both celecoxib and valdecoxib, suggesting that CVD-associated risk may actually represent a ‘class effect’ of these drugs [42,43].

**Dyslipidemia**

The results of investigations examining the role of dyslipidemia in RA are somewhat contradictory but suggest that serum lipids oscillate according to the duration or severity of disease. In severe active RA, patients have been reported to have significantly lower serum levels of total cholesterol, HDL, and low-density lipoprotein (LDL), findings that may be associated with the relative malnutrition and cachexia observed in patients with advanced RA [12,44]. By contrast, RA patients with early untreated disease show significant declines in HDL with concomitant elevations in LDL and total cholesterol/HDL ratios, lipid profiles known to correlate with an increased CVD incidence [21]. In addition, the use of anti-inflammatory treatments (DMARDs with or without low-dose prednisolone) in previously untreated RA patients has been associated with substantial improvements in serum lipoprotein values, including a 21% increase in HDL ($P < 0.001$), a 23% increase in apolipoprotein A-I ($P < 0.001$), and a 13% decrease in the LDL/HDL ratio ($P = 0.10$) [22]. The beneficial impact of anti-inflammatory treatment seems to be far more pronounced in those obtaining a clinical response in terms of arthritis activity, suggesting that dyslipidemia may represent a common pathway between inflammation and CVD early in the course of RA.

**Systemic inflammation**

Accumulating evidence suggests that atherosclerosis is an inflammatory disorder sharing a common pathobiology with the synovial inflammation and pannus formation that characterize RA [2,45]. Overlapping pathogenic features of the two diseases include the predominant role of pro-inflammatory cytokines (e.g., TNF-$\alpha$ and interleukin-6), elevated serum levels of acute phase reactants (including C-reactive protein [CRP], fibrinogen, and serum amyloid-A), neo-angiogenesis, T cell activation (with an increased Th1 to Th2 cell ratio), and the local expression of leukocyte adhesion molecules and endothelin [2,45–47].

Evidence supporting an association of inflammation with atherosclerosis comes from population-based investigations examining the association of elevated serum CRP levels and the subsequent development of CVD [48,49]. In a study involving more than 1000 otherwise healthy men, elevated baseline highly sensitive CRP levels served as an independent predictor of incident MI; those in the highest quartile of highly sensitive CRP were nearly three times more likely than those in the lowest quartile to have an MI [49]. Studies by Pasceri et al. [50,51] have suggested a more direct role of CRP in the CVD causal pathway by demonstrating CRP-induced expression of intercellular adhesion molecule-1 on endothelial cells as well as CRP-induced production of monocyte chemotactic protein-1 (MCP-1).

Endothelial dysfunction, characterized by reduced vasodilator function, is an early event in CVD pathogenesis [48,49]. A growing body of evidence suggests that systemic inflammation acts as an important mediator of endothelial dysfunction. For instance, pro-inflammatory cytokines have been shown to impair endothelial function both in animal models and in dissected human veins, an effect that seems to be mediated by reduced endothelial nitric oxide availability and increased oxidant stress [54–56].

In a small study involving individuals younger than 60 years, investigators assessed endothelial function in RA patients using noninvasive ultrasonography to measure brachial flow–mediated vasodilation (FMV) [26]. Compared with age- and sex-matched controls, RA patients with low levels of disease activity demonstrated significant reductions in brachial flow–mediated vasodilation ($P < 0.001$), reductions that were positively correlated with CRP values. Impaired endothelial function also is evident early in the course of RA. In a study involving 10 patients with newly diagnosed RA, Bergholm et al. [25] assessed endothelial function by measuring vasodilatory responses to intrabrachial artery infusions of acetycholine. Vasodilatory responses were 37 to 50% lower in RA patients than in control individuals.

If systemic inflammation promotes endothelial dysfunction and atherogenesis, then it follows that the use of potent anti-inflammatory agents in RA may decrease the CVD burden in this population. Two recent studies have shown that disease-modifying therapies, both conventional DMARDs and anti–TNF-$\alpha$ therapy, may improve measures of endothelial function in RA [25,57•]. Choi et al. [58] examined the impact of methotrexate (the most commonly used DMARD in the United States) on CVD-related mortality in RA. After adjustment for other CVD risk factors and confounding by indication, methotrexate was associated with a significant 70% reduction in the risk of CVD-related death ($RR = 0.3; 95\% \text{ CI} 0.2–0.7$). The associations of methotrexate and select DMARDs with overall mortality are summarized in Figure 2. These results suggest that the potent anti-inflammatory properties of methotrexate offer substantial protection against CVD, far outweighing the potential effect of hyperhomocysteinemia, which may result from its use [19,20].
In contrast to reports in other populations showing a positive association of glucocorticoid use and the development of atherosclerotic vascular disease, data from a retrospective cohort study suggest that low-dose glucocorticoid use may actually be protective against CVD development [31,59,60]. In an intriguing research letter, pulse therapy with glucocorticoids was recently associated with a significant 26% reduction in plasma homocysteine, an effect that was both rapid and durable over a 6-month follow-up period [61]. The results of this latter study must be interpreted with some caution because the investigation was open-label, uncontrolled, and involved only a few RA patients. Despite potential methodologic limitations, these studies are important and suggest that the deleterious effects of low-dose glucocorticoids may be outweighed by their potent anti-inflammatory effect in RA, again supporting the role of untreated inflammation in CVD pathogenesis.

Reducing cardiovascular disease burden in rheumatoid arthritis

It is critical that health care providers recognize RA patients at greatest cardiovascular risk and implement primary and secondary preventive measures aimed at reducing the effects of CVD (Table 1). With accumulating evidence suggesting that inflammation plays a pivotal role in CVD pathogenesis, cardiovascular protection provides further rationale for the paradigm shift in RA management toward earlier, more aggressive medical intervention. Preliminary reports suggest that this shift toward more aggressive RA management may already be paying dividends. In a recent study involving successive incidence and birth cohorts of RA patients, investigators noted temporal improvements in MI-related mortality [62••]. This decrease in CVD mortality was accompanied by a temporal increase in patients who entered the study receiving methotrexate (used as a surrogate for aggressive DMARD intervention).

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<th>Table 1. Cardiovascular disease risk factors in rheumatoid arthritis and possible preventive measures to reduce risk</th>
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CVD, cardiovascular disease; RA, rheumatoid arthritis; CHF, congestive heart failure; TNF, tumor necrosis factor; NSAID, nonsteroidal anti-inflammatory drug; HCQ, hydroxychloroquine; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MTX, methotrexate; SSZ, sulfasalazine.
Smoking cessation is also of paramount importance, because cigarette smoking not only increases the risk for coronary heart disease but also seems to increase RA disease severity [13,14,63]. In the absence of contradictory evidence, it seems appropriate to minimize exposure to both glucocorticoid and COX-2 NSAID and to institute low-dose aspirin in patients who are at the highest risk for CVD. Additionally, both NSAIDs (traditional and COX-2-specific) and TNF-α inhibitors should be avoided in RA patients with a history of severe CHF. Folate supplementation, routinely used to minimize methotrexate-associated toxicity, lowers serum homocysteine levels and may provide cardiovascular protection in the setting of ongoing methotrexate administration [64]. Homocysteine metabolism may be abnormal in RA patients even in the absence of methotrexate use, suggesting that serum levels should be measured routinely in all RA patients with significant elevations prompting appropriate folate supplementation [65].

Although hormone replacement therapy (HRT) was at one time advocated as a cardioprotective agent, recent data from the Women’s Health Initiative have shown an increased risk for both coronary heart disease (RR = 1.29; 95% CI 1.02–1.63) and stroke (RR = 1.41; 95% CI 1.07–1.85) for women receiving HRT [66,67]. Although HRT use is associated with significant declines in plasma homocysteine, it also leads to CRP elevation, an effect that may explain its apparent adverse effect on CVD risk [68]. By contrast, raloxifene, a selective estrogen receptor modulator (like HRT, often used for osteoporosis treatment or prevention) does not adversely affect CRP levels and, similarly to estrogen therapy, lowers serum homocysteine, raising speculation that this agent may have a preferential role in the primary and secondary prevention of CVD in RA patients [68]. Clearly, the small increase in CVD incidence that has been observed with estrogens and the small increase in deep venous thrombosis that has been seen with selective estrogen receptor modulators mandates that health care providers carefully examine the safety profile of these agents for the individual RA patient [69].

Given the prevalence of dyslipidemia in early disease, lipid measurements should be a routine component of health care screening in all patients with RA, with abnormal lipid values treated appropriately [21]. Of great interest, hydroxychloroquine, a commonly used antimalarial DMARD, may have a favorable impact on serum lipids, implying that this may be a preferred agent for RA patients with abnormal lipid profiles [70].

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), well recognized for their suppression of cholesterol production, may also have additional anti-inflammatory properties according to recent research, suggesting that these agents may play an important role in RA therapy [71–73]. The use of statins has been associated with protein immunomodulatory effects, including the inhibition of leukocyte-endothelial cell interactions; decreased expression of pro-inflammatory cytokines (interleukin-6, interferon, and TNF-α) and metalloproteinases; and reductions in T cell proliferation [74–83].

To date, the most comprehensive examination of statin therapy in RA comes from the recently published Trial of Atorvastatin in Rheumatoid Arthritis (TARA) [84••]. In this double-blind, placebo-controlled comparison, 116 RA patients were randomized to receive either atorvastatin (40 mg/day) or placebo in addition to stable background DMARD therapy. In this 6-month study, the disease activity score (DAS 28) improved significantly for the atorvastatin-treated group compared with the placebo group (group difference −0.52, \( P = 0.004 \)) in the absence of significant toxicity. CRP and erythrocyte sedimentation rate fell by 50% (\( P < 0.001 \)) and 28%

### Table 2. Changes in 6-month outcomes among rheumatoid arthritis patients receiving atorvastatin (40 mg/day) vs. placebo: results from the Trial of Atorvastatin in Rheumatoid Arthritis (TARA) trial

<table>
<thead>
<tr>
<th></th>
<th>Atorvastatin (n = 58)</th>
<th>Placebo (n = 58)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean change from baseline</td>
<td>95% CI</td>
<td>Mean change from baseline</td>
</tr>
<tr>
<td>DAS 28</td>
<td>−0.50</td>
<td>−0.75 to −0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>−5.03</td>
<td>−8.4 to −1.62</td>
<td>1.91</td>
</tr>
<tr>
<td>CRP, log mg/L</td>
<td>−0.46</td>
<td>−0.64 to −0.28</td>
<td>0.12</td>
</tr>
<tr>
<td>Swollen joint count</td>
<td>−2.69</td>
<td>−3.81 to −1.53</td>
<td>−0.53</td>
</tr>
<tr>
<td>Tender joint count</td>
<td>−1.21</td>
<td>−3.28 to 0.88</td>
<td>0.38</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>−1.40</td>
<td>−1.63 to −1.17</td>
<td>−0.07</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>0.03</td>
<td>−0.03 to 0.09</td>
<td>−0.04</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>−0.38</td>
<td>−0.69 to −0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>Interleukin-6, pg/mL</td>
<td>−6.6</td>
<td>−13.2 to 0.01</td>
<td>3.84</td>
</tr>
</tbody>
</table>

DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; LDL, low density lipoprotein; HDL, high-density lipoprotein; significant differences favoring atorvastatin also seen with total cholesterol (\( P < 0.001 \)), triglycerides (\( P < 0.001 \)), and plasma viscosity (\( P < 0.001 \)); non-significant differences seen with morning stiffness, visual analog score, patient global assessment, physical function, Von Willebrand factor, and intracellular adhesion molecule-1. From [84].
Systemic inflammation in rheumatoid arthritis and cardiovascular disease

Conclusion
Cardiovascular disease remains the single largest contributor to excess mortality in the context of RA. An increasing body of evidence points to ‘unchecked’ systemic inflammation as the major culprit driving increased CVD risk in RA. The association of inflammatory pathways with CVD is complex and is composed of several intermediate factors, including dyslipidemia, homocysteinemia, insulin resistance, and endothelial dysfunction. Given the enormous CVD burden in RA, it is paramount that health care providers caring for RA patients adopt a more comprehensive approach, one that accounts for this lethal extra-articular disease manifestation and one that includes early and aggressive control of vascular inflammation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest


11 Kremers H, Nicola P, Crowson C, et al. Prognostic importance of low body mass index in relation to cardiovascular mortality in rheumatoid arthritis. Arthritis Rheum 2004; 50:3450–3457. This observational study from the Rochester Epidemiology Project showed that low body mass index (as opposed to obesity) serves as a significant determinant of CVD mortality among patients with RA.
18 Solomon D, Curhan G, Rimm E, et al. Cardiovascular risk factors in women with and without rheumatoid arthritis. Arthritis Rheum 2004; 50:3444–3449. This study showed that traditional cardiac risk factors are not disproportionately prevalent in women with RA, suggesting that RA is an ‘independent’ risk factor for CVD.
Clinical therapeutics


This observational cohort study showed temporal improvements in MI-related mortality among RA patients.


van der Wal AC, Becker AE, van der Loos CM, et al. Site of initial rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994; 89:36–44.


Gonzalez-Juanatey C, Testa A, Garcia-Castello A, et al. Active but transient improvement of endothelial function in rheumatoid arthritis patients undergoing long-term treatment with anti-tumor necrosis factor alpha antibody. Arthritis Rheum 2004; 51:447–450. The results of this study showed that aggressive intervention with therapies targeting TNF-α may improve measures of endothelial dysfunction.


Rheumatoid arthritis: non–tumor necrosis factor targets
Louise Pollard and Ernest Choy

Purpose of review
The treatment of rheumatoid arthritis has been revolutionised in recent years with the advent of biologic treatments. The purpose of this review is to outline new treatments that target the inflammatory pathway in rheumatoid arthritis other than tumor necrosis factor-α.

Recent findings
As the use of anti–tumor necrosis factor-α treatment has become more widespread, the number of patients in whom this treatment is unsuccessful has also accumulated. Contraindications such as infection and cardiac failure further add to the number of patients who need alternative treatment. A better understanding of the inflammatory pathway in rheumatoid arthritis has led to interest in other therapeutic targets. Promising treatments such as interleukin-6 antagonists (MRA), CTLA4Ig (abatacept), and anti–B cell therapy (rituximab) have already been tested in randomized controlled trials over the past year. Other cytokines have been identified and have been shown to be of benefit in animal models, including interleukin-15, interleukin-17, and interleukin-18, and clinical trials of these agents are currently under way.

Summary
For patients with rheumatoid arthritis that does not respond to anti–tumor necrosis factor-α treatment, the promising alternatives MRA, abatacept, and rituximab have been tested. It is hoped that these agents will become available shortly.

Key Words
anti-CD20, cytotoxic T-lymphocyte–associated antigen 4-IG, interleukin, rheumatoid arthritis

Introduction
Rheumatoid arthritis (RA) is globally the most common chronic inflammatory polyarthritis. Disease is lifelong, and disability after 10 years is frequent [1]. With the introduction of anti–tumor necrosis factor (TNF)-α agents, the treatment of RA has improved significantly over the past decade. These new agents improve signs and symptoms and also halt joint damage [2]. Anti–TNF-α treatment itself has limitations, however: not all patients respond, and there is increased susceptibility to infection. Reactivation of latent tuberculosis has been a particular problem, so that patients have to be screened before the commencement of treatment [3]. Although TNF-α is an important cytokine, other proinflammatory cytokines are attractive therapeutic candidates. This review will concentrate on new non–TNF-α treatments for RA.

Interleukin-1
It is thought that RA is driven by T cells and B cells, which produce proinflammatory mediators. Macrophages and fibroblasts release monokines, TNF-α, and interleukin (IL)-1 and -6 when stimulated by activated T cells. TNF-α and IL-1 are thought to be the two most important mediators of inflammation, and IL-1 is thought to be important in joint damage [4–5]. IL-1 receptor antagonist (IL-1Ra) is an endogenous, competitive antagonist of the IL-1 receptor. It competes with IL-1 for binding to IL-1 receptors but does not transduce signal [6]. The only drug licensed for use in RA that blocks IL-1 specifically is anakinra (Kineret, Amgen, recombinant-methionyl-human IL-1Ra). There have been five randomized placebo-controlled clinical trials of anakinra [7–10,11••]. The full results of a multicentre double-blind placebo-controlled trial of anakinra in combination with methotrexate were reported earlier this year [11••]. This study examines the efficacy of combining anakinra at the recommended dose of 100 mg/day. Although there was a significant improvement in American College of Rheumatology (ACR) response rates, they were less impressive than those seen with anti–TNF-α in combination with methotrexate [12]. The place of IL-1Ra in the treatment of RA is not clear. It has been postulated in the past that patients who do not respond to anti–TNF-α may have disease in which IL-1 is the dominant mediator of inflammation; however, a study of 26 patients with RA in whom anti–TNF-α treatment was unsuccessful did not show any significant response to IL-1Ra [13•]. Also, it has been postulated that blocking TNF-α and IL-1 would give superior efficacy than blocking TNF-α alone; however, the results of a randomized controlled trial of adding...
anakinra to etanercept over monotherapy showed no added benefit but increased toxicity [14**].

The lack of efficacy compared with TNF-α may be related to receptor occupancy issues and rapid clearance in vivo. Therefore, other methods to block IL-1 are currently under evaluation. The IL-1 trap comprises two IL-1 cell surface receptors combined into a single bivalent construct that exhibits a high affinity binding of IL-1 and therefore better IL-1 neutralization [15]. It has been evaluated in a phase II trial [16]. There was clinical improvement (ACR 20 response of 46% compared with 30.9% with placebo) with few adverse events, but the number of patients studied was small, and the difference was not statistically significant.

**Interleukin-6**

Interleukin-6 is a pleiotropic cytokine, which stimulates B cells to mature and generate immunoglobulin, megakaryocytes to form platelets, osteoclasts to resorb bone, and hepatocytes to produce acute-phase proteins. IL-6 is found in abundance in the synovial fluid and serum of patients with active RA [17]. Patients with RA have higher levels of IL-6 and its receptor than do control individuals, and the level is higher in synovial fluid than in serum, suggesting that there is local production of IL-6 in the synovium [18]. Furthermore, serum levels of IL-6 correlate with clinical and laboratory indices of disease activity [19].

MRA is a humanized antihuman IL-6R monoclonal antibody that inhibits the binding of IL-6 to cell surface IL-6R or soluble IL-6R. It is administered as an intravenous infusion. The first open-label trial of MRA in 15 RA patients showed improvement in ACR 20 and ACR 50 as well as improvement in inflammatory markers. There were no serious adverse events, but a rise in cholesterol was frequently observed, which was in keeping with previous studies showing a decrease in cholesterol in patients given recombinant IL-6 in cancer [20].

The first double-blind placebo-controlled trial of IL-6R antibody showed an improvement in signs and symptoms of RA and normalisation of acute-phase reactants [21]. The acute-phase reactants reduced to a normal level after only one dose of MRA. MRA was well tolerated, with only minor disturbances in liver enzymes and decreased leukocyte counts.

In a double-blind placebo-controlled trial, 164 patients with RA were randomized to receive either MRA (4 mg/kg or 8 mg/kg) or placebo [22**]. Treatment was given every 4 weeks for 3 months. MRA reduced disease activity in a dose-dependent manner. In the 8-mg group, 78% of the patients achieved at least an ACR 20 response. The efficacy was apparent at week 4 and most pronounced at week 12. There were three serious adverse events in the treatment group, compared with two in the placebo group. One patient in the treatment group died as a result of reactivation of chronic active Epstein-Barr virus. Otherwise, adverse events were generally mild.

Another randomized controlled trial examined the effect of MRA monotherapy and combination therapy with methotrexate [23]. In all, 359 RA patients with active disease were randomised into seven treatment arms: 3 doses of MRA (2, 4, and 8 mg/kg) either as monotherapy or in combination with methotrexate. The comparator was 10 to 25 mg of methotrexate plus placebo. MRA was efficacious when given as monotherapy or in combination with methotrexate, although methotrexate seemed to enhance the benefit of MRA. The ACR 20 response in the methotrexate group was 41%, compared with 61% and 63% in the 4-mg/kg and 8-mg/kg monotherapy groups, respectively. In combination with methotrexate, the ACR 20 response was 64%, 63%, and 74% in the 2-, 4-, and 8-mg/kg groups, respectively. Serious adverse events in the MRA-treated groups included five cases of infection and five cases of hypersensitivity.

It seems that MRA is generally well tolerated and reduces the signs and symptoms of RA. Although further work is needed on the effect of MRA on radiologic progression in RA as well as to investigate its long-term effects, it seems to hold promise as a new therapeutic agent in RA.

**Cytotoxic T-lymphocyte–associated antigen 4**

Cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) is expressed on the surface of T-cells. CTLA4 is the high avidity receptor for CD80 and CD86. Engagement of CD80/CD86 provides essential secondary costimulatory signals for T cell activation. In the absence of secondary signals, T-cells become anergic when stimulated by antigenic peptides. CTLA4Ig (abatacept, Bristol-Myers Squibb) is a fusion protein consisting of the extracellular domain of human CTLA4 and a fragment of the Fc-domain IgG1 [24]. It is the first in a new class of drugs for RA known as co-stimulation blockers. Abatacept is administered as a single 30-minute IV infusion. It inhibits T cell function but does not deplete T cells.

A 3-month pilot study of abatacept in RA showed it to be safe and well tolerated, and to demonstrate efficacy, although the number of patients was small [25]. More recently, the results of a phase II trial were published [26**]. This 6-month, double-blind, randomized, placebo-controlled study of abatacept included a total of 339 patients with RA taking concurrent methotrexate. Patients received 2 mg/kg or 10 mg/kg of abatacept or placebo. The main aim of the study was to compare the safety, efficacy, and immunogenicity of abatacept with those of placebo in patients with active RA. The ACR 20 responses
in the group given 10 mg/kg were significantly higher than those the placebo group from month 2 through month 6 (60% compared with 35.3% at month 6). The ACR 50 and ACR 70 responses were significantly higher in both treatment groups compared with placebo. Abatacept was well tolerated, and there were no reports of opportunistic infections. There were fewer serious adverse events in the 10-mg/kg group than the placebo group. The response rate was similar to that in patients receiving methotrexate after treatment with infliximab (10 mg/kg every 4 weeks) for 30 weeks [12].

Phase III trials of abatacept in patients with inadequate response to methotrexate or anti–TNF-α were recently reported [27–28]. Of 547 RA patients with an inadequate response to methotrexate, 67.9% receiving abatacept achieved an ACR 20 response, compared with 39.7% receiving placebo. ACR 50 and ACR 70 responses in the abatacept group were 48.3% and 28.8%, respectively, compared with 18.2% and 6.1% in the placebo group. There was also a significant reduction in joint damage assessed radiologically. In 391 patients who did not respond to anti–TNF-α, 50.4% of patients receiving abatacept achieved an ACR 20 response compared with 19.5% receiving placebo. ACR 50 and ACR 70 responses in the abatacept group were 20.3% and 10.2%, respectively, compared with 3.8% and 1.4% in the placebo group. Abatacept had a significant clinical benefit in patients who had an inadequate response to methotrexate or who had not responded to anti–TNF-α.

### B cells

Edwards and Cambridge [29] first published the beneficial effect of B cell depletion in RA. This brought renewed interest in the role of B cells in RA. In this open-label study, five patients were given rituximab (Roche, a genetically engineered chimeric anti-CD20 monoclonal antibody), which is licensed for the treatment of non-Hodgkin lymphoma. They also received concomitant high-dose steroids and cyclophosphamide, a therapeutic regimen used in non-Hodgkin lymphoma, so the exact effect of rituximab was unclear. There have since been several studies, however, and this year the full results of the first randomized, double-blind, placebo-controlled trial of rituximab in RA were published [30**]. This multicentre trial included 161 patients with active RA. The patients were assigned into one of four groups: methotrexate alone, rituximab (100 mg on days 1 and 15), rituximab plus cyclophosphamide (750 mg on days 3 and 17), or rituximab plus methotrexate. The patients were given 24 weeks of treatment and followed up for 48 weeks. In addition to the above treatment, all patients including those in the methotrexate group, were given a 17-day course of corticosteroids, including intravenous methylprednisolone and high-dose oral steroids. The ACR 20 and 50 responses were significantly higher in all the rituximab groups than in the control group. Only the rituximab plus methotrexate group had significantly higher ACR 70 responses (23% compared with 5% in the control group). The response rates compared favourably with those of anti–TNF-α. There were several serious adverse events in the rituximab groups (highest in the rituximab plus cyclophosphamide group) and one fatality. Therefore, close monitoring for infection is needed in patients receiving rituximab. The responses to a single course of rituximab compared favourably at 48 weeks, with only the rituximab and methotrexate group retaining significance in all ACR responses.

From these results, rituximab may have a place in the treatment of RA. In view of the increased infections in the cyclophosphamide group and the favourable results from the rituximab and methotrexate group, this would seem a more logical combination. The issue of the high-dose steroids needs further clarification.

### Other cytokines as targets

Although therapies aimed at IL-1 and IL-6 have been developed, other cytokines are involved in the inflammatory process, and they have become the focus of new research. Other cytokines being targeted in animal experiments and clinical trials include interferon-α, interferon-γ, and IL-15, 17, 18, and 23.

### Interleukin-15

Interleukin-15 enhances synovial T cell proliferation and cytokine release, and optimises interactions between T cells and macrophages, which in turn cause monokine release, including TNF-α [31]. A recent study using an IL-15 receptor antagonist in murine collagen-induced arthritis (CIA) showed therapeutic benefit [32**]. The study used a recombinant fusion protein consisting of a point mutated IL-15 and the constant region of murine IgG2a. This IL-15 mutant/Fcy2a fusion protein (CRB-15) binds the IL-15 receptor with high affinity. The authors observed a reduction in expression of IL-1β, TNF-α, IL-6, and IL-17. Overall treatment with CRB-15 led to a reduction in synovial inflammation, bone resorption, and cartilage destruction.

AMG 714 (Amgen) is a human anti–IL-15 monoclonal IgG1 antibody that is administered by subcutaneous infusion. In a early phase II clinical trial, 110 patients with active RA despite treatment with one or more disease-modifying antirheumatic drugs were randomized to placebo or one of four doses of AMG 714 (40, 80, 160, and 280 mg) [33]. The ACR 20 response in the highest-dose group was 62%, compared with 26% in those receiving placebo at 12 weeks. The treatment was well tolerated. Further studies with AMG714 will further elucidate its role in the treatment of RA.
Interleukin-17
Interleukin-17 is a T cell–derived cytokine, which has been found in high levels in synovial fluid in RA [34]. A recent study using rabbit antimurine IL-17 antibody in mice showed a benefit in early onset of CIA [35]. The authors demonstrated a reduction in IL-6, IL-1β, and receptor activator of nuclear factor κB ligand (RANKL) positive cells. After one infusion of the antibody, there was a reduction in synovitis, erosions, and cartilage destruction. The authors also gave the antibody at a later stage of CIA and found that it slowed progression of disease. Previous studies have suggested that IL-17 can work synergistically with other cytokines such as IL-1 and TNF-α on synoviocytes [36]. Therefore, it may be that IL-17 will have a place in the treatment of RA, possibly in combination with other cytokine inhibitors.

Interleukin-18
Interleukin-18 is a member of the IL-1 cytokine family. IL-18 promotes inflammation by enhancing several cytokines, including TNF-α, IL-1, and IL-6 [37]. A recent study in CIA found that small doses of IL-18 increased the incidence and activity of arthritis and activity and that its effect was attenuated with neutralising antibodies. Interestingly, higher doses of IL-18 reduced arthritis, suggesting a bimodal response. Further research is needed, but the results so far suggest that IL-18 antagonists may be useful as a treatment for RA [38*].

Conclusion
Although TNF-α and IL-1 are important in the pathogenesis of RA, targeting other inflammatory mediators has promising therapeutic effects. Other treatments are needed in RA, because despite its undoubted benefit there are a proportion of patients who do not respond to anti–TNF-α treatment or who have problems with infections and other contraindications. Anti–IL-6R monoclonal antibody, CTLA4Ig, and anti-CD20 monoclonal antibody all showed promising results in late phase II or phase III trials, but the safety of these agents will need to be addressed in long-term follow-up studies.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

15 Animal models have shown a benefit in blocking both TNF and IL-1. This study showed that there is no advantage in blocking both cytokines and this strategy simply increases the risk of infection.
17 Regeneron Reports Phase II. Results for IL-1 Trap Clinical Program in RA. www.regeneron.com/investor/press_detail.asp?v_c_id=184.
24 This was the first multicentre trial of anti-IL-6, which was well tolerated and achieved good response rates. The results are promising, and anti-IL-6 has potential as a new therapeutic agent in RA.
Clinical therapeutics


This study of IL-18 showed that administration of small doses of IL-18 can worsen disease in collagen-induced arthritis, and disease can be attenuated with IL-18 antibodies. Higher doses of IL-18 caused a reduction in disease activity.
Treatment update on spondyloarthritis

Allen Anandarajah and Christopher T. Ritchlin

Purpose of review
The unexpected success of the tumor necrosis factor antagonists in ankylosing spondylitis and psoriatic arthritis has generated considerable enthusiasm regarding the therapeutic potential of these drugs. By contrast, concerns regarding the high cost and long-term safety of the tumor necrosis factor blocking agents have prompted investigators to take a closer look at more traditional anti-inflammatory agents and to explore novel therapeutic targets. The purpose of this review is to summarize treatment advances in spondylarthropathy over the past year and to discuss potential future therapies.

Recent findings
Recent studies indicate that the morbidity of ankylosing spondylitis and PsA are considerably higher than previously reported. Etanercept, infliximab and adalimumab safely and effectively relieved the signs and symptoms of psoriatic arthritis patients in phase III trials. Etanercept and infliximab were also effective in phase III trials in ankylosing spondylitis. Etanercept slowed radiographic progression in psoriatic arthritis trials, but it is not known whether tumor necrosis factor antagonists can prevent structural damage in ankylosing spondylitis. One trial showed that methotrexate may be effective for relieving the pain of axial disease in ankylosing spondylitis but these findings contradict two previous studies. For reactive arthritis and undifferentiated spondylarthropathy, a combination of antibiotics may be more effective than a single antibiotic for the relief of musculoskeletal symptoms. Last, potential therapeutic targets include interleukin-1, interleukin-12, B lymphocytes, accessory molecules on T lymphocytes, and angiogenic factors.

Summary
Phase III trials have confirmed that tumor necrosis factor antagonists are effective and safe for the treatment of ankylosing spondylitis and psoriatic arthritis. For patients who do not respond to tumor necrosis factor blockade, several treatment options are under study. Information from these trials will more clearly define the role of disease-modifying antirheumatic drugs, novel therapeutic agents, and antibiotics in the treatment of spondylarthropathy.

Keywords
ankylosing spondylitis, anti—tumor necrosis factor-α, methotrexate, physiotherapy, psoriatic arthritis, reactive arthritis, spondyloarthritis, therapy

Abbreviations
AS ankylosing spondylitis
BASDAI Bath Ankylosing Spondylitis Disease Index
BASFI Bath Ankylosing Spondylitis Functional Index
DMARD disease-modifying antirheumatic drug
IL interleukin
NSAID nonsteroidal anti-inflammatory drug
PASI Psoriasis Area and Severity score
PsA psoriatic arthritis
RA rheumatoid arthritis
SpA spondyloarthropathy
TNF tumor necrosis factor
VEGF vascular endothelial growth factor

Introduction

[Some] cases…are confined mainly to the vertebral column…. It leads very gradually and painlessly to a complete ankylosis of the entire spinal column, and the hip joints, so that the head, trunk, and thighs are firmly united and completely stiffened, while all other points retain their normal mobility.

Ernst Adolph Gustav Gottfried von Strumpell, A Textbook of Medicine for Students and Practitioners, 1887

The spondyloarthropathies (SpAs) are a group of chronic interrelated rheumatic disorders of the axial and peripheral joints that are characterized by common clinical, radiologic, and genetic features. Included among this group are ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis, enteropathic arthritis, and undifferentiated SpA [1]. PsA was initially thought to be a benign arthropathy, but a recent 2-year follow-up study of a cohort of patients with early PsA revealed that a majority of patients were taking disease-modifying antirheumatic drugs (DMARDs) and that 47% manifested erosions on plain radiographs [2*]. Likewise, studies exploring the impact of AS on work performance and long-term disability showed a high level of functional impairment and a surprisingly low quality of life in many patients [3].

Taken in isolation, these studies paint a bleak picture for many patients with PsA and AS, but the remarkable success of the anti—tumor necrosis factor (TNF) agents in
phase II trials generated new hope for the possibility that biologic agents could effectively and safely relieve signs and symptoms and perhaps even limit disease progression. The promising results from these trials created great enthusiasm and fostered the concept that TNF blockade was a tremendous therapeutic breakthrough for patients with SpA, but several important questions remained unanswered. Are anti-TNF agents safe and effective over the long term? What is the role of traditional DMARDs in PsA and AS? Do anti-TNF agents inhibit disease progression in both the axial skeleton and the peripheral joints? Do anti-TNF agents lead to clinically meaningful improvements in quality of life, and what fraction of patients does not respond to these compounds? Last, are these compounds cost effective for the treatment of AS and PsA?

Over the past year, several phase III trials that examined the efficacy and safety of anti-TNF agents in PsA and AS have been completed. In addition, investigators have continued to examine the role of traditional agents, including nonsteroidal anti-inflammatory drugs (NSAIDs) and DMARDs, in the treatment paradigm. Last, small pilot studies, in many cases designed on the basis of expanding knowledge about disease mechanisms, were carried out in an attempt to discover novel therapeutic targets in the SpA. In this review, we shall summarize the data from pivotal SpA clinical trials over the past year, which provide at least partial answers to many of the questions posed above, and we shall highlight new therapeutic targets that are under consideration.

**Ankylosing spondylitis**

Ankylosing spondylitis is a chronic inflammatory disease that involves the axial skeleton and generally begins in the sacroiliac joints. Prevalence figures in the United States are not firmly established. Reports from Western Europe, however, indicate that 0.5 to 1% of the adult population have the disorder [4]. The prevalence of AS is directly linked to the frequency of the HLA-B27 gene in a given population, and concordance is observed in family members [5,6]. Axial pain, stiffness, and fatigue are well-recognized symptoms of the SpAs. Over the past several years, the Assessment in Ankylosing Spondylitis (ASAS) Working Group has developed domains and instruments for assessment of AS patients in clinical trials.

**Outcome measures**

Several different instruments have been developed for the assessment of disease in AS and some of these are listed in Table 1 [7]. The ASAS 20 and the Bath Ankylosing Spondylitis Disease Index (BASDAI) have gained widespread acceptance, and versions of the BASDAI have been validated in several languages. The Bath Ankylosing Spondylitis Functional Index (BASFI) provides a patient assessment of function, and the AS-specific quality of life and the Patient Generated Index are disease-specific instruments for the assessment of quality of life. The Maastricht AS Enthesitis Score is a valid and feasible instrument for the assessment of enthesitis and is preferred over the Mander Enthesitis Index because it reduces the number of measured sites from 66 to 13.

**Disease-modifying antirheumatic drugs**

The conventional treatment of AS has centered on the combination of exercise with NSAIDs [1]. Exercise has been stressed as an important adjunct to medications in the treatment of patients with AS. A recent systematic review, however, concluded that only limited evidence suggests a benefit of supervised physiotherapy over individualized home programs [8]. Furthermore, no randomized trials of specific physiotherapy interventions in the treatment of patients with AS have been published.

Patients with more aggressive or severe AS have been treated with DMARDs on the basis of the efficacy of these agents in treating RA. Sulfasalazine is the most extensively studied of these agents, but its efficacy is modest and is limited to the peripheral joints [9]. A recent meta-analysis

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**Table 1. Outcome measures in ankylosing spondylitis**

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<thead>
<tr>
<th>Instrument</th>
<th>Outcome measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment in Ankylosing Spondylitis (ASAS) 20</td>
<td>A composite of disease activity and disability</td>
</tr>
<tr>
<td></td>
<td>An improvement of ≥20% and absolute improvement of ≥10 units on a 0–100 scale in ≥3 of the following 4 domains:</td>
</tr>
<tr>
<td></td>
<td>1. Patient global assessment (by VAS global assessment)</td>
</tr>
<tr>
<td></td>
<td>2. Pain assessment (the average of VAS total and nocturnal pain scores)</td>
</tr>
<tr>
<td></td>
<td>3. Function (represented by BASFI)</td>
</tr>
<tr>
<td></td>
<td>4. Inflammation (the average of the BASDAI’s last two VAS concerning morning stiffness, intensity, and duration)</td>
</tr>
<tr>
<td></td>
<td>And an absence of deterioration in the potential remaining domain (deterioration is defined as 20% worsening)</td>
</tr>
<tr>
<td>Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)</td>
<td>A composite index that includes questions on fatigue, axial pain, peripheral pain, stiffness, and comfort, measured on a VAS of 0–10</td>
</tr>
<tr>
<td>Bath Ankylosing Spondylitis Functional Index (BASFI)</td>
<td>A self-assessment instrument consisting of 8 questions regarding function in AS and 2 questions assessing patient’s ability to cope with activities of daily living, recorded on a VAS of 0–10</td>
</tr>
</tbody>
</table>

VAS, visual analog scale.
of two randomized controlled trials that evaluated the efficacy and toxicity of methotrexate for the treatment of AS was published by the Cochrane Database for Systematic Reviews [10]. In these two studies, a total of 81 patients were assessed for outcome measures of pain, function, peripheral arthritis, morning stiffness, patient and physician global assessments, and inflammatory markers. No significant benefits were shown in the methotrexate groups. The authors concluded that these studies did not provide enough evidence to support the use of methotrexate in the treatment of AS.

Gonzalez et al. [11] recently reported on a randomized, double-blind, placebo-controlled trial that assessed the efficacy of low-dose methotrexate in AS patients. Patients with AS based on the modified New York criteria were randomized to receive either methotrexate 7.5 mg/week (n = 17) or placebo (n = 18) for 24 weeks. A response to a prespecified composite index was the primary endpoint. An improvement of 20% or more in at least five of the following measures—(1) severity of morning stiffness, (2) physical wellbeing, (3) disease activity as measured by the BASDAI, (4) functioning as evaluated by the BASFI, (5) Health Assessment Questionnaire, (6) physician global assessment, and (7) patient global assessment—was considered a positive response. In the methotrexate group, 53% of patients had a response by week 24, and this was significantly more than that in the placebo group (11%) (P = 0.01). The proportion of patients who experienced side effects was similar in both groups. The mean score for spinal pain, evaluated by use of the BASDAI, decreased significantly in both the treatment and placebo groups.

This is the first trial to show efficacy for a DMARD in treating axial disease; however, these results should be interpreted with caution. The apparent efficacy of low-dose methotrexate and the relatively rapid response stand in sharp contrast to the results reported in the previous meta-analysis. Furthermore, the inclusion of a nonvalidated outcome measure, the high percentage of patients with peripheral arthritis, and the impressive BASDAI response rate in the placebo group may explain the discrepant results. Certainly, larger trials that use higher doses of methotrexate and incorporate validated outcome measures combined with radiographic endpoints are needed to adequately assess the efficacy of methotrexate in the treatment of AS.

An open label trial of leflunomide in AS patients was recently reported [12]. Twenty patients were given a loading dose of 100 mg of leflunomide for 3 days, followed by 20 mg/day for 6 months. A BASDAI 25%, the primary endpoint, was achieved by 7 of 20 patients, and 5 patients achieved a BASDAI 50 at the end of the study. Of note, this same level of improvement has been reported in patients receiving placebo in previous AS trials [13, 14]. By contrast, a significant improvement was noted in the peripheral arthritis patients. The mean number of swollen joints decreased from 1.7 at baseline to 0.2 at week 24. Only 50% of patients completed the study. Six patients dropped out because of the side effects of medication and the other 4 because of inefficacy or noncompliance. The data from this small open-label did not show that leflunomide is effective for spinal symptoms, but this agent may be an option for treating peripheral joint inflammation in AS patients.

Anti—tumor necrosis factor agents

Previous trials have established the efficacy and safety of etanercept in treating AS over the short term [15, 16]. Calin et al. [17*] reported on a multicenter, double-blind, randomized, placebo-controlled study of etanercept in AS. Eighty-four patients with AS were randomized to receive either etanercept (n = 45) 25 mg twice a week or placebo (n = 39) for 24 weeks. An ASAS 20, the primary endpoint, was achieved by 60% of patients in the etanercept group compared with 23% in the placebo group (P < 0.001 at week 12). In keeping with previous studies, improvement was noted as early as 2 weeks after etanercept was started, and the response was maintained throughout the trial. Significant improvements were also recorded for acute-phase reactants. The treatment was well tolerated, and no patient discontinued the drug for safety reasons. Injection site reactions were significantly more common in the etanercept group. Overall, the safety profile of etanercept was similar to that reported in rheumatoid arthritis (RA) and PsA studies. This European phase III trial confirmed that etanercept is an effective and well-tolerated agent in the short-term treatment of AS.

Phase III trials in the United States and Europe showed that etanercept significantly reduced symptoms and improved function in many AS patients, but continuous treatment is required to sustain a therapeutic response. Brandt et al. [16] earlier reported that 75% of AS patients experienced a relapse at a mean of 6 weeks, and almost all patients experienced relapse 24 weeks after stopping etanercept. Recently, the same authors reported on the results of readministration of etanercept in AS patients who experienced relapse after discontinuation of the drug [18*]. Patients were enrolled in a 6-month trial of etanercept compared with placebo, were observed for 52 weeks while not receiving medication, and were retreated with etanercept if they experienced flare. Twenty-six of 29 patients who completed the controlled trial and experienced flare (defined as a BASDAI score of >4) during the observational period were enrolled into this study. These patients were retreated with etanercept. The primary response was a 50% improvement or better in BASDAI. Nearly 60% of patients achieved the BASDAI 50 at week 54, and 46% achieved this target by week 6. Most patients (83%) were able to completely
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discontinue NSAIDs. Twenty-three patients completed the study, and only one patient dropped out because of an adverse event. This study confirmed that etanercept is efficacious and safe over 12 months and also showed that patients who experience flare while not receiving the drug often respond when they are retreated.

The Ankylosing Spondylitis for the Evaluation of Recombinant Infliximab, a multicenter, randomized, placebo-controlled study that evaluated the efficacy of infliximab in 279 patients, was reported in abstract form at the European League Against Rheumatology (EULAR) meeting in Berlin [19*]. In this large phase III trial, 277 patients were assigned to receive either infliximab 5 mg/kg at 0, 2, and 6 weeks and then every 6 weeks, or placebo. The ASAS 20 response at week 24 was the primary endpoint, and 123 patients (61%) were considered responders in the treatment group compared with 15 of 78 (19%) in the placebo group (P < 0.001). Efficacy was noted as early as 2 weeks after the beginning of therapy and was maintained for the 24-week period. None of 7 infliximab-treated patients who experienced serious adverse events discontinued the study. This is the first phase III trial demonstrating the safety and efficacy of infliximab in AS.

Brandt et al. [20] examined the parameters that would help predict a clinical response to TNF blockade in AS patients. Univariate analysis of two clinical trials conducted earlier by the same authors showed that the likelihood of achieving a major clinical response to etanercept and infliximab in active AS patients was significantly higher in younger patients with shorter disease duration, better functional status, elevated acute-phase reactants, and higher disease activity. By comparison, a study of Dutch rheumatologists found that most physicians based the decision to initiate therapy with TNF blockers on the following factors: rapid functional decline, radiographic progression, a high level of current and previous disease activity, and the presence of peripheral arthritis [21].

Kobelt et al. [22**] assessed the cost effectiveness of AS treatment on the basis of combining data from a large cross-sectional survey in the United Kingdom with data from the first controlled trial of infliximab in this disorder. The outcome and cost-effectiveness of infliximab therapy were estimated for unremitting AS by use of two outcome models. The authors concluded that the cost of treatment with infliximab was partly offset by reductions in the cost of the disease and by improvements in the patients’ quality of life. Unexpectedly, a majority of the costs were related to nonmedical expenses and production losses. The cost per quality-adjusted-life-year (QALY) gained ranged from 30,000 to 40,000 pounds in the long term and potentially less than 10,000 pounds in the long term. To place these numbers in perspective, 30,000 pounds per QALY has been selected as the threshold value for determining whether infliximab is cost effective for treating AS from a societal perspective.

Psoriatic arthritis

Psoriatic arthritis is an inflammatory arthropathy that can involve both the peripheral and the axial skeleton [23]. In general, psoriatic skin disease precedes the arthritis by several years, but occasionally patients experience joint symptoms before they manifest cutaneous features. Similarly to the other SpAs, enthesitis is often a prominent feature, which can lead to pain and physical impairment. Over the past 2 years, the Group for the Assessment of Psoriasis and Psoriatic Arthritis has been working to develop international consensus on domains and instruments for assessing PsA in clinical trials [24*].

Outcome measures

Gladman et al. [25**] completed an extensive review of instruments included in PsA trials, and the measures in widespread use are listed in Table 2. The modified American College of Rheumatology (ACR) response criteria combine assessment of the distal joints of the hands and feet and the first carpal metacarpal joints with the traditional joint count that is performed in RA patients. Another measure of clinical response, the Psoriatic Arthritis Response Criteria (PsARC), has been included as a primary or secondary outcome measure in many PsA trials. Instruments for the assessment of enthesitis and spondylitis are identical to those measures developed for AS but they have not been validated in the PsA population. The Health Assessment Questionnaire was widely used to evaluate health status, physical disability and pain in most PsA trials conducted over the last twelve months. Recently, a PsA quality of life (PsAQoL) instrument with excellent internal consistency, test-retest reliability (0.89), and validity was reported by McKenna et al. [26].

Disease-modifying antirheumatic drugs

Traditionally, NSAIDs are prescribed to treat mild joint disease and DMARDs are reserved for patients with more aggressive PsA [27]. Methotrexate has been the preferred DMARD among many rheumatologists, despite lack of evidence for its efficacy in PsA, from large randomized controlled trials. A systematic review of therapies in PsA concluded that only parenteral high dose methotrexate salazopyrin and possibly azathioprine and etretinate were statistically better than placebo for treatment of PsA [28]. Currently a large randomized controlled trial to assess the efficacy of methotrexate in PsA is being conducted in the United Kingdom.

A study by Kane et al. [29] provided insights into a potential mechanism of action of methotrexate in PsA. The authors analyzed the effects of methotrexate on cellular infiltration, on the extent of vascularity and on cytokine
and metalloproteinase gene expression in the synovium of PsA patients. Ten patients with active PsA, not taking DMARDs, were started on methotrexate (dose increased from 7.5mg/wk to 15mg/wk). Arthroscopic needle biopsy of the knee was done before methotrexate was given and between 6 and 12 months after therapy. A significant improvement was seen in the various measures of disease activity (Ritchie Articular Index, swollen joint count and Disease Activity Score, DAS) after methotrexate treatment (median 11.5 months). All patients had macroscopic and microscopic features of synovial inflammation at baseline arthroscopy. After methotrexate treatment, a significant reduction was noted in the synovial T cell and macrophage infiltrate and proinflammatory cytokine gene expression. Interestingly, methotrexate did not reduce hypervascularity. This last finding is at odds with a previous report which found that methotrexate did inhibit vasculature.

Kaltwasser et al. [32*] reported on the phase III trial of leflunomide (TOPAS) in the treatment of PsA. Patients with active PsA were randomized to receive leflunomide 20 mg/d (n = 98) or placebo (n = 92), for 24 weeks. A total of 58 leflunomide treated patients and 41 placebo treated patients completed the study. During the treatment phase, significantly fewer patients discontinued treatment in the leflunomide group compared with the placebo group (n = 38 vs. n = 51 respectively). The major reason for withdrawal was lack of efficacy. At 24 weeks, 58.9% of the patients on leflunomide were considered responders by the Psoriatic Arthritis Response Criteria (PsARC) compared with 29.7% in the placebo group (p = <0.0001). Also, significantly more patients achieved a modified ACR 20 response in the treatment group (36.3%) than in the placebo group (20%) (P = 0.01). In the treatment group, 30.4% achieved the PASI 50 compared with 18.9% in the placebo group (P = 0.05). Potential drug-related adverse events were noted in 61 patients in the leflunomide group and 37 patients in the placebo group. Notably, a higher incidence of diarrhea, elevated alanine aminotransferase levels, tiredness, and lethargy was observed more commonly in the leflunomide group. Seventeen of 96 patients in the leflunomide arm were

Table 2. Outcome measures in psoriatic arthritis

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Outcome measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Activity Score (DAS)</td>
<td>Calculated using the formula $0.54 \times \text{RAI} + 0.065 \times \text{SJC} + 0.33 \times \ln \text{ESR} + 0.0072 \times \text{GH}$</td>
</tr>
<tr>
<td>Psoriasis Area and Severity Index (PASI)</td>
<td>Area of psoriatic involvement in 4 areas of the body (head, trunk, upper and lower extremities) are graded numerically (0–6) and the severity (degree of erythema, infiltration, and desquamation) is assessed on a scale of 0–4.</td>
</tr>
<tr>
<td>Psoriatic Arthritis Response Criteria (PsARC)</td>
<td>Improvement in at least 2 of following 4 measures, one of which must be joint pain/tenderness or swelling, with no worsening in any of the 4 measures: 1. Patient self assessment: 5-point scale 2. Physician self assessment: 5-point scale 3. Joint pain/tenderness score: 4-point scale 4. Swollen joint score: 4-point scale</td>
</tr>
<tr>
<td>Modified American College of Rheumatology 20 (ACR20)</td>
<td>20% improvement in tender joint count and 20% improvement in swollen joint count (including all DIP and first CMC joints) and 20% improvement in at least 3 of the following: 1. Patient global assessment (10-cm VAS) 2. Physician global assessment (10-cm VAS) 3. Pain assessment (10-cm VAS) 4. Disability (Health Assessment Questionnaire) 5. Acute phase reactant (ESR or CRP)</td>
</tr>
</tbody>
</table>

RAI, Ritchie articular index; SJC, swollen joint score; GH, general health status; VAS, visual analog scale; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DIP, distal interphalangeal joint; CMC, carpal metarpal joint.
reported to have transaminase elevations, and 5 had alanine aminotransferase elevations more than two times the upper limit. These data suggest that although leflunomide may be a convenient and cost-effective alternative to biologic agents in PsA, significant safety concerns remain, particularly in regard to hepatotoxicity.

**Anti—tumor necrosis factor agents**

The phase II trials of etanercept (25 mg subcutaneously twice a week) and infliximab (5 mg/kg body weight every 6 weeks) have demonstrated these agents to be effective therapies in the management of PsA and psoriasis [33,34]. Mease *et al.* [35**] reported the results of a phase III trial of etanercept in the treatment of PsA. In this multicenter, double-blind, placebo-controlled trial, 205 PsA patients with a mean disease duration of 9 years were randomized to receive etanercept 25 mg subcutaneously twice a week (n = 101) or placebo twice a week (n = 104) for 24 weeks. At 24 weeks, all patients were eligible to enroll in a 48-week open-label extension. At 12 weeks, 59% of patients in the etanercept group and 15% of those in the placebo group achieved the ACR 20 (P < 0.0001), the primary endpoint of the study. A PsARC response of 72% and 70% was recorded at weeks 12 and 24, respectively, in the treatment group compared with 31% and 23% in the placebo group. Psoriatic skin disease also improved significantly in the etanercept group. Forty percent of patients in the treatment group and 19% in the placebo group achieved ‘clear’ or ‘minimal’ scores in the dermatologist’s static global assessment of target lesions, and 47% achieved the PASI-50 endpoint at week 24, compared with 18% in the placebo group.

At 12 months, joint damage, assessed by measurement of radiographic disease progression by use of a modified Sharp score, decreased in the etanercept group (−0.03 unit) compared with worsening (+1.00 unit) in the placebo group at 12 months (P = 0.0001). A significant difference in modified Sharp score between the etanercept and placebo groups was noted as early as 6 months after therapy was started. Serious adverse events were similar in both groups; injection site reaction was the only adverse event that was significantly more frequent in the etanercept group. This study confirmed the results of the phase II trial and provided the first evidence that etanercept is safe and effective for the treatment of the joints and skin in PsA over 2 years. This study was also the first to show that TNF blockade can retard radiographic progression in PsA.

A phase III trial of infliximab (5 mg/kg body weight every 6 weeks) in PsA (IMPACT 2) was presented in abstract form at the EULAR meeting, in Berlin [36*]. Two hundred patients with PsA for at least 6 months, with an inadequate response to NSAIDs, were enrolled in this study. At 24 weeks, 54%, 41%, and 27% achieved the ACR 20, 50 and 70 respectively, in contrast to an ACR 20 of 16% for placebo. The percentage of patients who reached the PASI 75 at week 14 was 63.9% and 2.3% in the infliximab and placebo groups respectively.

Recent studies have investigated the early effects of infliximab treatment on skin and synovial tissue of PsA patients. Goedkoop *et al.* [37] demonstrated a significant reduction in the number of T cells and macrophages in the synovium of PsA patients 48 hours after infliximab infusion. A significant decrease in the T cell numbers was also noted in the skin of these patients. Interestingly, the number of apoptotic cells in the skin and synovium was unaltered with infliximab therapy. This is similar to the results of synovial biopsy in RA patients receiving infliximab but contrasts with the findings in Crohn disease, wherein an increase in the number of apoptotic cells in the lamina propria of the gut has been reported after infliximab therapy [38*].

The same authors also reported on the effect of infliximab on angiogenesis in psoriatic skin and synovium. In a 24-week, single-center, prospective, open-label trial, patients receiving stable doses of methotrexate were given infusions of infliximab (3 mg/kg) at baseline and at weeks 2, 6, 14, and 22 [39*]. Skin and synovial biopsies were performed at baseline and 4 weeks after the initiation of infliximab therapy. A decrease in vascularity and neo-angiogenesis was noted in both the synovium and the skin after 4 weeks of therapy with infliximab. Interestingly, expression of the adhesion molecules (E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1) was reduced in both the skin and the synovium. Given that these molecules facilitate the migration of inflammatory cells to inflamed areas, these observations provide a mechanism to explain how TNF blockade reduces inflammatory cell infiltration and new vessel formation.

Canete *et al.* [40] also reported on the anti-angiogenic effects of infliximab therapy in the synovium of PsA patients. Nine patients with PsA were treated with infliximab (5 mg/kg) at baseline and at weeks 2 and 6. Synovial biopsies were performed at baseline and week 8. Immunohistochemical analysis was performed by staining the biopsy specimens with antibodies to vascular endothelial growth factor (VEGF), VEGF-receptor-2, and stromal cell–derived factor-1. At week 8, all patients had a significant improvement in clinical parameters and acute phase reactants, compared with baseline. A marked reduction in the vascular area was noted, and the expression of VEGF, VEGF-receptor-2, and stromal cell–derived factor-1 declined significantly after therapy. Furthermore, a significant reduction in mean scores for synovial infiltration was reported. These data show that infliximab can suppress angiogenesis in inflamed synovium and suggest that the reduction may be modulated by key growth factors that orchestrate new vessel formation.
A phase 3 study of adalimumab in PsA was presented at the ACR meeting in October 2004 [41*]. At week 24, 57% of patients receiving adalimumab achieved the ACR 20, in contrast to 15% of those receiving placebo, 39% achieved the ACR 50 in contrast to 6% of those receiving placebo, and 23% achieved the ACR 70 in contrast to 1% of those receiving placebo. The results were consistent with those observed in the etanercept and infliximab trials. With respect to the PASI scores, among patients with psoriasis that affected at least 3% of body surface area, adalimumab treatment led to PASI 50, PASI 75, and PASI 90 responses in 75%, 59%, and 42% of the patients receiving adalimumab compared with 3%, 1%, and 0% of patients receiving placebo, respectively. A PASI 90 is nearly equivalent to freedom from all skin lesions. The drug was well tolerated in this short-term trial.

Undifferentiated spondyloarthropathy

The concept of undifferentiated SpA emerged with the use of the European Spondyloarthropathy Study Classification criteria. Although undifferentiated SpA is second only to AS in prevalence among the SpAs, therapy of this condition has not been analyzed in a large randomized trial.

Brandt et al. [42*] reported on an open-label trial of etanercept in undifferentiated SpA. Ten patients with severe undifferentiated SpA, with mean disease duration of 5.9 years, were treated with 25 mg of etanercept twice a week for 12 weeks. Of the 9 patients who completed the trial, the primary endpoint (BASDAI 50) was achieved by 60%. Six of these patients showed substantial improvement as early as 1 week after the first injection. Significant improvements were also noted in the BASFI and the mean pain values. After cessation of treatment, 4 of 8 patients experienced relapse within 3 months, and 3 patients experienced relapse at later time points. The results of this study, taken together with those from a positive trial of infliximab in undifferentiated SpA, suggest that anti-TNF therapy may be beneficial for patients with active undifferentiated SpA, but larger randomized controlled trials are needed to confirm these preliminary results [43].

Reactive arthritis

Reactive arthritis is a form of inflammatory arthritis that follows certain infections, mainly of the genitourinary or gastrointestinal tract [44]. Bacterial antigens and DNA/RNA have been detected in the joints of patients with reactive arthritis many months after the initial infection, suggesting the possibility of a persistent infection eliciting an immune reaction. On the basis of the concept of persistent infection, antibiotics have been prescribed in an attempt to eradicate the organism and lessen joint inflammation.

Most trials to date have not demonstrated that antibiotic therapy is effective for reactive arthritis except for patients with Chlamydia infection [45]. Kvein et al. [46] conducted a 6-month, double-blind, placebo-controlled study of 152 patients with reactive arthritis. Patients with a diagnosis of reactive arthritis who had an acute inflammatory arthritis of less than 2 months duration and as many as three single swollen joints were enrolled in the study. The patients were given 1 g of oral azithromycin weekly for 12 weeks or placebo. NSAIDs were allowed as needed. The primary efficacy measures were physician and patient assessment of disease activity, number of tender and swollen joints, and time to resolution of arthritis. Improvements were recorded in both groups, and no statistically significant difference was noted between the two groups, although mild adverse events were more frequent in the treatment group. This large trial of patients from 12 countries suggests that reactive arthritis is not mediated by persistent infection or, alternatively, that organisms associated with reactive arthritis have low susceptibility to antibiotics commonly used to treat acute chlamydial infections.

The latter theory is supported by the results of a study by Carter et al. [47*] that compared the use of doxycycline alone against doxycycline and rifampin in patients with a diagnosis of undifferentiated SpA, although 30% of patients had documented immediately preceding infections with C. pneumoniae or C. trachomatis. Regrettably, definitive proof of infection was absent. The authors reported a significant improvement in the amount of back pain as assessed by a visual analog scale, the primary endpoint, in the doxycycline and rifampin groups compared with the doxycycline group. A significant difference was also noted for the improvement in duration of morning stiffness and swollen and tender joint scores in the patients receiving combination therapy, in contrast to those receiving monotherapy. Overall, 11 of 15 patients in the combined group were considered responders, in contrast to only 2 in the doxycycline group ($P < 0.003$). This open-label study suggests that traditional antimicrobials that are thought to be effective in treating acute Chlamydia infections may not be effective for the treatment of chronic Chlamydia infections. Moreover, the resistance to conventional antimicrobial therapy may be overcome by the addition of rifampin. Of particular importance, this is the first study to demonstrate that prolonged combination antimicrobial treatment is beneficial in undifferentiated SpA, and these data lend support to the concept that chronic infection can lead to sustained axial inflammation.

New therapeutic targets

The impressive results observed with anti-TNF agents in SpA have created additional momentum to the development of novel biologic agents for the treatment of inflammatory arthritis. Several compounds with therapeutic potential in the SpA cluster of diseases are listed in Table 3. T lymphocytes are an attractive target based on the
Table 3. Potential therapeutic agents for the treatment of spondylarthropathy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Disease</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td>Anakinra</td>
<td>IL-1</td>
<td>AS</td>
<td>Open label trials</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>IL-12</td>
<td>PsA</td>
<td>Open label trial</td>
</tr>
<tr>
<td>Anti-IL-12</td>
<td>IL-12</td>
<td>PsA</td>
<td>Future?</td>
</tr>
<tr>
<td>Rituximab (anti-CD20)</td>
<td>B lymphocyte</td>
<td>PsA</td>
<td>Planned</td>
</tr>
<tr>
<td>Abatacept CTLA4-Ig</td>
<td>T lymphocyte</td>
<td>PsA</td>
<td>Future?</td>
</tr>
<tr>
<td>Alefacept LFA3Ig</td>
<td>T lymphocyte</td>
<td>AS</td>
<td>Future?</td>
</tr>
<tr>
<td>AMG-162 Anti-RANKL antibody</td>
<td>RANKL</td>
<td>PsA</td>
<td>Phase IIb trial in progress</td>
</tr>
</tbody>
</table>

strong class I MHC associations with both AS and PsA [48]. In particular, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) provides a pivotal costimulatory signal in T-cell activation. CTLA-4-Ig (abatacept) attenuates CD80/CD86–CD28 costimulation of T lymphocytes and has proved effective in psoriasis and RA [49,50]. Another agent that targets T lymphocytes, human lymphocyte function-associated antigen 3/immunoglobulin 1 fusion protein (alefacept), binds to CD2 molecules on the surface of activated T cells, selectively targeting memory-effector (CD45RO+) T cells [51]. Phase II trials are currently in progress in PsA. The contribution of B lymphocytes to the pathobiology of PsA and AS has not been established, but a trial designed to test the efficacy of rituximab (anti-CD19) in PsA is planned. The Th1 cytokines interleukin (IL)-12 and IL-23, which share a p40 subunit, are compelling molecules because of their ability to induce a Th1 response and activate T lymphocytes [52]. A study designed to evaluate the efficacy of anti–IL-12 antibody in psoriasis is under way. The prominent bone resorption characteristic of aggressive PsA (arthritis mutilans) may respond to anti-Receptor Activator of NF-κB ligand (RANKL) antibody, a molecule that dramatically inhibits osteoclastogenesis [53]. Finally, molecules that target angiogenic factors may be particularly effective in PsA, given the extensive degree of new vessel formation in the psoriatic synovial membrane [54].

Interleukin-10 is a known inhibitor of TNF-α and other pro-inflammatory cytokines [55]. Levels of IL-10 are low in psoriatic plaques, although levels in the joints can be quite elevated [56]. In-vitro studies [57] have shown that delipidated, deglycolipidated, heat-killed Mycobacterium vaccae (PVAC) increased IL-10 levels by stimulating dendritic cell activity. Two small studies [58,59] have shown that PVAC therapy improved plaque inflammation in psoriasis. Dalbeth et al. [60] conducted a 24-week double-blind, placebo-controlled, randomized study to assess the safety and efficacy of this agent in PsA. No significant difference was noted in the primary (PsARC scores) or secondary endpoints (ACR 20 and PASI) between the treatment and placebo groups, suggesting that immunotherapy, in the form of a mycobacterial vaccine, is not effective in the treatment of PsA.

Conclusion

Over the past year, therapeutic advances in the SpAs have provided additional treatment options for patients with these disorders. For example, traditional DMARDs such as methotrexate, which was thought not to be helpful in AS, may have a therapeutic role in this disease, although additional controlled trials are required. Likewise, the utility of methotrexate in PsA has not been established in a clinical trial, but mechanistic studies showed that it lessened psoriatic synovitis. Leflunomide also suppressed joint inflammation in PsA, although hepatotoxicity was a concern. The phase III trials of anti-TNF agents demonstrated sustained efficacy with relatively little toxicity in PsA, and both etanercept and infliximab slowed radiographic progression. The beneficial effects of TNF-blocking agents on signs and symptoms has been confirmed in all AS trials, but the ability of this class of drugs to slow disease progression remains to be determined; however, well-designed studies that incorporate longitudinal MRI outcome measures will undoubtedly provide answers to this important question in the near future. Finally, the success of combination antibiotics for treatment of undifferentiated SpA has rekindled interest in the association between chronic infection and axial disease with the hope that antibacterial therapy may yet have a role in the treatment of this poorly understood group of disorders.

Despite the encouraging news from these trials, we must not forget that approximately 50% of AS patients do not achieve the BASDAI 50, and 40% of PsA patients do not reach the modified ACR 20 endpoint. From a clinical perspective, failure to attain these important endpoints underscores the unmet need for new therapies to treat spondyloarthritis. Furthermore, TNF blockade suppresses disease activity, but continuous therapy is required to maintain a response with these agents. Thus, although anti-TNF agents have been remarkably effective in many patients, their cost-benefit ratio over the long term has not been established. In the meantime, the continued emergence of new therapeutic targets, fueled by a rapidly expanding knowledge of the innate and acquired immune response, will provide even more treatment choices for SpA patients.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


First European controlled trial of etanercept in AS.


This trial demonstrated that continuous therapy with etanercept is required to control disease activity in AS.


Phase III trial of infliximab in AS. Abstract was presented at the 2004 EULAR meeting.


Carter J, Valeriano J, Vasey F. Doxycycline versus doxycycline plus rifampicin in undifferentiated spondyloarthritis, with special reference to chlamydia-induced arthritis. J Rheumatol 2004; 31:1973–1980. This was the first study to show that treatment of undifferentiated SpA with a combination of antibiotics can relieve musculoskeletal signs and symptoms.


Randomized controlled trials in vasculitis associated with anti–neutrophil cytoplasmic antibodies
Oemer N. Goeka and John H. Stoneb

Purpose of review
To review three major randomized clinical trials in forms of vasculitis associated with anti–neutrophil cytoplasmic antibodies.

Recent findings
The design features, results, and context of the Cyclophosphamide versus Azathioprine for the Remission Phase of Vasculitis, Non-renal AAV alternatively treated with Methotrexate, and Wegener Granulomatosis Etanercept Trial are reviewed.

Summary
Until recently, therapies for Wegener granulomatosis and microscopic polyangiitis have been based primarily on a relatively small number of nonrandomized studies. During the past 18 months, three randomized, controlled trials (one double-blinded) have been performed in vasculitis associated with anti–neutrophil cytoplasmic antibodies. Careful comparisons of the results of these trials yield insights into new standards of care for vasculitis associated with anti–neutrophil cytoplasmic antibodies. This paper summarizes the designs of these three trials; highlights their principal conclusions, strengths, and shortcomings; and distills from their results several recommendations on major questions related to the therapy of vasculitis associated with anti–neutrophil cytoplasmic antibodies.

Keywords
anti–neutrophil cytoplasmic antibodies, azathioprine, cyclophosphamide, etanercept, vasculitis

Introduction
Wegener granulomatosis (WG) and microscopic polyangiitis (MPA) are the most common forms of vasculitis associated with anti–neutrophil cytoplasmic antibodies (AAV). Until now, therapy for these diseases has been based primarily on a relatively small number of prospective but nonrandomized studies, conducted primarily in WG. These studies, which have defined the standard of care, demonstrated that AAV patients may achieve disease remission through regimens consisting of glucocorticoids plus either cyclophosphamide (CYC) or methotrexate (MTX), depending on their level of disease severity.

During the past year, the results of three prospective, randomized, multicenter trials were published [1–3]. CYCAZAREM and NORAM were both undertaken by the European Union Vasculitis Study Group. The third trial, WGET, was conducted by American centers within the International Network for the Study of the Systemic Vasculitides. These trials examined the effects of different treatment regimens in several clinical trial settings. Thorough comparisons of the results of these trials provide insights into new standards of care for AAV.

In this review, we compare CYCAZAREM, NORAM, and WGET in light of their different trial designs (Tables 1 and 2). We outline their occasionally contrasting conclusions (Table 3) and consider potential reasons for the disparate results. To put their conclusions into a broader perspective, we also discuss several clinically relevant questions, the answers to which are informed by these trials' results.

Cyclophosphamide versus Azathioprine for the Remission Phase of Vasculitis
In the CYCAZAREM trial, it was hypothesized that azathioprine (AZA) is equivalent to CYC as an agent for the maintenance of disease remission in patients with WG or MPA.

Patient population
One hundred forty-four patients were randomized, 95 of whom (61%) had clinical diagnoses of WG [4]. The remaining 60 (39%) had clinical diagnoses of MPA.

Trial-specific definitions
‘Generalized’ vasculitis: AAV manifestations that threatened vital organ function. Such manifestations included glomerulonephritis, alveolar hemorrhage, vasculitic neuropathy, and similar complications.
Remission: The Birmingham Vasculitis Activity Score (BVAS) was the basis for the definition of disease remission [5]. In CYCAZAREM, patients who did not have persistent disease activity in more than one BVAS item were defined to be in remission.

Minor relapse: Recurrence of at least three BVAS items. Consequently, a patient could have two minor items present (e.g., arthritis plus palpable purpura) and still not be considered to have a relapse.

Major relapse: Recurrence or first appearance of at least one item on the BVAS that was indicative of a threat to the function of a vital organ.

Adverse events grading: According to predefined criteria as mild, moderate, severe, or life threatening.

Primary outcome
Relapse rate after achievement of remission in both treatment arms.

Design overview
CYCAZAREM, a prospective, randomized, multicenter trial that was not blinded, enrolled patients with new diagnoses of WG or MPA. The remission induction regimen consisted of daily CYC (2 mg/kg/day) and prednisolone (starting at 1 mg/kg/day) for at least 3 months (5 mg prednisolone = 4 mg prednisone). The design of CYCAZAREM is summarized in Figure 1.

Patients who achieved disease remission were randomized to either continuing CYC at a lower dosage (1.5 mg/kg/day) or beginning AZA at 2 mg/kg/day for a total of 12 months. After randomization, prednisolone was continued at a fixed dosage of 10 mg/day for the entire 12-month period. Twelve months after randomization, all patients were switched to AZA 1.5 mg/kg/day and a fixed dosage of 7.5 mg of prednisolone per day. The overall length of follow-up was 18 months.

Major results
Remission induction was achieved in 144 of the 155 patients (93%). These 144 patients were randomized. The relapse rate at 18 months was not significantly different in the two treatment arms: 16% of the patients in the AZA group experienced relapse, compared with 14% of those in the CYC group ($P = 0.65$). The number of severe or life-threatening events during the remission induction

Table 1. Summary of designs of the CYCAZAREM, NORAM, and WGET trials

<table>
<thead>
<tr>
<th>Trial name</th>
<th>CYCAZAREM</th>
<th>NORAM</th>
<th>WGET</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. randomized</td>
<td>144</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>Trial design</td>
<td>Randomized</td>
<td>Unblinded</td>
<td>Randomized</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td>WG + MPA</td>
<td>WG + MPA</td>
<td>WG only</td>
</tr>
<tr>
<td>ANCA status</td>
<td>AZA group</td>
<td>ANCA positive by either IF or ELISA</td>
<td>Etanercept group</td>
</tr>
<tr>
<td>CYC group</td>
<td>54% PR3-ANCA +</td>
<td>71% PR3-ANCA +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39% MPO-ANCA +</td>
<td>17% MPO-ANCA +</td>
<td></td>
</tr>
<tr>
<td>Disease severity</td>
<td>Severe (generalized)</td>
<td>Limited (early systemic AAV) with serum creatinine &lt; 150 µmol/L</td>
<td>71% Severe</td>
</tr>
<tr>
<td>Onset of disease</td>
<td>Newly diagnosed (no cytotoxic medications in previous year)</td>
<td>Newly diagnosed</td>
<td>44% newly diagnosed</td>
</tr>
<tr>
<td>Duration of follow-up</td>
<td>18 months (anniversary closeout)</td>
<td>18 months (anniversary closeout)</td>
<td>Mean of 27 months (common closeout date)</td>
</tr>
<tr>
<td>Definition of remission induction</td>
<td>BVAS = 0 or 1 persistent symptom</td>
<td>BVAS = 0 or 1 persistent symptom</td>
<td>BVAS/WG = 0</td>
</tr>
<tr>
<td>Definition of relapse</td>
<td>Major: 1 point BVAS incr. and vital organ involved</td>
<td>Major: vasculitis activity, threatening vital organ function</td>
<td>BVAS/WG increase of 1 point</td>
</tr>
<tr>
<td>Minor: 3 point BVAS incr. and no vital organ involved</td>
<td>Minor: disease activity requiring increased prednisolone dosage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary endpoint</td>
<td>Relapse rate at 18 months</td>
<td>Remission rate at 6 months</td>
<td>Sustained remission: BVAS/WG = 0 for at least 6 months</td>
</tr>
</tbody>
</table>

ANCA, anti-neutrophil cytoplasmic antibodies; IF, immunofluorescence; ELISA, enzyme-linked immunosorbent assay; CYC, cyclophosphamide; MTX, methotrexate; AZA, azathioprine; BVAS, Birmingham Vasculitis Activity Score; BVAS/WG, Birmingham Vasculitis Activity Score for Wegner’s granulomatosis.
phase was 10%. During the remission maintenance phase, the occurrence of severe or life-threatening adverse events did not differ between the CYC-treated and AZA-treated groups: 10% compared with 11%, respectively ($P = 0.94$). Thus, during the combined remission induction and maintenance phases, approximately 20% of patients in both groups experienced severe or life-threatening adverse events.

**Conclusions**

After a 3- to 6-month induction period with glucocorticoids and CYC, AZA plus a maintenance dosage of glucocorticoids is as effective as CYC plus maintenance glucocorticoids in sustaining disease remission in AAV. The duration of exposure to CYC at the initiation of treatment for AAV may therefore be reduced safely.
Non-renal AAV alternatively treated with Methotrexate

In the NORAM trial, it was hypothesized that MTX is as efficacious as CYC in both the induction and maintenance of disease remission for patients with non-renal AAV.

Patient population
Eighty-nine patients with WG and 6 with MPA, all of whom had newly diagnosed ‘early generalized disease’ (see below), were treated as randomized. Five patients, 2 in the MTX group and 3 in the CYC group, were withdrawn before receiving treatment.

Trial-specific definitions

*Early systemic AAV:* Manifestations of vasculitis that do not threaten vital organ function. Patients with serum creatinine levels greater than 150 μmol/l (1.7 mg/dl) or urinary red blood cell casts were excluded. Other exclusions were severe hemoptysis (associated with bilateral pulmonary infiltrates), cerebral infarction due to vasculitis, orbital pseudotumor, and rapidly progressive neuropathy.

*Remission:* Absence of clinical disease activity as defined by the absence of new or worse clinical disease activity. As a disease activity measure, the investigators used a modification of the original BVAS [5]. Persistent disease activity in one item was considered compatible with remission, provided that the BVAS was 2 or less.

*Minor relapse:* Relapses were defined on clinical grounds in NORAM. A minor relapse was the recurrence of any disease activity without organ- or life-threatening manifestations that warranted an increase of the prednisolone dosage.

*Major relapse:* Recurrence or first appearance of vasculitis activity, threatening vital organ function.

*Disease extent index:* Extent of disease was evaluated with the Disease Extent Index [6].

Primary outcome
Remission rate at 6 months of treatment.

Important secondary outcome
Relapse rate after 18 months of treatment.

Design overview
The NORAM was a prospective, randomized, unblended clinical trial that involved 26 centers in 10 European countries. Ninety-five patients with newly diagnosed limited AAV were treated with glucocorticoids plus either MTX or CYC for remission induction. A schematic of the NORAM trial is shown in Figure 2. All patients received 1 mg/kg/day prednisolone, tapered to 15 mg/day by 3 months, 7.5 mg/day by 6 months, and 0 by 12 months. In the CYC treatment arm, the dosage of CYC was 2 mg/kg/day for 3 to 6 months, decreased by 25 mg/day for patients older than 60 years. After remission induction, CYC was reduced to 1.5 mg/kg/day until month 10, after which it was tapered off within 2 months. Patients randomized to MTX began with an initial dosage of 15 mg/week, increased over 12 weeks to 25 mg/week. This MTX dosage was maintained until month 10, whereupon a rapid taper led to cessation by the end of month 12. As in CYCAZAREM, patients were followed up for 18 months after randomization.
Major results
The percentages of patients achieving remission at 6 months were not significantly different: 44 of the 49 patients in the MTX group (90%) and 43 of the 46 in the CYC group (94%) achieved this measure ($P = 0.78$). Among the patients who achieved remission, however, 32 of the 46 in the MTX group (70%) experienced relapse during the trial, compared with 20 of 43 patients (47%) in the CYC group ($P < 0.05$). The only differences in adverse events between the two groups included a higher incidence of leukopenia among those treated with CYC, and a higher incidence of liver function test abnormalities among those treated with MTX. No Pneumocystis infections were reported, even though the protocol did not require prophylaxis against such events.

Conclusions
Methotrexate is as effective as CYC for the induction of remission within 6 months of treatment, but more relapses occurred in MTX than in CYC treatment after remission induction.

Wegener Granulomatosis Etanercept Trial
In the WGET trial, it was hypothesized that etanercept, a soluble TNF receptor fusion protein, is a safe, effective medication for the maintenance of remission in WG.

Patient population
One hundred eighty patients with WG, including 128 (71%) with severe disease and 52 (29%) with limited WG, were enrolled [3,7]. In contrast to the CYCAZAREM and NORAM studies, which enrolled only newly diagnosed patients, 56% of the patients in WGET were enrolled during relapses that followed periods of quiescent disease, and only 44% of the patients had newly diagnosed disease.

Definitions
Severe WG: Disease that poses an immediate threat to either the patient’s life or vital organ function.
Limited disease: Manifestations of WG that do not pose threats to either the patient’s life or vital organ function at randomization.
Remission: Birmingham Vasculitis Activity Score for Wegener granulomatosis (BVAS/WG) of 0 [8]. BVAS/WG, adapted from the original BVAS, was validated specifically for use in WG before WGET began [5].
Sustained remission: BVAS/WG of 0 for more than 6 months.
Relapse: increase of one or more items on the BVAS/WG.

Primary outcome
The percentages of patients in each group who achieved sustained remission.

Design overview
In contrast to CYCAZAREM and NORAM, WGET was a randomized, double-blind, placebo-controlled trial. The trial schema is shown in Figure 3. In addition to standard immunosuppressive regimens (outlined below), 180 WG patients were randomized to receive either 25 mg of etanercept or placebo twice weekly. Follow-up for all patients continued until a common closeout date, 1 year after the final patient had been randomized. The mean follow-up time was 27 months.
All patients were treated with a standard remission induction regimen of prednisone (1 mg/kg/day), tapered off completely within 6 months. Patients with severe disease could receive methylprednisolone (1 g/day for 3 days) at the discretion of the investigator. The standard immunosuppressive agents were either CYC (2 mg/kg/day) for severe disease or MTX (0.25 mg/kg/wk), increased to 25 mg/wk over only 2 weeks, for patients with limited disease. All patients with severe disease received at least 3 months of treatment with CYC. After 3 to 6 months, patients who had achieved remission were switched to MTX. Patients with impaired renal function, signified by a serum creatinine higher than 2.0 mg/dl, received AZA (2 mg/kg/day) in lieu of methotrexate. Twelve months after remission induction and treatment with MTX or AZA, these medications were tapered by 2.5 mg or 25 mg per month, respectively.

**Major results**
The percentages of patients who achieved sustained remission were not different: 70% in the etanercept group and 75% in the control group ($P = 0.39$). In the WGET cohort overall, only 49% of patients achieved disease remissions and maintained them throughout the trial. There were no between-group differences in sustained periods of low disease activity (BVAS/WG ≤2) (87% compared with 91%). Disease flares were common in both groups, with a total of 118 flares in the etanercept group (23 severe, 95 limited) and 134 in the control group (25 severe, 109 limited) (relative risk of disease flare/100 person-years: 0.89; $P = 0.54$). Overall, 50% of the patients experienced at least one severe or life-threatening adverse event or died ($n = 6$). The overall numbers of adverse events in the two groups were not different, but solid malignancies developed in 6 etanercept-treated patients (in contrast to 0 control individuals) ($P = 0.01$).

**Conclusions**
Etanercept is not superior to placebo for the maintenance of disease remission. Although only etanercept was studied in WGET, the results of this trial cast significant doubts about the effectiveness of anti-TNF approaches in WG in general. The findings emphasize that a therapy efficacious in one inflammatory disease, such as rheumatoid arthritis, may not always be extrapolated to others. Disease remissions, achieved at least temporarily in the great majority of patients, came at a high cost of treatment-induced morbidity and were followed eventually by flares in many cases. Much of the treatment-related morbidity seemed to be related to conventional therapy rather than to etanercept. The combination of etanercept and CYC, however, may be associated with an increased risk of solid malignancies; this potential association requires further study.

**Discussion**
Below we discuss several questions, which are pertinent to the treatment of AAV.

What accounts for the differing percentages of patients who experienced disease flares in the three trials?
The percentages of patients who experienced disease flares in these three trials were strikingly different: fewer
than 15% of all patients in CYCAZAREM experienced relapses, contrasted with more than 30% in both NORAM and WGET. Understanding the probable reasons for these differences may offer insights into strategies for remission maintenance. Several factors may account for the differences observed in these trials.

**Longer glucocorticoid use in CYCAZAREM**

Among the three trials, both the shortest duration of glucocorticoid treatment (6 months) and the highest percentage of patients experiencing disease relapses were observed in WGET. In NORAM, the period of glucocorticoid use (off prednisolone by 12 months) was intermediate in length to the other trials, but the percentage of patients experiencing disease flares was closer to the percentage in WGET. In CYCAZAREM, after initially high dosages of prednisolone that were comparable to those used in the other two trials, patients did not taper off glucocorticoids entirely. Rather, they received maintenance dosages of prednisolone not less than 7.5 mg/day throughout the 18-month observation period. The CYCAZAREM investigators observed a correspondingly much lower flare rate compared with WGET and NORAM. The prolonged use of glucocorticoids in CYCAZAREM is probably the greatest reason that fewer disease flares were observed in that trial.

**Definitions of disease flare**

The CYCAZAREM also had a significantly higher threshold for classifying a clinical event as a disease relapse. In CYCAZAREM, a ‘relapse’ required a minimum of three BVAS items. Thus, many minor relapses were probably not counted in CYCAZAREM. This is probably the second most important contributor to the lower rate of disease flares reported in that trial. By comparison, only one new or recurrent BVAS/WG item constituted a relapse in WGET. Similarly, any increase in clinical activity leading to an increase in glucocorticoid use was considered a relapse in NORAM.

**Distributions of Wegener granulomatosis and microscopic polyangiitis among the trials**

The WGET enrolled WG patients only. By contrast, 39% of the patients in CYCAZAREM and 6% of those in NORAM had MPA. Because MPA is believed to have a lower relapse rate compared with WG, the different trial populations likely contributed to the comparatively low relapse rate of CYCAZAREM.

**Percentages of patients with ‘limited’ rather than ‘severe’ disease**

Finally, the trials differed substantially with regard to disease severity among enrolled patients. CYCAZAREM, for example, enrolled only patients whose disease was classified as ‘generalized’ (approximately equivalent to the ‘severe’ term used in WGET). By contrast, 30% of the patients in WGET had ‘limited’ disease, and the conventional treatment regimen was different for this subset of patients. NORAM also enrolled many patients who would have fit the ‘limited’ definition used by the WGET investigators. Differential outcomes in severe and limited disease, with limited WG patients being perhaps less likely to achieve sustained remissions, may also partly explain the lower flare rate in CYCAZAREM.

**What is the optimal approach to remission induction in early generalized disease? In limited disease?**

The NORAM was designed to answer this question specifically by the head-to-head comparison of CYC with MTX in patients with early generalized AAV. The remission rates at 6 months after enrollment were not different between the two groups. Given the lower long-term toxicity risks of MTX, MTX plus glucocorticoids is a rational choice as the remission induction regimen for early generalized AAV. The spectra of disease signified by the terms ‘limited’ and ‘early generalized’ do not overlap entirely, but previous case series (uncontrolled studies) have also indicated that the same is true for limited WG. In WGET, 88% of the patients with limited disease (in the etanercept and comparison groups combined) achieved at least a transient disease remission: a BVAS/WG of 0. As both NORAM and WGET demonstrate, the principal challenge in these diseases is not how to induce remission, but rather how to maintain it once the glucocorticoids are tapered. In NORAM and WGET, well over half of the patients with early generalized or limited disease experienced relapse after the achievement of remission.

**How long should cyclophosphamide be maintained for patients with severe or generalized disease?**

The results of CYCAZAREM refute the notion that longer use of CYC equates to better disease control. A 12-month course of CYC was no more effective than 3 months of CYC followed by AZA in leading to sustained disease remission. The long-term reduction in toxicity resulting from shorter duration of CYC treatment is not trivial. The results of CYCAZAREM therefore retire the concept – still prevalent among many practitioners – that the use of CYC should continue until patients have been in remission for 1 full year. For most patients with severe or generalized disease, replacement of CYC with either AZA or MTX is reasonable (albeit MTX has not been tested in a randomized, controlled trial). Before instituting AZA, clinicians should ensure that patients have a full complement of thiopurine methyltransferase alleles and can therefore metabolize the medication. MTX must be used with extreme caution in patients with significant renal dysfunction (e.g., serum creatinine >2 mg/dl).
How long should remission maintenance therapy in vasculitis associated with anti-neutrophil cytoplasmic antibodies be continued?

On this question, the three trials do not provide clear guidance; none was designed to address it. These trials and other clinical experience indicate, however, that prolonged states of disease quiescence result in some patients treated with high-dose glucocorticoids and 3 to 6 months of CYC who then undergo a year or so of maintenance therapy with AZA or MTX. In the absence of any symptoms or signs of active disease for many months, it is reasonable to consider discontinuing AZA or MTX, particularly if these medications are tolerated poorly. (Some evidence indicates that ANCA negativity at this time point – 15 to 18 months after the start of treatment – provides encouragement but no guarantee that the patient will do well after treatment ceases.) Conversely, for patients whose disease has demonstrated a propensity for recurrence, continuing patients with a remission maintenance agent may be reasonable, provided that the medication is well tolerated. Given the importance (evident in the contrasting results of these three trials) of low-dose glucocorticoids in maintaining disease remission in many cases, some patients may benefit from the continued use of this therapy as well, alone or in addition to AZA or MTX, until new approaches to the treatment of AAV are available.

Conclusions

The great majority of patients with AAV achieve at least temporary disease control with currently available immunosuppressive therapy.

Even the shorter courses of CYC now used for the induction of remission are associated with substantial treatment-induced morbidity.

Disease relapses are common, whichever regimen is used for remission induction. Discontinuation of glucocorticoids seems to correlate well with disease relapses in many patients.

In the remission maintenance phase, AZA or MTX can generally be used safely as substitutes for the more toxic CYC.

For remission induction in patients with ‘limited’ or ‘early generalized’ WG or MPA, MTX is similar in its efficacy to CYC. Ultimately, however, disease relapses are more common among MTX-treated patients.

Etanercept is not effective for the maintenance of remission in patients with WG. Further investigation of the potential association among TNF inhibition, CYC, and the induction of malignancy is appropriate.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

** of outstanding interest


Pediatric clinical research
Daniel J. Lovell and Natasha M. Ruth

Purpose of review
This review will focus on childhood-onset systemic lupus erythematosus, juvenile idiopathic arthritis, and juvenile dermatomyositis, with special interest on strategies to improve the health-related quality of life in these conditions.

Recent findings
The contribution of plasma insulin levels, lipoproteins, markers of oxidized state (including nitric oxide metabolites, isoprostanes) and autoantibodies to oxidized low-density lipoprotein to risk for atherosclerosis has been studied in childhood-onset systemic lupus erythematosus. Elevated serum levels of myeloid-related protein-8 (also called S100A8) and myeloid-related protein-14 (S100A9) in children with juvenile idiopathic arthritis can indicate clinically occult disease activity. Serum levels of S100A12 correlate with disease activity in juvenile idiopathic arthritis. Magnetic resonance imaging T2 relaxation times in weight-bearing cartilage in patients with juvenile idiopathic arthritis may help with early detection of cartilage changes. Quantitative computed tomography commonly shows decreased muscle mass and abnormal bone geometry in juvenile idiopathic arthritis patients. In patients with juvenile idiopathic arthritis who do not respond to oral methotrexate, subcutaneous methotrexate dosing was frequently successful. Duration of inactive disease while a patient is receiving methotrexate does not decrease the frequency of flaring of disease once methotrexate is discontinued. Residual synovial inflammation seems to be a stronger influence on the rate of relapse. In juvenile dermatomyositis, the quantitative magnetic resonance imaging T2 relaxation time and overexpression of Class I major histocompatibility complex in early juvenile dermatomyositis are reported. Intravenous cyclophosphamide in refractory juvenile dermatomyositis and tacrolimus ointment for the dermatologic manifestations of juvenile dermatomyositis seem promising.

Summary
Progress has been made in the diagnosis and treatment of childhood-onset systemic lupus erythematosus, juvenile idiopathic arthritis, and juvenile dermatomyositis.

Keywords
juvenile dermatomyositis, juvenile idiopathic arthritis, juvenile rheumatoid arthritis, pediatrics, systemic lupus erythematosus

Introduction
In the realm of childhood-onset systemic lupus erythematosus (cSLE), clinical research over the past year has focused on investigations describing abnormalities that may contribute to accelerated atherosclerosis. Myocardial infarction and cerebrovascular events are increasingly being recognized as serious problems in this patient group. Studies have also focused on quality of life in these patients. New advances have also been seen in juvenile idiopathic arthritis (JIA). The revision of diagnostic criteria as well as diagnostic procedures including serum levels of myeloid-related protein (MRP)-8 (S100A8) and MRP14 (S100A9) and neutrophil activation by S100A12 serum concentrations were described this year. Imaging using MRI T2 relaxation times and quantitative CT were investigated. The prolonged use of methotrexate (MTX) treatment after induction of remission and how it influences the subsequent duration of remission was assessed along with benefits of subcutaneous MTX after oral therapy. Genetics, transitional care, and quality of life were also studied. Intravenous cyclophosphamide showed promise in the treatment of patients with severe juvenile dermatomyositis (JDM) and topical tacrolimus was noted to be helpful in the treatment of the skin manifestations of JDM. MR imaging and major histocompatibility complex (MHC) Class I overexpression were described as useful diagnostic procedures in JDM.

Childhood-onset systemic lupus erythematosus
Cardiovascular disease has been shown to be a significant problem in patients with SLE. Quality of life issues have also grown in importance since early diagnosis and early interventions have led to increased survival in this patient population.
Cardiovascular disease
Posadas-Romero et al. [1] examined low-density lipoprotein (LDL) susceptibility to oxidation, and plasma insulin levels in 59 cSLE patients and 59 healthy, age-matched control individuals. LDL size, LDL oxidizability, and plasma levels of fasting insulin, glucose, lipids, lipoproteins, apolipoproteins B and A-I, and fatty acids were measured. In comparison with control individuals, cSLE patients showed significantly higher plasma insulin levels and increased susceptibility of LDLs to oxidation. Patients with active disease were more likely than patients with inactive disease or control individuals to have small, dense LDL subclass, elevated total cholesterol levels, elevated LDL cholesterol levels, elevated triglyceride levels, and low levels of high-density lipoprotein cholesterol. Prednisone dosage explained only 15.6% of the variance in insulin levels. The authors concluded that these abnormalities may contribute to the accelerated atherosclerosis observed in patients with SLE.

Soep et al. [2] investigated atherosclerotic risk factors and endothelial function in cSLE. In 33 cSLE patients and 30 healthy control individuals, lipoproteins, oxidized state, autoantibodies to oxidized low-density lipoprotein, and endothelial function (brachial artery reactivity) were measured. The cSLE patients had significantly decreased mean levels of high-density lipoprotein cholesterol and apolipoprotein A-I; no differences in mean levels of markers of oxidized state (nitric oxide metabolites, isoprostanes, and oxidized LDL) or endothelial function; and increased median anti-oxidized LDL IgG and IgG immune complexes with LDL. The authors concluded that the cSLE patients demonstrated abnormalities in several known factors related to increased risk for atherosclerosis.

Quality of life in childhood-onset systemic lupus erythematosus
Ruperto et al. [3] studied health-related quality of life (HRQL), disease activity, and damage in 297 patients with cSLE. HRQL was assessed by use of the Child Health Questionnaire (CHQ), disease activity using the Systemic Lupus Erythematosus Disease Activity Index, and accumulated damage using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index. The mean ± SD physical and psychosocial summary scores of the CHQ were 40.2 ± 15 and 44.8 ± 10.7, respectively. The most impaired CHQ subscales were global health, general health perceptions, and parent impact—emotional. This study showed that patients with cSLE have significant impairment of HRQL, particularly in the physical domain.

Juvenile idiopathic arthritis
Revisions in diagnostic criteria as well as advancements in new diagnostic procedures and therapeutic approaches have been made in JIA. Genetics influences have been better characterized, and special interest has been seen in the realm of transitional care in adolescents as well as in the quality of life of patients with JIA.

Diagnostic criteria
The International League of Associations for Rheumatology met in Edmonton in 2001 to delineate, for research and clinical purposes, relatively homogenous, mutually exclusive categories of idiopathic childhood arthritis based on predominant clinical and laboratory features [4]. They pointed out the importance that their classification be used with accuracy. The authors also explained that their classification requires validation before it can be used routinely in the clinical setting. ‘JIA’ is the preferred classification throughout much of the world and was recently approved for use in trials by the US Food and Drug Administration.

Diagnostic procedures
A. Schulze zur Wiesch et al. [5] determined whether the serum level of the complex of S100A8/S100A9 is a reliable predictive marker for the risk of relapse in clinically inactive JIA. Serum concentration of S100A8/S100A9 was determined by enzyme-linked immunosorbent assay and correlated with laboratory and clinical parameters for disease activity in patients with JIA. In all, 29 patients with changing disease activity were followed up for a mean time of 2.9 years [2]. Two groups of patients — one before relapse (mean 3.7 months) but without clinical signs of disease reactivation, and one in remission for 12 months — were compared. S100A8/S100A9 serum level in patients before relapses were significantly higher than the level in patients in stable remission for 1 year, whereas no differences were found for C-reactive protein and erythrocyte sedimentation rate between the groups. Serum level of S100A8/S100A9 can give a hint to clinically occult disease activity, in this way helping the adjustment of therapy in times of low disease activity.

Foell et al. [6] studied serum concentrations of S100A12, a pro-inflammatory protein secreted by human neutrophils, in 124 patients with chronic active polyarticular, oligoarticular, or systemic-onset JIA and synovial fluid from 22 JIA patients. The level of S100A12 was approximately 10-fold higher in synovial fluid than in serum, indicating release at sites of local inflammation. Serum levels decreased in response to different anti-inflammatory therapies. The investigators found that S100A12 serum concentrations indicate neutrophil activation in JIA and correlate with disease activity. It is possible that S100A12 may indicate synovial inflammation even when other signs of arthritis are absent. The authors thought that its function as a pro-inflammatory factor secreted by activated neutrophils makes this protein a potential target for future therapies.
Therapeutic approaches

Foell et al. [9**] investigated whether the duration of MTX treatment after the induction of remission influences the subsequent duration of remission in patients with JIA and the usefulness of S100A8/S100A9 as a predictive marker for the stability of remission when MTX is withdrawn. In 25 JIA patients, MTX treatment was stopped after a mean of 3.8 months (group 1) or 12.6 months (group 2) after remission was documented. No difference was found in the number of relapses in the two groups. The patients who were in stable remission had significantly lower MRP levels when MTX was discontinued than did patients demonstrating relapses. Using a cutpoint for serum S100A8/S100A9 level of 250 ng/mL, sensitivity and specificity for prediction of those who would experience relapse after stopping MTX were 100% and 70%, respectively. This study suggests that the presence of residual subclinical synovial inflammation (as measured by S100A8/S100A9), not duration of MTX treatment after the induction of remission, influences the rate of relapse after discontinuation of MTX. Normal serum concentration of S100A8/S100A9 in clinical inactive arthritis may help to identify patients in whom MTX can be safely withdrawn after remission is achieved [9**].

Subcutaneous administration of MTX in children with JIA who have not responded to oral MTX was studied in 61 children with JIA [10**]. Outcome variables included the physician’s assessment of global disease activity, number of active joints, number of joints with limited range of motion, duration of morning stiffness, and erythrocyte sedimentation rate. Improvement was defined as an improvement of at least 30% from baseline in three of five variables in the core set, with no more than one of the remaining variables worsening by more than 30%. The JIA disease subtypes were systemic 8, polyarticular 25 (12 rheumatoid factor positive), oligoarticular 14, enthesitis related arthritis 5, and unclassified 4. In 31 patients switched to subcutaneous MTX (reason for switching: 13 not improved, 11 nausea, 7 insufficient clinical improvement), after 3 months 76% were classified as improved and 23% as not improved. Toxicity with subcutaneous MTX was less than in those receiving oral MTX. The authors concluded that for patients who do not respond to oral MTX because of either inefficacy or toxicity, the use of subcutaneous MTX has a high likelihood of success without clinically significant toxicity.

Genetics

Evidence suggests that JIA is a complex genetic disorder that is influenced by multiple genetic and environmental factors. A genetic study investigated affected sibpairs (ASP) with JIA and compared clinical phenotypes of the ASP cohort with those of JIA patients with sporadic disease and investigated whether there is greater sharing of specific clinical features within rather than between sibpairs [11]. The most common JIA onset type among the 164 nontwin ASPs was oligoarticular (65% overall). Fifty-three percent of the ASPs were concordant for oligoarticular-onset JIA; 19% were concordant for a polyarticular disease onset. Among patients with polyarticular-onset disease, significantly more joints were involved at onset in simplex patients than in ASPs. The difference in age at JIA onset within sibpairs (sibling 1 rather than sibling 2) was not significantly different. Disease developed in ASPs at a mean real-time difference of 5.1 years apart. Familial aggregation was found for tenosynovitis, leukocytosis, rheumatoid factor, anemia, and antinuclear antibodies. This study confirmed the findings of earlier studies showing that a high proportion of ASPs overall show concordance of disease-onset type, except for the subset of patients with systemic disease, and that disease does not develop in nontwin ASPs at the same point in real time. The authors concluded that JIA and its clinical manifestations do not differ substantially between ASPs and the simplex population. Familial aggregation of clinical features among ASPs added strong evidence for a genetic background in this disease.

After the above information had been gathered, a genome scan was performed to detect linkage to JIA in 121 families containing 247 affected children in the JIA ASP Registry [12**] for HLA-DR and 386 microsatellite markers subjected to multipoint nonparametric linkage analysis. Linkage of JIA to the HLA region was confirmed (logarithm of odds [LOD] score 2.26). Additional evidence supporting linkage of JIA was observed at 1p36 (D1S214; LOD 1.65).
Transitional care for adolescents

Three studies explored the transitional needs of adolescents with JIA. The investigators distributed 1670 postal surveys to key professionals employed in health, social support, education, and vocation, and 478 were completed [13]. In all, 91% of the respondents were active in the care of adolescents with JIA. The respondents rated a wide range of resources to be important in supporting adolescents, including self-medication teaching packages and social skills training. Several barriers to providing transitional care were identified, including inadequate resources, coordination, and training. The authors concluded from the initial study that transitional care in the context of JIA is perceived as necessary by a wide range of professionals.

The same authors used focused group discussions in adolescents with JIA, young adults with JIA, and parents to examine how the transitional needs relating to physical, social, psychologic, and vocational areas for adolescents with JIA could be addressed [14]. The participants (n = 55) called for developmentally appropriate care based on shared decision making, continuity of health professionals, and wider access to information and community services.

The ideal program of transitional care for adolescents with JIA as perceived by users and providers and the feasibility of achieving this within the UK National Health Service context was addressed in another study by the same group [15]. A modified two-stage Delphi study was undertaken with rheumatology health professionals, young people with JIA (aged 12–25 years), and their parents. The participants rated statements about transitional care in relation to both best practice and feasibility. Items strongly agreed to constitute both best practice and highly feasible included ‘addressing young people’s psychosocial and educational/vocational needs,’ ‘providing honest explanations of the adolescent’s condition and health care,’ ‘providing opportunities for adolescents to express opinions and make informed decisions,’ ‘having continuity in health personnel,’ and ‘giving adolescents the option of being seen by professionals without their parents.’

Quality of life in juvenile idiopathic arthritis

Malleson et al. [16] explored predictors of pain in children 8 years old and older with established JIA. Pain was measured by a self-administered 10-cm visual analog scale. In a multiple regression model, active disease duration, physician global assessment, and age at study were independent predictors explaining 22% of the variation in pain scores. Stratified analyses showed an effect of age in the 8- to 15-year-old group but not in older patients. The disease-related factors explained only a small proportion of the variation in pain scores. The authors concluded that further studies were needed to assess the impact of psychosocial factors and how these factors relate to pain in JIA patients.

Brunner et al. [17] examined the relations of different measures of HRQL (including the Pediatric Quality of Life Questionnaire [PedsQL], the Juvenile Arthritis Quality of Life Questionnaire [JAQQ], and visual analog scale) and disability, pain, and well being in children with chronic arthritis. The three measures of HRQL were moderately to highly correlated with one another and were significantly decreased with increasing disability. Parents were moderate to good proxy reporters of HRQL, disability, and well being of children with chronic arthritis.

Juvenile dermatomyositis

Juvenile dermatomyositis has seen advances in diagnostic procedures such as MRI and MHC Class I overexpression and in therapeutic approaches including intravenous cyclophosphamide and topical tacrolimus.

Diagnostic procedures

In 10 children with active JDM, 10 with inactive JDM, and 20 healthy children, the MRI T2 relaxation times in the thigh muscle were significantly increased in patients with active JDM compared with those with inactive JDM and healthy children, indicating a detectable increase in inflammation within the muscles [18]. There were also good correlations between the MRI scores and measures of muscle strength and function but no correlation between the MRI and muscle enzymes. The study demonstrated that the MRI T2 relaxation time can be used as a quantitative measure of muscle inflammation and has good correlation with other measures of disease activity.

Light microscopic and immunohistochemical analysis of muscle biopsy specimens from 10 patients with JDM and 3 control individuals was performed to assess expression of MHC Class I genes in early JDM [19]. The mean duration from initial weakness to muscle biopsy was 2.8 months. At the time of biopsy, 9 patients had not received steroid treatment or immunomodulatory drugs. MHC Class I gene overexpression was evident on muscle fibers in all 10 JDM samples, even in a biopsy reported as normal by conventional histology. MHC Class I heavy chain and...
B2 microglobulin were overexpressed in an identical distribution. Variable infiltration of T cells and macrophages was seen in the JDM biopsy specimens, with minimal lymphocytic and monocytic infiltration in 4 cases, and none in 1 case. Only very occasional natural killer lymphocytes were identified. Neuronal cell adhesion molecule (CD56) staining of regenerating muscle fibers was seen in all samples, and these cells were confirmed as being of muscle origin by costaining for dystrophin. MHC Class I gene overexpression is an early event in JDM and may occur in the absence of lymphocytic infiltration and muscle damage. Immunostaining for MHC Class I genes could be used routinely in the assessment of muscle histology in JDM [19].

Therapeutic approaches
In a retrospective review study of 12 patients with severe refractory JDM after 6 months of treatment with intravenous cyclophosphamide (0.5–1 g/m²) administered monthly (except for severe cases when administered in the third week), 10 patients showed a significant improvement in muscle function as assessed by the Childhood Myositis Assessment Scale, muscle strength, global extramuscular disease score, skin disease severity, and lactate dehydrogenase level [20]. Clinical improvement was maintained after discontinuation of the cyclophosphamide (follow-up duration 0.5–7 years), and no severe side effects were seen. The authors concluded that the cyclophosphamide seemed to have provided major clinical benefit with no evidence of serious toxicity in the short term.

The dermatologic manifestations of JDM are often very difficult to treat. Topical tacrolimus 0.1% ointment was used to treat 6 patients with recalcitrant cutaneous lesions [21]. After 6 to 8 weeks of treatment, 6 of 6 patients experienced improvement; 2 of 6 had an excellent response (>90% improvement), 1 of 6 had a moderate response (40–90% improvement), and 3 of 6 had a minimal response (20–40% improvement). The results of this brief observational study were encouraging.

Conclusion
Patients with cSLE demonstrate several risk factors for atherosclerosis and significantly impaired HRQL. ‘JIA’ is the preferred term to classify chronic idiopathic arthritis in children. In children with JIA, S100A8/S100A9 is a sensitive laboratory measure for subclinical joint inflammation and thus serves as a sensitive and specific predictor of which patient will or will not do well after discontinuation of MTX. Subcutaneous MTX was shown to be effective in JIA patients in whom oral MTX has not been successful. Genetic studies highlighted the association of multiple genes in each of several JIA subtypes. JIA patients also have decreased HRQL, and the reported pain is only partially explained by disease-related parameters. In children with JDM, MRI T2 relaxation times indicate areas of muscle inflammation, and MHC Class I genes are overexpressed. In small open studies, intravenous cyclophosphamide (in severe treatment-resistant cases) and topical tacrolimus (for dermatologic manifestations) were effective treatments.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


The authors concluded that cSLE demonstrated abnormalities in several known factors related to increased risk for atherosclerosis.


Patients with cSLE had significant impairment of their HRQL, particularly in the physical domain.


MRP8/MRP14 correlates highly with clinical measures of disease activity in patients with JIA and demonstrates very good sensitivity and specificity for predicting disease flares in these patients.


S100A12 serum concentrations indicate neutrophil activation in juvenile RA and correlate with disease activity.


T2 relaxation time mapping may allow for early detection of cartilage changes and provide an objective, quantitative method of monitoring disease progression, with long-term potential to guide therapy.


Longer duration of MTX treatment after induction of inactive disease during therapy does not increase the likelihood that inactive disease will continue after MTX is discontinued in patients with JIA.


This study suggests that in patients for whom oral MTX is ineffective, because of either inefficacy or toxicity, the use of subcutaneous MTX has a high likelihood of success.


This study supports the hypothesis that multiple genes, including at least one in the HLA region, influence susceptibility to juvenile RA.


The MRI T2 relaxation time can be used as a quantitative measure of muscle inflammation and correlates highly with other measures of disease activity.


In this cohort of children with severe and refractory JDM, cytoxan seemed to provide major clinical benefit with no evidence of serious short-term toxicity.

Rheumatic arthritis (RA) remains a significant cause of morbidity and disability associated with high health economic costs to society. Recent advances in therapy arising primarily from detailed pathogenetic studies offer an exciting glimpse of what can be achieved in the clinical domain when appropriate inflammatory pathways are suppressed or abrogated [1]. The success of TNF blockade has proved a catalyst for unprecedented efforts to develop novel agents targeting either TNF itself or related pathways, together with a wide range of novel moieties and cellular targets. This wealth of emerging therapeutics is discussed in detail in this section of Current Opinion in Rheumatology, both at the cellular and cell-signalling level. However we have chosen also to highlight key developments in the pathogenetic impetus that has informed these novel therapeutic developments. Thus is included an evaluation of the genetic and cell-signalling level. However we have chosen also to highlight key developments in the pathogenetic impetus that has informed these novel therapeutic developments. Thus is included an evaluation of the genetic factors currently understood to play a role in RA, the epideiologic and clinical datasets that may explain increased mortality, state-of-the-art imaging studies that will in due course direct our interventions in a timely and appropriate manner and crucially novel studies that redefine the physiologic environment in which the ongoing synovitis inflammatory process occurs.

Although thus far anti-inflammatory interventions have proven most successful, RA has long been considered an autoimmune disorder. The ‘holy grail’ of therapeutics should therefore be antigen specific, individually tailored re-establishment of immune tolerance with consequent removal of the factors that drive the chronic inflammatory response. The theoretical advantage of this approach is the maintenance of host defense intact whereas autoimmune self-harm is abrogated. Several recent studies have reiterated the autoimmune component in both T cell and B cell compartments of the acquired immune response. The re-emergence of lymphocyte mediated effector function is of particular interest in the context of this autoimmune response. Singh et al. [2] here review the clinical data supporting the development of co-stimulator pathway blocking agents, including CTLA4-Ig. That this strategy has been successful is intriguing since they strongly implicate a T cell mediated (CD28 dependent) component to chronic synovial inflammation. Similarly targeting CD20 with depleting antibodies has proven of long term benefit in RA – originally developed on the basis of eradicating autoantibody formation, these studies now raise the possibility that immune tolerance can be in some way regulated by auto-reactive B cells perhaps via antigen processing function in synovium or beyond. B cells also produce cytokines rendering this therapeutic approach both locally anti-inflammatory and immune regulatory. Therapeutic advances continue in the field of cytokine targeting – IL-6 receptor inhibition for example leads to significant amelioration in RA disease activity [3]. Other cytokine targets such as IL-12, IL-15, IL-18 and IL-17 are under intense examination [4]. There has also been increased drive to characterise the signalling pathways that subserve cytokine regulatory and effector function. Whereas there has been much interest in blocking TNFR and IL-1R superfamily signalling, particularly via MAPK and NF-kB, less attention until recently has focused on the JAK/STAT signalling pathways in RA. By virtue of their immune developmental, immune regulatory as well as inflammatory roles they offer possibilities across a range of autoimmune disorders. Their biology and inhibition is described herein by O’Shea et al. [5]. Together with an excellent overview of JAK/STAT biology, this article argues persuasively for potential advantages in targeting intracellular pathways, e.g., to capture broader immune regulatory effects than single cytokine targeting agents. A feature of cytokine targeting thus far has been synergism with conventional immunosuppressive disease modifying agents, such as methotrexate, suggesting that only partial disease modification is possible with single agent targeting. It will be of interest to determine whether ‘rational’ combination of immune modulatory and cytokine targeting therapy, encompassing signal inhibition, can substantially increase the remission rate achieved in the clinical setting, and indeed whether therapy withdrawal without flare over time is achievable.

There has been much interest recently in angiogenesis as a therapeutic target in both cancer and inflammation.
Taylor and Sivakumar [6] here review the expanding literature that describes not only the crucial role played by neovascularisation in synovitis, but also recent studies that define the synovium as a profoundly hypoxic environment. These observations provide an elegant example of in vitro studies combined with in vivo advances in imaging technology together yielding important observations. Since much immune biology is examined under normoxic conditions in vitro and in acute models in vivo, the discovery that synovium contains areas of marked hypoxia has important implications for pathogenesis studies, beyond simply elaborating novel therapeutic interventions.

Immune modulatory approaches achieved (via altered immune regulation, inflammation suppression or blockade of angiogenesis) will inevitably be patient subset and disease kinetic dependent. To this end exciting advances in articulating imaging are relevant. Keen et al. [7] have compared and contrasted recent data addressing the potential for ultrasound, magnetic resonance imaging and conventional radiography, particularly in early RA. New technologies require careful validation against existing measures and may offer distinct information in discrete tissues within the joint. Importantly, they may show early changes predictive of disease progression to allow earlier more aggressive intervention in appropriate cases. The same may be true in predicting disease suppression at an early stage after a given intervention, thus allowing shorter clinical trials, perhaps comparison with placebo in an ethical manner and likely reduced costs both clinical and financial to the research and biotech community. Taken together, it is becoming clear that integration of clinical, imaging, and serology evaluation together with intervention will be necessary to optimise treatment of individual patients over time.

In this context and in the wider pathophysiologic area, genetic studies remain a major focus in RA research. It is of interest that in their genetic update, Huizinga et al. [8] highlight the potential role for PTPN22 in RA that in turn can influence the T cell receptor activation threshold and potential for auto-reactivity. Beyond this, their review thoughtfully explores recent linkage studies, candidate based analyses and includes discussion of phenotypic-genotypic analyses. They provide examples, whereby genetic studies extending beyond the HLA locus suggest novel therapeutic targets in the organic cation transporter gene SLC22A4 that is expressed exclusively in immunologic and hematologic tissues, in which a disease associated SNP altered binding of the transcription factor RUNX1. Intriguingly the latter itself contains disease associated SNPs which when combined with those in SLC22A4 significantly increased the odds ratio for disease susceptibility. They also highlight the necessity to examine multiple populations citing the variable role potentially played by PAD4 in Japanese [9] as opposed to Caucasian populations. Post-human genome project, the necessity for carefully phenotyped patient cohorts and careful comparative approaches, remains vital.

Recently enhanced mortality has been added to the physical dysfunction anticipated in the natural history of RA. Standardised mortality rates for RA are significantly higher than those of comparable control populations. This has been explained on the basis of therapies used, such as non-steroidal anti-inflammatory drugs, but more recently has been attributed to the effects of long-term effects of poorly controlled inflammation. The evidence for this proposal has been reviewed by Sattar et al. [10,11]. These data are important not only because explanation for increased mortality should provoke intervention studies with firm cardiovascular endpoints, but also because they predict that intensive control of inflammatory synovitis should in turn reduce the mortality associated with RA. That methotrexate therapy reduces mortality over time in RA particularly via reduced vascular deaths lends credence to this hypothesis [12]. The effects of TNF blockade in this area are eagerly awaited. These studies exemplify cross talk in biomedical research; it is likely that RA studies will inform the wider cardiovascular community.

In summary, these remain exciting times for RA research with significant advances ongoing across a broad front. We will shortly be presented with a range of novel interventions to target inflammatory synovitis, articular destruction and disease-associated co-morbidities. The challenge is clearly to allocate the necessary intervention(s) to the appropriate patient in a timely manner according to the optimal strategy. The combination of novel imaging technologies, genetic evaluation and clinical phenotyping will be essential in bringing closer the objective of long term remission in RA.

References


Purpose of review
Biologic therapy for rheumatoid arthritis targets specific molecules, both cell-bound and soluble, that mediate and sustain the clinical manifestations of this complex disease. The aim of all the therapeutic strategies is to achieve complete and sustained suppression of inflammation, in the absence of unacceptable short-term and long-term toxicity. Despite the success of the currently available biologic inhibitors of tumor necrosis factor-α and interleukin-1, a substantial number of rheumatoid arthritis patients are refractory to these treatments. The purpose of this review is to highlight recent clinical trials of emerging biologic treatments for rheumatoid arthritis.

Recent findings
T cell co-stimulation has been targeted by the use of cytotoxic T lymphocyte-associated antigen 4-Ig, a genetically engineered fusion protein. In a large controlled clinical trial, this nondepleting approach was shown to achieve impressive clinical responses, without evidence of short-term toxicity. Likewise, rituximab, a B cell-deleting monoclonal antibody, was shown in a controlled clinical trial to have sustained benefit in patients with refractory rheumatoid arthritis. Despite profound B cell depletion with rituximab, there was an acceptable safety profile with this treatment. MRA, a monoclonal antibody that inhibits interleukin-6 by binding to its receptor interleukin-6R, demonstrated clinically significant improvement in rheumatoid arthritis and a particularly impressive reduction in the acute phase response.

Summary
The response of rheumatoid arthritis to a wide spectrum of therapeutic strategies attests to the complexity and heterogeneity of the disease and provides further impetus for studies that use these therapies to enhance our understanding of disease pathogenesis.

Keywords
B cells, biologic agents, cytokines, rheumatoid arthritis, T cells

Introduction
The pathogenesis of rheumatoid arthritis (RA) remains incompletely understood. It involves complex interactions between T and B lymphocytes, macrophages, and fibroblast-like synoviocytes, involving a network of cytokines acting in an autocrine and paracrine manner [1]. In recent years, the development of biologic agents that target specific soluble or membrane-bound molecules has revolutionized the treatment of RA. The success of tumor necrosis factor-α (TNF-α) inhibition, and to a lesser extent interleukin (IL)-1 inhibition, has firmly established these therapies in the clinical management of RA. Moreover, this approach has allowed unprecedented opportunities for developing a better understanding of the pathogenesis of this complex and heterogeneous disease. Despite the success particularly of the TNF-α inhibitors, data from both clinical trials and real-life clinical experience have clearly suggested that a substantial proportion of RA patients either do not respond, or lose their initial responses, to these agents [2••]. Thus, there continues to be a compelling need for the development of new therapeutic strategies. This review highlights recent research activity in the clinical development of novel biologic therapies for RA, focusing on therapies that target specific immune cells, and therapies that target cytokines.

Cell-targeted therapies
There has been a long-standing interest in manipulating cells of the immune system to achieve control of RA. Because of the prominence of T lymphocytes in rheumatoid synovitis, early attempts focused on the depletion of this cell population in the hope of ameliorating the disease. Clinical experience with T cell–depleting agents such as the CAMPATH-1H antibody was disappointing and was associated with long-lasting lymphopenia, although not an excess of morbidity or mortality after prolonged follow-up [3]. Interestingly, the T cells persisted in the synovial
membrane despite profound peripheral lymphopenia [4].
A host of other T cell–depleting strategies were associated with either unacceptable toxicity or modest efficacy or both. More recently, interest has focused on modulating T cell function rather than depleting large number of T cells or subsets of T cells. The important role of co-stimulation in the activation of T cells is now well understood, and this process has been targeted therapeutically with the cytotoxic T lymphocyte–associated antigen 4-Ig (CTLA4Ig) fusion protein, which interferes with co-stimulation without depletion of T cells.

In contrast to T cells, B cells had largely been ignored in RA pathogenesis until recently. After a period of prolonged indifference, the potential therapeutic utility of manipulating B cells in RA has been explored in recent years. These cells are well known to be responsible for producing rheumatoid factors (RF) and other RA-associated autoantibodies such as anti-cyclical citrullinated peptide. Importantly, B cells, which are abundant in the synovium of most patients with well-established RA, also act as highly efficient antigen-presenting cells (APC) to T cells and thus may play an important role in synovial T cell activation [5]. It has thus been postulated that depletion of B cells or modulation of their function may be associated with clinical benefit in RA.

Biologic therapies targeting T cells and B cells have been developed over the past 5 years and have been evaluated in well-designed clinical trials in patients with RA.

Inhibition of T cell co-stimulation: abatacept

T cells require two signals from APC for complete activation [6]. The first signal is antigen specific and occurs between a T cell receptor and the major histocompatibility complex—peptide complex on the APC. A second co-stimulatory signal occurs between CD28 molecules on T cells and CD80 or CD86 molecules on APCs. These two signals cause T cell proliferation and cytokine production, which in turn activate other inflammatory cells. If the second co-stimulatory signal is missing, the T cells may be poorly responsive to stimuli, and apoptosis may occur.

Cytotoxic T lymphocyte–associated antigen 4 (CTLA4) is an immunoregulatory protein expressed on the T cell surface after activation. It binds to CD80 or CD86, blocks their interaction with CD28, and thus acts as an off-switch for cell activation. CTLA4Ig is a genetically engineered fusion protein that consists of a human CTLA4 portion fused to a constant IgG1 region. This molecule binds to CD80 and CD86 and thereby inhibits T cell co-stimulation. On the basis of the central role of T cell activation in the pathogenesis of RA, it was hypothesized that inhibition of this process using CTLA4Ig would achieve clinically meaningful improvement in RA [7].

An initial 3-month, dose-finding pilot study of CTLA4Ig therapy in RA had demonstrated that at a 10 mg/kg dosage given every 2 weeks, 53% of patients had an ACR 20 response, whereas 16% had an ACR 50 response after 85 days of therapy [8]. The treatment was well tolerated, with no evidence of major toxicity. This study led to a larger clinical trial that was published in late 2003. In this publication, Kremer et al. [9] reported the results of a 6-month randomized, double-blinded, placebo-controlled trial studying the efficacy of CTLA4Ig in RA. All 339 patients in the study had active RA despite taking methotrexate (10–30 mg weekly). Two hundred fifty-nine patients completed 6 months of treatment. Methotrexate was continued in all patients, but all other disease-modifying antirheumatic drugs (DMARDs) were discontinued. Stable low-dose corticosteroids (prednisolone ≤10 mg/d) and nonsteroidal anti-inflammatory drugs (NSAIDs) were allowed. The patients were randomly assigned to three arms: placebo with methotrexate, CTLA4Ig (2 mg/kg) with methotrexate, and CTLA4Ig (10 mg/kg) with methotrexate. CTLA4Ig or placebo was infused at days 1, 15, and 30 and thereafter monthly for 6 months. ACR 20, 50, and 70 were measured at 6 months to assess clinical response. Responses to the Short Form Health Survey-36 were also assessed at baseline, 90 days, and 180 days. ACR 20 responses in the CTLA4Ig 10 mg/kg group were improved from months 2 to 6. There was no statistically significant difference in ACR 20 response between the placebo group and the CTLA4Ig 2 mg/kg group at 6 months ($P = 0.31$). ACR 50 and ACR 70 responses were higher at 6 months in both CTLA4Ig groups than in the placebo group. There was also significant improvement in all Short Form Health Survey-36 subscales in the CTLA4Ig 10 mg/kg group ($P < 0.05$), but no statistically significant difference between the CTLA4Ig 2 mg/kg and placebo groups. The drug was well tolerated. No deaths, malignancies, or opportunistic infections were reported. The most commonly reported adverse symptoms included headache, upper respiratory tract infection, musculoskeletal pain, nausea, and vomiting. The rate of serious side effects was actually lower in the CTLA4Ig 10 mg/kg group than in the other two groups. These data suggested that the 10 mg/kg dose has a favorable benefit-to-toxicity ratio and is the most suitable for clinical use.

As a follow-up to this study, Kremer et al. [10] and Dugados et al. [11] published data from a 1-year open-label extension of this study in abstract form. In this study, ACR 20, 50, and 70 values and DAS-28 suggested that efficacy was maintained at 2 years. Moreland et al. [12] presented data indicating similar rates of serious adverse effects when the CTLA4Ig 10 mg/kg (plus methotrexate) and the control (methotrexate alone) groups were compared. Together, these data suggest that abatacept (Bristol-Myers Squibb, Princeton, NJ, USA) combined
with methotrexate demonstrates sustained ACR response at 2-year follow-up and is well tolerated.

This promising strategy is now being evaluated in large phase III trials. It remains unclear whether CTLA4Ig therapy in RA should be considered for patients who are refractory to TNF-α inhibitors, or whether there is a subset of patients who are particularly well suited for treatment with this approach.

**B cell depletion: rituximab**

The CD20 antigen is present on the cell surface of all pre-plasma cell stages of B cell differentiation, although the role of this molecule remains unclear. The mature plasma cell loses the CD20 antigen, and thus it serves as a relatively specific marker for B cells [13]. Rituximab (Roche Pharmaceuticals, Basel, Switzerland; Genentech, South San Francisco, USA; IDEC Pharmaceuticals, San Diego, USA), a genetically engineered human-mouse chimeric monoclonal antibody against the CD20 antigen, has been used successfully in the treatment of B cell malignancies like non-Hodgkin lymphoma, chronic lymphocytic leukemia, and others. Rituximab binds to the CD20 antigen on the B cell surface and efficiently depletes B cells by antibody-dependent and complement-dependent cell lysis [13,14].

In an initial report, Edwards and Cambridge [15] described an open-label study of rituximab in combination with cyclophosphamide and prednisolone in five patients with refractory RA. These patients all demonstrated dramatic and sustained clinical improvement, with two of the patients continuing to show ACR 70 responses at 1 year. In these patients, RF had become undetectable. Despite profound B cell depletion, no significant toxicity was seen. This group published an expanded series of 22 patients that suggested the need for a dosage of at least 600 mg/m² to achieve clinical benefit [16]. A subsequent analysis of the serologic effects of this treatment suggested that IgA-RF, IgG-RF, and anticyclic citrullinated peptide antibodies decreased out of proportion to a decrease in total serum immunoglobulins, and in antibodies to specific pathogens [17]. Moreover, the decrease in autoantibodies paralleled a decrease in C-reactive protein, and the disease relapsed when autoantibody levels increased again, although the return of B cells was unpredictable.

These data led to a controlled phase II trial, the results of which were published in 2004 [18••]. In this multicenter, double-blind, controlled study by Edwards et al. [18••], 161 patients with methotrexate-refractory RA were randomized into four treatment groups: continuing oral methotrexate (≥10 mg/wk), rituximab (1000 mg on days 1 and 15), rituximab and cyclophosphamide (750 mg on days 3 and 17); and rituximab and methotrexate. No other DMARD was allowed during the trial. Stable doses of NSAIDs and corticosteroids (prednisolone ≥12.5 mg/d or equivalent) were allowed. The primary endpoint of the study was the ACR 50 response at week 24. The ACR 50 response for rituximab combination therapy with either methotrexate or cyclophosphamide was significantly higher than the control methotrexate group. ACR 50 responses for methotrexate, rituximab, rituximab plus methotrexate, and rituximab plus cyclophosphamide were 13%, 33%, 41%, and 43%, respectively. ACR 50 response differences between methotrexate and rituximab monotherapy did not reach statistical significance (P = 0.059) but did trend towards increased values. Interestingly, the ACR 20, ACR 50, and ACR 70 responses even at week 48 for the rituximab plus methotrexate group were 65%, 35%, and 15% respectively (P ≤ 0.001, P = 0.002, P = 0.03, respectively). This demonstrates a sustained clinical response after just two doses of rituximab.

Rituximab was associated with almost complete depletion of peripheral B cells. The greatest decline in peripheral B cells was noted in the rituximab-cyclophosphamide group. The control group showed initial decline followed by rebound in cell numbers. Rituximab treatment groups also showed a rapid decline in RF levels. The methotrexate (control) group experienced an initial decrease, but the RF levels returned to baseline by week 24. Immunoglobulin levels did not change significantly. Despite the profound peripheral B cell depletion in the rituximab groups, the overall incidence of infection was similar in all groups at weeks 24 and 48. All the treatment groups had a similar overall incidence of adverse effects; however, the highest incidence of serious adverse events was noted in the rituximab plus cyclophosphamide group. Serious infections occurred in one patient in the control group, two patients in the rituximab monotherapy group, and two patients in the rituximab plus cyclophosphamide group. Fatal bronchopneumonia occurred in a patient in the rituximab monotherapy group.

An extension to the trial was published in abstract form by Emery et al. [19]. The patients were evaluated at week 104. ACR 50 values for the rituximab plus methotrexate and methotrexate (placebo) groups were 21% and 11%, respectively. Also, 13% of the rituximab plus methotrexate group reached a major clinical response (defined as ACR 70 maintained for ≥6 months). No significant differences in infections were noted between the different treatment groups.

Two recent case reports provide further evidence on the potential role of rituximab in DMARD-refractory RA. Kramm et al. [20] reported five patients with RF-positive erosive arthritis. All five patients experienced lack of efficacy with multiple DMARDs, including anti-TNF therapy. The DMARDs being taken at the time of the trial were continued. All five patients were given four weekly doses...
of 375 mg/m² of rituximab. Swollen and tender joint counts were evaluated before and after the rituximab therapy. Four of the five patients achieved remission lasting 5 to 12 months. All patients experienced relapse after a mean duration of 8 months after rituximab therapy.

Similarly, Kneitz et al. [21] performed an open study of five patients with refractory RA. The patients had been unsuccessfully treated with at least three other DMARDs. All DMARDs except methotrexate were stopped at least 2 weeks before the patients entered the study. Methotrexate was continued at the same dosage if a partial response had been previously observed. Four of the five patients had not responded to anti–TNF-α therapy. All five patients reached the primary efficacy point (improvement in Disease Activity Score 28 $\geq 1.2$).

These studies suggest that B cell depletion may be effective in treating refractory RA and in producing prolonged and sustained improvement in disease activity parameters. It has been speculated that RF-positive patients would potentially be the most responsive to this approach, although this contention remains unanswered. A theoretic risk of immune-system reaction against chimeric antibodies exists, but no significant reactions of this nature have been reported to date in RA patients [22]. Fully human monoclonal antibodies are, however, currently being developed.

**Cytokine-targeted therapy**

Cytokines are molecules that play both pro-inflammatory and anti-inflammatory roles. Indeed, our knowledge of their central role in inflammation has been used for therapeutic benefit with the advent of TNF-α and IL-1 blocking agents in RA. Other cytokines have been evaluated as potential therapeutic targets.

**Inhibition of interleukin-6 using MRA, an anti-interleukin-6 receptor antibody**

Interleukin-6 is a glycoprotein composed of 184 amino acids. Numerous cells can produce this inducible cytokine, including macrophages, B cells, T cells, fibroblasts, endothelial cells, mesangial cells, and many types of tumor cells [23]. IL-6 gene expression is regulated primarily through the nuclear factor-κB pathway, which is activated by both TNF-α and IL-1, along with several other pro-inflammatory stimuli [1]. IL-6 signaling is inhibited by suppressor of cytokine signaling and the protein inhibitors of activated STATS [23]. The effects of IL-6 are pleiotropic, occurring at both a systemic and a local tissue level, and involving a wide variety of cells (Fig. 1). Of particular relevance to RA are the effects on the differentiation of B and
T lymphocytes, as well as the differentiation of macrophages, megakaryocytes, and osteoclasts. IL-6 is now known to be the primary regulator of the hepatic acute-phase response, stimulating hepatocytes to produce C-reactive protein, fibrinogen, serum amyloid A protein, and a spectrum of other acute-phase proteins, while suppressing albumin production.

Interleukin-6 is elevated in the serum and synovial fluid in RA patients [24,25]. The excessive production of IL-6 is postulated to play a role in the pathogenesis of several inflammatory diseases such as RA, Crohn disease, and juvenile idiopathic arthritis [26]. In RA, IL-6 participates in immune cell activation and autoantibody production, osteoclastogenesis, and bone loss, and the often debilitating systemic and constitutional symptoms associated with the acute-phase response. It also plays a role in activating the hypothalamic-pituitary axis, leading to the release of anti-inflammatory hormones [27].

MRA (Chugai Pharmaceutical Co. Ltd., Tokyo, Japan) is a humanized anti–IL-6 receptor antibody that inhibits the binding of IL-6 to its receptor IL-6R and prevents IL-6 signal transduction. The ultimate development of this approach as an effective treatment for RA by Nishimoto and his colleagues in Japan has been a spectacular example of the path from bench to bedside. In 2004, Nishimoto et al. [28] published the results of a multicenter, double-blind, placebo-controlled trial with 162 patients with active RA who had been unsuccessfully treated with at least one DMARD or immunosuppressant. The study population represented a group of patients with refractory RA for whom four to five DMARDs had been tried in the past without success. No DMARDs, immunosuppressants, or parenteral or intraarticular corticosteroids were allowed, but stable doses of oral corticosteroids (prednisolone ≤ 10 mg/d) and NSAIDs were. Patients were divided into three groups: placebo, MRA 4 mg/kg, and MRA 8 mg/kg; they were administered the allotted study drug three times every 4 weeks for 3 months. The primary end point was ACR 20 measured at week 12. Twenty-five of the 53 patients in the placebo group withdrew. The reasons included exacerbation of disease requiring DMARD (12 patients), patients’ request (3 patients), lack of efficacy and patients’ request (6 patients), and adverse events (4 patients).

There was significant improvement in ACR 20, 50, and 70 with MRA treatment compared with placebo. The efficacy was initially noted at week 4 and continued to increase up to week 12. The ACR 20 responses were 11.3%, 57.4%, and 78.2% for placebo, MRA 4 mg/kg, and MRA 8 mg/kg, respectively. The ACR 50 and ACR 70 responses for the MRA 8 mg/kg group were 40% and 16.4%, respectively. Similarly, improvements in DAS-28 were also noted. Furthermore, normalization of the C-reactive protein level occurred in 76% and 26% of patients in the MRA 8 mg/kg and 4 mg/kg groups, respectively. Only 1.9% of the placebo group patients showed C-reactive protein normalization. A decrease in RF titers was seen in the MRA 8 mg/kg group but no correlation was seen between decrease in RF titer and ACR response rate in this study.

The incidences of adverse events in the placebo, MRA 4 mg/kg, and MRA 8 mg/kg groups were 56%, 59%, and 51% respectively. Three serious adverse events were noted in the MRA group. One of the patients died of reactivation of chronic Epstein-Barr virus infection and hemophagocytosis syndrome after receiving a single dose of MRA 8 mg/kg. Other serious side effects noted in the MRA groups were allergic pneumonitis and infection of a leg burn. Increases in lipid levels (total cholesterol, triglycerides, and high-density lipoprotein cholesterol) were common in the MRA groups. There was no increase in cardiovascular complications associated with the increase in lipid levels, although the trial was too short and too small to enable adequate assessment of this. An elevation of transaminases was observed in 12.8% of the MRA group. Leukopenia was also noted. Both these abnormalities were transient. Anti-MRA antibodies were detected in 2 patients who received MRA. These patients were withdrawn from the study.

In a published abstract, Nishimoto et al. [29] reported a study in five patients who had achieved ACR 50 or 70 responses with MRA treatment. The MRA treatment was suspended until the patients no longer fulfilled ACR 50 criteria. ACR 50 criteria lasted for 3, 6, 9, and 22 months in four of the five patients. The authors also showed a correlation between reductions in IL-6 levels after MRA treatment and ACR 70 response. They concluded that once the IL-6 levels are normalized, the efficacy could be sustained even after MRA cessation.

Other cytokine targets

Preliminary trials targeting other cytokines including IL-12, IL-15, and IL-18 are under way. AMG 714 (Genmab, Copenhagen, Denmark) is a human monoclonal antibody that binds to IL-15 and inhibits its signaling. In a published abstract, McInnes et al. [30] demonstrated that patients receiving AMG 714 had clinically meaningful improvement compared with placebo, providing a first proof of the concept that IL-15 may be a rational target in the treatment of RA. In preclinical studies, an anti–IL-17 antibody significantly reduced the severity of collagen-induced arthritis [31].

Conclusion

Despite the success of TNF-α and IL-1 inhibitors, a substantial proportion of RA patients remain refractory to the available therapeutic modalities. There continues to be considerable investigative activity to develop new strategies
for treating RA patients. In the past year it has been shown that depletion of B cells with the monoclonal antibody rituximab results in sustained improvement in the signs and symptoms of RA, even after only two doses of this agent. Moreover, there is little evidence of excessive toxicity despite profound depletion of B cells. Likewise, the inhibition of T cell co-stimulation by the use of CTLA4Ig demonstrates impressive clinical efficacy and acceptable toxicity. This is in sharp contrast to the previously observed unfavorable risk-to-benefit ratio associated with T cell–depleting agents such as Campath. Effective inhibition of IL-6, a central pro-inflammatory cytokine in RA, produces clinically meaningful improvement in the disease state, particularly in the acute-phase response associated with RA.

The response of RA patients to this wide spectrum of therapeutic strategies attests to the complexity and heterogeneity of the disease and provides further impetus for studies that use these therapies to enhance our understanding of disease pathogenesis.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest

This is a state-of-the-art review of drug therapy in RA
This randomized, double-blind trial compared oral methotrexate, rituximab, rituximab plus methotrexate, or rituximab plus cyclophosphamide in 161 randomly assigned RA patients. The trial showed that rituximab, alone or in combination, provided significant and prolonged improvement in symptoms.
This multicenter placebo-controlled trial demonstrated a reduction in disease activity with MRA, an anti-human IL-6R monoclonal antibody
How should we manage early rheumatoid arthritis? From imaging to intervention
Helen I. Keen and Paul Emery

Purpose of review
Rheumatoid arthritis is a chronic systemic and progressive inflammatory disorder of the synovium characterised by destruction of bone and cartilage. It is associated with significant morbidity and economic costs. Recent advances have shown that early diagnosis and timely, intensive therapy of rheumatoid arthritis can modify disease outcomes.

Recent findings
Current investigations into the role of ultrasonography and magnetic resonance imaging in early rheumatoid arthritis suggest these modalities will provide information to assist in the early diagnosis of rheumatoid arthritis, identify poor prognostic factors, and aid in the monitoring of response to therapy. New developments in pharmacologic therapy, particularly the development of biologic agents, allow better disease control than was previously achievable, and the early application of these drugs in combination with conventional disease-modifying antirheumatic drugs seems to produce the best outcomes.

Summary
The application of novel imaging techniques will aid the target application of biologic therapy within the window of opportunity and aid in the monitoring of response to therapy. This is likely to significantly decrease the rate of structural damage and offers hope of a future when the normal outcome for rheumatoid arthritis will be remission.

Keywords
combination therapy, drug therapy, early arthritis, magnetic resonance imaging, rheumatoid arthritis, ultrasonography

Introduction
Traditionally, a pyramidal approach to the pharmacologic therapy of rheumatoid arthritis (RA) was applied. Initial treatment consisted of nonsteroidal anti-inflammatory drugs, which have no disease-modifying effects. Once patients had demonstrated radiographic damage, therapy progressed to more toxic disease-modifying antirheumatic drugs (DMARDs). These drugs have been shown to improve outcomes in RA, in particular slowing the rate of radiographic progression. Radiographic damage occurs early in RA, and the greatest rate of radiographic progression occurs in the first 2 years of disease, even allowing for artefacts of scoring [1,2].

Imaging in early rheumatoid arthritis
Early RA can be difficult to differentiate from self-limiting or other forms of inflammatory arthritis [5]. In practice, diagnosis is based on the experienced clinician’s acumen, but the clinical picture at presentation can be confusing, and some patients with early RA have no radiographic changes at baseline [1,2]. In addition, conventional radiography does not provide information about soft tissue structures or synovium. The role of ultrasonography and MRI in early RA suggest that these novel imaging techniques will provide information to assist in the early diagnosis of RA, identify poor prognostic factors, and aid in the monitoring of response to therapy. Here we review the role of novel imaging techniques in the diagnosis and management of rheumatoid arthritis, and optimal therapeutic regimens in this setting, with a focus on recent publications.

Ultrasoundography
Recent publications have demonstrated the potential for ultrasonography to be widely used in the clinics devoted to the treatment of early arthritis. Ultrasonography has consistently been more sensitive than conventional radiography in detecting erosions in peripheral joints in RA and is able to follow the progression of erosions over time [6–8,*9–11*]. Hoving et al. [11*] monitored a cohort of
patients with early RA for 6 months with MRI, ultrasonography, and conventional radiographic assessment. All three modalities noted progression in size and number of erosions; MRI was the most sensitive for the presence of erosions, and ultrasonography performed less well than radiography. Ultrasonography was found to be more useful than the other modalities in identifying joint and tendon sheath effusions. A cross-sectional study of the metatarsophalangeal joints in established cases of RA found that ultrasonography detected more erosions than MRI or conventional radiography [10].

Ultrasonography has consistently been shown to be more sensitive than clinical examination in detecting small and large joint synovitis [10,12,13]. In a recent publication from an early arthritis clinic, ultrasonography detected subclinical synovitis in almost two thirds of patients with a clinical diagnosis of oligoarthritis, and the diagnosis was revised to polyarthritis in one third [13]. Another study of 60 patients with heterogeneous knee disorders showed ultrasonography to be more sensitive and accurate than clinical examination [14]. When arthroscopy was used as the gold standard, the validity of ultrasonography in detecting knee synovitis was demonstrated, and a high level of inter-observer and intra-observer reproducibility was documented. This confirms several previous studies validating ultrasonography against a variety of imaging techniques, including MRI [15]. The ability of ultrasonography to accurately and reproducibly detect erosions, synovitis, and tendon abnormality clearly has potential implications in the early diagnosis and ongoing management of rheumatoid arthritis.

**Power Doppler ultrasonography**

The addition of power Doppler to conventional grey scale ultrasonography allows assessment of soft tissue vascularity and potentially can provide information about synovial vascularity, morphology, and temporal changes [16]. The technique has been validated against histopathology and MRI, and it seems to correlate with synovial inflammation and to be sensitive to change [17–20,21]. A recent study demonstrated the prognostic potential of grey scale and power Doppler ultrasonography in early RA. Patients with early erosive disease, taking stable doses of methotrexate, were randomised to receive infliximab or placebo infusions [22]. Both grey scale and power Doppler ultrasonography were more sensitive than traditional clinical outcome measures in discriminating between the treatment groups. In the methotrexate monotherapy arm, synovial thickening and vascularity as demonstrated by grey scale and power Doppler ultrasonography at baseline were predictive of radiographic scores at 12 months. This finding is consistent with our view, which is discussed later, that biologic therapy in combination with methotrexate within the window of opportunity has the greatest potential to alter prognosis. Presently, power Doppler ultrasonography remains a research tool.

**Magnetic resonance imaging**

Magnetic resonance imaging is able to identify erosions at an earlier stage than conventional radiography [23,24]. As discussed earlier, whether MRI is more sensitive to erosive changes than ultrasonography has been investigated, with conflicting results [10,11]. MRI has been demonstrated to show progression of rheumatoid erosions with time, despite improvements in clinical parameters due to treatment [25]. Investigation is currently ongoing to develop a reliable system to quantitatively score MRI-detected erosions and temporal changes [26]. This process may prove invaluable in the clinical trial setting.

Magnetic resonance imaging useful in the imaging of soft tissue structures such as synovium and tendons, and, when it is used with a contrast agent such as gadolinium diethylenetriamine pentaacetic acid, synovial inflammation can be differentiated from fat. Like ultrasonography, MRI has been shown to be more sensitive than clinical examination in detecting synovitis [27,28,29]. In a recent study of patients with early RA and no radiographic erosions on radiographs of the hands or feet, MRI was able to detect synovitis or bone oedema in the feet despite the absence of abnormalities in the hand. In addition, most of these patients had no clinical evidence of metatarsophalangeal joint swelling [30].

Magnetic resonance imaging may also have a prognostic role, because synovitis and bone oedema can predict the future development of erosions, and the absence of MRI-detectable erosions at baseline has been associated with the absence of radiographic erosive changes at 1 year [25,31]. MRI synovitis scores at the knee in 30 patients with early rheumatoid arthritis correlated with both MRI and conventional radiography erosion scores at 3-year follow-up [29]. In addition to prediction of structural outcomes, in contrast to conventional radiography, baseline findings of bone oedema and total MRI scores correlated with functional outcomes 6 years later [32].

Although MRI seems to be sensitive to changes in early RA, Ejbjerg et al. [33] question the specificity of MRI changes. They demonstrated that standard MRI images, scored according to the latest Outcome Measures in Rheumatology Group (OMERACT) consensus, occasionally depict changes that resemble mild synovitis or erosions in healthy individuals. The findings highlight the fact that issues of MRI specificity need to be further addressed before this modality can reliably be used diagnostically, and that investigations need to be interpreted within a clinical context.

The ability of MRI to detect bony and soft tissue abnormalities in the absence of radiographic abnormalities, and
the discordance between MRI findings and clinical parameters, suggest a potential role for MRI in guiding management decisions in early RA.

Imaging in the clinic
The application of ultrasonography and MRI to early RA is not yet fully refined and requires further investigation and validation, but it is likely that rheumatologists will begin to use these modalities in early arthritis clinics more commonly. D’Agostino et al. [34] recently demonstrated the relative ease with which rheumatologists inexperienced in ultrasonography were able to detect synovitis in the small joints of the hands. This demonstration of skill acquisition despite limited training raises important issues regarding the use of these imaging techniques in rheumatology clinics. If these modalities are to be used in rheumatology clinics, then issues such as the development of adequate training programs, curriculum, and assessment of competency needs to be addressed. Other issues include clinical governance, allocation of time, and how developing these skills as rheumatologists will affect our relationships with radiologists. Nevertheless, these modalities seem very promising and should have a role in the future management of early arthritis.

Intervention
By definition, DMARDs reduce the rate of radiographic progression of RA and result in improvement in outcome measures. Methotrexate, leflunomide, sulphasalazine, corticosteroids, cyclosporine, parental gold, and auranofin have all been shown to reduce radiographic progression [35].

Although the introduction of DMARDs at diagnosis is now standard practice in RA, the optimal therapeutic approach has yet to be determined. Strategies are diverse and involve serial monotherapy or combination therapy, which may be step-down or step-up. Combination DMARD therapy in conjunction with corticosteroids seem to outperform monotherapy. As an example, the Combinatietherapie Bij Rheumaatide Arthritis (COBRA) study compared a step-down protocol of short-term high-dose steroids, methotrexate, and sulphasalazine with sulphasalazine monotherapy in early RA [3]. At medium-term follow-up, a sustained reduction in the rate of radiographic progression was seen independently of DMARD therapy after the initial combination treatment regimen [36]. The step-down therapeutic approach uses intensive therapy early, with the aim of achieving disease control – ideally, remission – followed by less intensive maintenance therapy. The benefits of step-down combination and corticosteroid therapy over monotherapy have been confirmed recently by several studies [3,37,38]. In both the Tight Control of Rheumatoid Arthritis (TICORA) and Finnish Rheumatoid Arthritis Combination Therapy (FIN-RACo) studies, patients receiving combination therapy had improved short-term clinical and radiographic outcomes [3,37]. The long-term FIN-RACo findings were consistent with those of the COBRA study; early treatment with sulphasalazine, methotrexate, and corticosteroids resulted in better radiographic outcomes at 5 years, independently of DMARD therapy, after the initial period of combination therapy [39]. This study also documented economic benefits from this therapeutic strategy, because intensive therapy given early resulted in less long-term loss of productivity [40].

In contrast to the intensive early use of corticosteroid in a combination step-down regimen, prolonged low-dose steroid was recently shown not to confer added clinical or radiographic benefits over DMARD monotherapy [41].

The aim of intensive early therapy is to capitalise on a ‘window of opportunity’. It is hypothesised that there is a time frame early in RA in which intensive therapy may produce disproportionate and sustained responses not seen when treatment is applied later. Some evidence supports this concept. The FIN-RACo study showed that in the monotherapy arm, symptoms lasting longer than 4 months were a marker of poor prognosis [3]. In a parallel combination therapy arm, delay to institution of therapy did not restrict remission rates, suggesting that aggressive therapy may be able to alter baseline prognosis. The COBRA study, discussed earlier, showed that intensive therapy reduced the prognostic impact of the shared epitope [4,36]. These findings may be due to corticosteroid use, but the use of these drugs is not ideal because of the associated adverse effects.

The therapeutic possibilities have been significantly altered by the development of biologic agents. Biologic agents improve disease control in patients with established RA who have previously not responded adequately to conventional DMARDs. A recent study confirms that combination therapy with entanercept and methotrexate outperforms either drug as monotherapy in established RA [42]. A subanalysis of Anti–Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT), a study of the impact of combination infliximab and methotrexate therapy in RA, found that patients with early disease received the greatest benefits [43]. Several studies have now shown that biologic agents in DMARD-naive RA result in qualitatively better outcomes than does conventional DMARD therapy. The Early Rheumatoid Arthritis (ERA) study compared methotrexate monotherapy with etanercept monotherapy in early RA. Etanercept resulted in a more rapid clinical response and continued to show better clinical response rates at 2 years [44,45]. Etanercept was associated with reductions in the rate of radiographic progression, and it significantly reduced erosion and total Sharp scores. More recently, the
Active Controlled Study of Patients Receiving Infliximab for Treatment of Rheumatoid Arthritis of Early Onset (ASPIRE) study compared infliximab and methotrexate with methotrexate monotherapy in early RA and found that infliximab resulted in better disease control and better radiographic and functional outcomes at 12 months [46••]. Similarly, PREMIER compared adalimumab in combination with methotrexate with both drugs as monotherapy in early RA [47]. The monotherapy arms showed similar functional and Disease Activity Score (DAS) outcomes, but adalimumab monotherapy produced better radiographic outcomes. The combination arm showed significantly improved clinical and radiographic outcomes compared with both monotherapy arms. These studies of biologic therapy in early RA show that combination therapy with biologic agents and methotrexate produces the best results and that biologic monotherapy differs little from DMARD monotherapy except in reducing structural damage in early RA. Whether these findings will equate to long-term benefits is yet to be shown.

The potential of high-dose biologic therapy as an induction agent within the window of opportunity has been also addressed. The hypothesis that high-dose short-term anti—tumor necrosis factor agents could induce remission that could then be sustained without biologic therapy has been tested. Five patients with early RA and a poor prognosis were treated with high-dose infliximab for 3 months [48]. Clinical remission was achieved in only one patient, and imaging-defined remission was not achieved in any patients. Retreatment did not improve the outcomes, and all patients went on to require further therapy. Induction therapy with 12 months of standard-dose infliximab induction has also been studied. Combination therapy with infliximab and methotrexate followed by methotrexate maintenance was compared with methotrexate monotherapy [49]. The group treated with combination therapy showed rapid benefit in Health Assessment Questionnaire and Rheumatoid Arthritis Quality of Life scores, which were sustained for 1 year after the end of biologic therapy. There was no significant difference in DAS scores; however, the study was powered to demonstrate MRI and not clinical outcome endpoints. Importantly, no patients in the combination therapy arm experienced flares once infliximab was withdrawn.

The most effective way of suppressing radiographic progression in RA seems to be early use of corticosteroids or biologic agents. The Behandel-Strategieen (BeST) study compared step-down approaches of high-dose prednisolone or infliximab with sequential monotherapy or step-up combination therapy in early RA [50]. The step-down regimens performed better in clinical, functional, and radiographic outcomes; half the patients in the infliximab arm were able to have the drug withdrawn because of a sustained clinical response. Larger studies are required to investigate whether induction therapy with biologic agents will induce remission that is then maintainable with DMARDs.

**Conclusion**

The advent of new drug therapies and the use of earlier and more aggressive pharmacologic intervention have resulted in better outcomes for our patients. Newer imaging techniques are likely to be used in rheumatology clinics in the future and are likely to aid the early diagnosis of RA and to allow targeted intensive combination therapy within this window of opportunity. Although coming at a financial cost, induction with biologic therapies is likely to offer the best hope of halting radiographic progression and of drug-induced remission. If this is achieved, the financial cost may be justifiable.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

9 Lopez-Ben R, Bennreuter WK, Moreland L, et al. Ultrasound detection of bone erosions in rheumatoid arthritis: a comparison to routine radiographs of the hands and feet. Skeletal Radiol 2004; 33:80–84. This report documented the sensitivity of ultrasonography at detecting erosions in the metatarsophalangeal joints and also confirmed its ability to detect erosive disease in the metacarpophalangeal and metatarsophalangeal joints when conventional radiographs are normal.
Rheumatoid arthritis


This paper further defined the validity of MRI and ultrasonography compared with conventional radiography and demonstrated the ability of MRI and ultrasonography to track radiographic erosions with time. MRI was more sensitive to the presence of erosions than conventional radiography or ultrasonography; however, ultrasonography was demonstrated to be most useful at detecting joint and tendon sheath effusions.


This paper demonstrated the ability of ultrasonography to detect significant amounts of subclinical synovitis and provides information that can alter the clinical diagnosis. This clearly has implications in the management of inflammatory arthritis.


This report confirmed the superiority of MRI over clinical examination in detecting synovitis of the knee and also demonstrated the potential of MRI to predict structural outcomes.


This study was large compared with previous similar studies and confirmed the superiority of ultrasonography over clinical examination at detecting knee joint synovitis. It demonstrated the accuracy of the modality through validating ultrasonography against arthroscopy, and demonstrated the precision of ultrasonography through high inter-observer and intra-observer reproducibility.


This report further defined the validity of MRI and ultrasonography compared with conventional radiography in early RA.


This study demonstrated the ability of grey scale sonography and power Doppler sonography to detect changes in the corticosteroids over time and suggests a potential role for ultrasonography in the monitoring of response to therapy in RA.


This study demonstrated that the potential prognostic role of ultrasound as both grey scale and power Doppler findings at baseline were predictive of radiographic changes at follow-up in the less intensive treatment arm. It also gave further weight to the concept of the window of opportunity, in which early intensive therapy can overcome poor prognostic baseline features.


This study demonstrated the use of a quantitative scoring system to follow the progress of erosions.


This study demonstrated the ability of ultrasonography to detect significant amounts of subclinical synovitis and provides information that can alter the clinical diagnosis. This clearly has implications in the management of inflammatory arthritis.


This report confirmed the superiority of MRI over clinical examination in detecting synovitis of the knee and also demonstrated the potential of MRI to predict structural outcomes.


This study demonstrated the potential role of MRI in aiding the diagnosis of early RA, showing large amounts of abnormalities on MRI in the feet when radiographs of hand and feet and MRI of the hands are normal.


This report demonstrated a potential prognostic role of MRI because baseline MRI findings predicted of medium-term functional outcomes. It also confirmed previous studies showing no relation between baseline conventional radiographic findings and medium-term functional outcomes.


This study demonstrated MRI abnormalities in healthy individuals and questioned the specificity of MRI-detected changes.


This study confirmed the superior efficacy of combination DMARD therapy in comparison with monotherapy in early RA. It also demonstrated the importance of tight control; the use of frequent assessments and objective outcome measures to guide management decisions resulted in better outcomes than those provided by routine care.


This study demonstrated that intensive combination therapy given early results in prolonged benefits.


This study confirmed the findings of the COBRA study that early treatment with sulfasalazine, methotrexate, and corticosteroids resulted in better radiographic outcomes at medium-term follow-up, independently of DMARD therapy, after the initial period of intensive combination therapy and corticosteroids. It adds further weight to the concept of a window of opportunity.


This excellent study showed that early intensive therapy reduces long-term work disability and lost productivity. This has important health economic implications. Similar studies need to be undertaken, addressing whether biologic agents can further improve these outcomes.

This study demonstrated that the addition of routine low-dose steroids to DMARD monotherapy in early RA does not result in improved radiographic or clinical outcomes. This is in contrast to other studies in which early use of high-dose steroids with combination DMARD therapy conferred radiographic benefits, and again suggests that there may be a window of opportunity in which intensive induction therapy produces the best results.


Although this study was in patients with established RA, it was a head-to-head study of etanercept and methotrexate therapy compared with either drug as monotherapy. The primary radiographic endpoint of change in total radiographic damage at 52 weeks was achieved. Combination therapy proved to be better than either monotherapy in established RA.


This subanalysis of the ATTRACT study showed that combination therapy is likely to be most beneficial in early RA.


In accordance with the ERA study, this study demonstrated the efficacy of biologic and methotrexate therapy over methotrexate monotherapy in early RA with regard to functional, clinical, and radiographic outcomes. Given that methotrexate monotherapy has been considered the gold standard of treatment for RA, these findings have an important implication as to how patients should be treated in early disease, when a window of opportunity is available.


Vascular comorbidity in rheumatoid arthritis: potential mechanisms and solutions
Naveed Sattar and Iain B. McInnes

Purpose of review
To summarise recent evidence for elevated risk of coronary heart disease (CHD) in rheumatoid arthritis (RA) and explore explanatory mechanisms and modalities that may lessen such risk.

Recent findings
Evidence for elevated CHD risk in RA is convincing. On current estimates, individuals who have had RA for several years have around a twofold higher risk for CHD compared with non-RA persons after taking account of most traditional risk factors. Such excess risk appears to be driven by systemic inflammation both directly via its deleterious effects on blood vessels (endothelial dysfunction inclusive of myocardial microvascular abnormalities) and indirectly by its accentuation of multiple risk pathways including lipid abnormalities. Established therapies that lessen RA disease activity and systemic inflammation will likely lessen CHD risk, although there remains considerable scope for more robust studies employing better measures of vascular disease (e.g., carotid intima–media thickening). Other emerging evidence indicates statins may have dual effects in RA, with a modest disease-modifying effect (requiring confirmation) and significant lipid-lowering action. The latter finding is particularly important because extrapolation of data from all statin endpoint trials suggests that the extent of low-density lipoprotein cholesterol reduction may account for most statin clinical benefit.

Summary
Systemic inflammation is the major driver for excess vascular comorbidity in RA. Controlling systemic inflammation should lessen vascular risk but complete, long-term suppression of articular inflammation is rarely achieved. Thus, the use of conventional CHD risk reduction strategies, in particular statins, should be considered in patients with RA with prevalent CHD or at elevated risk.

Keywords
endothelial dysfunction, epidemiology, lipids, statins, systemic inflammation

Abbreviations
CHD coronary heart disease
CRP C-reactive protein
DMARD disease-modifying antirheumatic agent
HDL high-density lipoprotein
IMT intima–media thickening
LDL low-density lipoprotein
RA rheumatoid arthritis
TNF-α tumor necrosis factor-α

Introduction
The excess risk of vascular disease in rheumatoid arthritis (RA) has been known for many decades [1] but at the clinical level, coronary heart disease (CHD) risk assessment or treatment in RA has been less well characterised. Increasing awareness over the past decade of inflammation as a novel player in the origin of cardiovascular disease in the general (non-RA) population [2] has stimulated resurgent interest in potential mechanisms underpinning excess CHD risk in RA. This review summarises recent evidence in this field and, by extrapolating from work in the cardiovascular arena, discusses how best to incorporate findings into clinical practice to improve management of CHD risk in RA.

Epidemiologic assessments of coronary heart disease risk in rheumatoid arthritis
Van Doornum et al. [3] summarised evidence from 21 observational studies published prior to and including 2000 examining CHD risk levels in RA. They noted that 17 of the 21 studies show an increased standardized mortality ratio in RA and that life expectancy is shortened by 3–18 years. Pooled analysis of these studies suggests a 70% increased risk of death in RA patients. Most such studies examined RA patients with duration of disease of 10 years or longer. Recent studies have corroborated and extended earlier evidence. For example, Solomon et al. [4] compared incidence rates of myocardial infarction and stroke in persons with and without RA among the 114,342 women in the Nurses’ Health Study. Multivariate pooled logistic regression was used to adjust for potential cardiovascular risk factors. A total of 527 incident cases of RA and 3622 myocardial infarctions and strokes were confirmed during 2.4 million person-years of follow-up. The adjusted relative risk of myocardial infarction in women with RA compared with those without was 2.0 (95% CI, 1.23–3.29). Women who had RA for at least 10 years had a risk for myocardial
infarction of 3.10 (95% CI, 1.64–5.87). Importantly, the endpoint data were prospectively gathered and validated with record acquisition. In addition, the risk for myocardial infarction was hardly attenuated following adjustment for most traditional risk factors, although it should be noted that high-density lipoprotein (HDL) cholesterol was not included as a potential confounder, and future epidemiologic studies should address this deficiency. Similarly, analysis of the relevant data from the UK General Practice Research Database (in total, >2 million patients) confirms elevated CHD risk in RA patients [3]. Finally, investigators in Malmo [5*] recently reported a standardized mortality ratio of 176 for myocardial infarction in their RA patients compared with the general population. Relevant evidence from all studies suggest CHD risk is equally elevated in men and women with RA and increases with disease severity and evidence of extra-articular disease and with disease duration [3,4].

**Inflammation and atherogenesis in the general population**

Interest in understanding CHD risk in RA has been stimulated by recognition of the inflammatory basis for cardiovascular disease in general. For example, it is known that plaque composition of unstable coronary lesions includes an abundance of inflammatory moieties and immune cells at the shoulder region, which contribute to erosion of the collagen cap that separates the atheromatous material of the plaque from the lumen [2]. This appearance is strikingly similar to the phenotype of inflammatory synovitis in RA [6]. Although elevated systemic markers of inflammation, albeit at considerably lower levels than those apparent in RA, independently predict CHD events in the general population, the level of independent prediction afforded by, for example, C-reactive protein (CRP) is debated [7*]. There also remain uncertainties about causal relationship of the ‘low-grade’ inflammation in the atherogenic process. Some argue that the inflammatory features within an evolving plaque may largely be consequent to entry of atherogenic low-density lipoprotein (LDL) species into the vessel wall, which, in turn, sets in motion a chain of molecular events leading to macrophage recruitment and subsequent foam cell formation [8]. Thus elevated systemic inflammatory markers may arise from diseased blood vessels — so-called reverse causation. Alternatively, elevated (low-grade) systemic inflammation levels may stem from multiple lifestyle factors that can increase CHD risk by mechanisms other than inflammation (Fig. 1). Indeed, adiposity especially [9*], but also smoking, poor diet, low physical activity, and deprivation are linked to elevated detectable inflammation in the general population, manifest by systemic CRP estimation, but such factors are also linked to many other atherogenic pathways. Of note, adiposity explains as much as 30% of the systemic inflammatory burden in population studies, and recent evidence indicates that obesity per se leads to excess and likely detrimental macrophage recruitment into adipose tissue [10]. Thus further studies are required to disentangle the link between ‘low-grade’ inflammation and cardiovascular disease, inclusive of genetic studies and clinical trials determining the vascular effects of specific anti-inflammatory agents.

**Inflammation and atherogenesis in rheumatoid arthritis**

In contrast to the complexities of linking low-grade inflammation and CHD pathogenesis, the inflammation–CHD link in RA is, paradoxically, easier to establish (Fig. 1). Systemic inflammation levels in individuals with RA are often far greater than in those without RA, well above levels attributed to lifestyle factors (e.g., obesity, smoking) or diseased blood vessels. Rather, the driver to systemic acute-phase reactants in RA is predominantly synovial inflammation and subsequent cross-talk with the liver via cytokine release. On the basis of several lines of evidence, we [11**] and others have argued that such ‘high-grade’ inflammation is likely pivotal to the accelerated CHD in RA. Such evidence includes the following:

- RA disease severity (however measured) predicts magnitude of CHD risk
- Excess CHD risk in RA is minimally attenuated by traditional risk factor adjustment
- Surrogate markers of vascular disease (carotid IMT, endothelial function measures) are linked to systemic inflammation markers
- Perturbations in many other pathways correlate to degree of systemic inflammation
- Dampening of inflammation in RA by a variety of therapies improve endothelial function, lipid profile (most studies), insulin action, and even homocysteine concentrations
- Cytokines are pleiotropic: they have well-documented metabolic as well as immune effects
- Animal data suggest pro-inflammatory cytokines can promote atherogenesis whereas inhibition of cytokines lessens this process.

**Details of evidence linking inflammation in rheumatoid arthritis to coronary heart disease**

Risk of CHD in RA is related to number of inflamed joints [10] and appears greatest in RA patients with extra-articular disease or those in tertiary referral centers.

Conventional risk factors do not account for excess CHD risk in RA patients. In a recent analysis from the Nurses’ Health Study, although inflammatory markers (inclusive of CRP, intercellular adhesion molecule-1) were substantially elevated in women with RA compared with women without RA, most traditional CHD risk factors were similar [12*]. In addition, this group and others [4,13] have recently demonstrated that adjustment for most
traditional risk factors minimally attenuates the RA to non-RA difference in CHD event rates.

Carotid artery intima–media thickening (IMT) (and plaque), a Food and Drug Administration–approved surrogate marker of vascular disease, was elevated in RA patients in association with elevated markers of inflammation [14]. More significantly, arterial thickening in women with RA, as measured by change in carotid IMT over 18–36 months, was accelerated compared with healthy controls and such increase in IMT was related independently to serum CRP concentration but not conventional risk factors [15].

Endothelial dysfunction has been suggested as both an early event in the atherogenic process and as a novel predictor of CHD events; recent longitudinal studies in the CHD arena provide some support for this proposition [16]. Several studies employing varying ‘direct’ measures of vascular function such as pulse wave analysis [17], flow-mediated vasodilation [18], or venous occlusion plethysmography [19] confirm endothelial dysfunction in RA patients. Where examined, such dysfunction has been linked to systemic inflammatory markers. Vaudo et al. [20] have provided recent evidence for endothelial dysfunction in young to middle-aged patients with low disease activity (disease activity score ≥3.2) and noted a strong association to average CRP levels. Interestingly, elevated LDL cholesterol was also an independent correlate to impaired vascular function in RA patients in the latter study.

Recent longitudinal studies report improved endothelial function following anti–tumor necrosis factor-α (TNF-α) therapy [21,22] but such benefit appears to be transient and linked to the pattern of change in systemic inflammatory markers upon TNF blockade. Treatment with disease-modifying antirheumatic drugs (DMARDs) also improves endothelial function in patients with RA [19].
so that the mechanism employed to achieve inflammatory control does not appear to be critical to improvement in endothelial function.

Numerous other risk factors beyond endothelial dysfunction can be directly and adversely influenced by the systemic inflammatory response in RA \cite{11**} (Fig. 1). Thus the lipid profile, despite showing lower LDL cholesterol, is more atherogenic (e.g., low HDL cholesterol, greater preponderance of smaller atherogenic LDL species); there is greater peripheral insulin resistance; altered body fat distribution (peripheral wasting, central accumulation); prothrombotic effects; pro-oxidative stress; and intriguingly, some evidence suggesting inflammatory cytokines may also elevate homocysteine concentrations in RA.

Cytokines released from inflamed synovial membrane can exert metabolic effects via effects on distant tissues including adipose tissues, skeletal muscle, liver, and vascular endothelium. One consequence of this functional pleiotropy is that the intensity of the metabolic adaptation could parallel other cytokine effects. Cytokine-induced metabolic effects, which include transient alterations in lipids and peripheral insulin resistance, are favourable in the short term and function as part of host response to infection and acute inflammation to target specific metabolic fuels to and from essential organs. Chronic elevation in cytokine levels, irrespective of magnitude or cause, however, is deleterious and promotes accelerated atherosclerosis via aggravation of several risk factor pathways as described above. From a developmental standpoint, cytokines or cytokinelike molecules such as interleukin-6 \cite{23} or leptin \cite{24} may have evolved to impart their systemic metabolic effects at very low levels, such that even minor degrees of chronic elevation are damaging. As a result, we recently argued \cite{11**} that both the magnitude and chronicity of systemic inflammation in RA are particularly deleterious. Thus, even during ‘quiescent’ phases of the disease, systemic levels of cytokines or their regulatory components often remain dysregulated relative to persons without RA and, as such, may continue to promote vascular disease \cite{11**}.

Numerous model data support a proatherogenic role for cytokines \cite{25,26}. A very recent study in apolipoprotein E knockout mice demonstrated reduced atherosclerosis via inhibition of TNF-α \cite{27}.

**Inflammation-mediated microvascular dysfunction as a cause of myocardial ischaemia in rheumatoid arthritis?**

Much recent evidence in the general CHD area suggests that patients with exercise-proven myocardial ischaemia do not necessarily demonstrate obstructed coronary vessels on angiography. Rather, as in women with cardiac syndrome X, myocardial ischaemia may manifest from dysfunctional endothelium in coronary vessels or in the myocardial microvasculature, which under conditions of stress vasoconstrict to cause symptoms. We recently suggested that independent of obstructive coronary disease, insulin resistance could be a major contributor to myocardial ischaemia via endothelial dysfunction \cite{28}. We proposed insulin sensitisation as a novel mechanism to lessen anginal symptoms in persons with and without diabetes. Such work is now ongoing in formal clinical trials. Consistent with this concept, Raza et al. \cite{29} recently reported complete reversal of significant myocardial ischaemia (proven by thallium scanning) in a 62-year-old man with RA upon intensive immunosuppression. Coronary angiography demonstrated no significant atheroma. This group argued that systemic inflammation-driven myocardial microvascular abnormalities may be as important to the pathogenesis of ischaemic heart disease in RA as atherosclerotic narrowing \cite{29}. Clearly future studies will be needed to develop these important findings but two additional factors merit comment. Firstly, recent data from noninvasive measurement of endothelial function and carotid atheroma burden in patients with coronary artery disease suggest that both structural and functional status of the vasculature are independent predictors of coronary events \cite{30}. Secondly, conventional coronary angiography can miss unstable lesions; it is now clear that arterial wall remodeling permits accumulation of a large atherosclerotic burden before there is any detectable narrowing of the vessel lumen by conventional angiography \cite{31}. Rather, novel imaging modalities such as intravascular ultrasound allow better detection of such ‘silent’ plaques \cite{31}.

**Lessening vascular risk in rheumatoid arthritis via inflammatory suppression**

The foregoing arguments suggest that absolute and long-term suppression of the systemic inflammatory response in RA should lessen CHD risk by improving risk factors. The overwhelming balance of evidence favours this likelihood including recent epidemiologic findings. In an 18-year follow-up of 1240 patients with RA, Choi et al. \cite{32} reported that methotrexate treatment, generally considered to be the most effective ‘nonbiologic’ DMARD, reduced overall mortality by 60% (95% CI, 20–80%) primarily by reducing CHD mortality by 70% (95% CI, 30–80%). Non-CHD mortality was not significantly altered. Others have shown that using one DMARD reduced risk of death in RA. The fact that methotrexate or indeed other DMARD therapy (in spite of some potentially toxic effects) appears to lessen CHD risk in RA clearly favours a dominant role of systemic inflammation in accelerating CHD in such patients. In addition, at the risk factor level, there is now accumulating evidence \cite{11**} for improvement in many pathways upon inflammatory suppression in RA. In particular evidence for improvement in endothelial function has been demonstrated.
for TNF blockade, DMARDs, and steroid therapy [19,21,22**,33].

There remain a number of cautionary points. Firstly, epidemiology does not always teach the correct answer; the recent example of HRT and CHD risk is noteworthy. Secondly, evidence of anti-inflammatory therapy benefits on endothelial function measures cannot be used as evidence of their cardioprotective ability because other pathways are often influenced simultaneously. Thirdly, two small, uncontrolled studies indicate a ‘reduction’ in the cardioprotective ability of HDL cholesterol concentrations with anti-TNF-α therapy [34*,35]. Finally, TNF-α blockade may lead to other toxic effects [36], including potential harm in patients with heart failure [37]. There is clearly a need for larger controlled studies to expand the evidence base in the RA therapies–CHD risk field, and wherever possible these should incorporate a wide range of risk factors and established vascular measures. The use of carotid IMT measurement in trials is to be encouraged and, in the near future, MRI and other novel imaging methods (e.g., intravenous ultrasound) may lead to significant advances in knowledge in the RA–CHD field.

**Statins to lessen coronary heart disease risk in rheumatoid arthritis?**

Since complete and absolute long-term suppression of systemic inflammation is rarely achieved in RA, even with the advent of more potent therapies, vascular risk will continue to be elevated. As a result, there is merit in considering other proven measures to lessen CHD risk in RA.

The lipid-lowering effect noted by us [40**] was in keeping with the magnitude of changes seen in the non-RA population [43]. This is important because, despite a tendency to lower LDL cholesterol in RA, the overall lipid pool in RA is highly atherogenic and, in addition, extrapolation from data from all statin endpoint trials suggests that the extent of LDL cholesterol reduction may account for most statin clinical benefit [44**]. It should also be noted that statin-induced reductions in CHD risk occur even when starting LDL cholesterol is considered ‘average’ or ‘low’ and that optimal LDL cholesterol levels may be around 1.3–1.8 mM [44**].

Of note, Van Doornum et al. have now confirmed that statins substantially improve RA lipids in a smaller uncontrolled study that also reported statin-induced reduction in arterial stiffness [45*], previously demonstrated to be elevated in RA. Clearly, endpoint studies with statins in RA would be ideal but in the interim phase, statin effects on progression of carotid IMT would be informative.

**How to assess and manage coronary heart disease risk in rheumatoid arthritis?**

Given the elevated risk of CHD in RA, it would seem worthwhile to incorporate CHD risk screening in this population by measuring traditional risk factors. In the United Kingdom, for example, the following parameters are used to assess risk in the primary prevention setting: age, sex, smoking, systolic blood pressure, and ratio of cholesterol to HDL cholesterol. Diabetes is unlikely to be in future
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primary prevention charts because patients with type 2 diabetes who are older than 40 years of age are now considered at sufficiently high risk to warrant statin therapy [47]. Thus, all that is required in RA patients in addition to available clinical data (age, sex, smoking history) is the measurement of systolic blood pressure and cholesterol and HDL cholesterol concentrations in serum or plasma. Moreover, with many laboratories now offering direct HDL cholesterol in nonfasting samples (cholesterol and HDL cholesterol levels are nearly identical whether fasting or not), such assessments can be made as part of routine RA assessment. The collection of such data will permit calculation of CHD risk based on existing charts — but, importantly, on the basis of current risk ratios, the figure derived from the chart could be multiplied by a factor of 1.7–2.0 to derive a better and more ‘accurate’ level of risk in RA patients. This takes account of evidence presented earlier [4,5,6] suggesting 70–100% higher CHD event rates in RA patients. Some investigators have reported higher CHD event rates in RA relative to non-RA persons but with wider confidence intervals [13]. In studies in which most traditional risk factors (with exception of HDL cholesterol) have been accounted for [4,13], such excess CHD risk has been minimally attenuated. Thus, if traditional risk factors suggest a 10-year CHD risk of 10% in an individual who has had RA for several years (perhaps ~10 years), then the actual risk is more likely closer to 20%. Currently in the United Kingdom, statin therapy is targeted to those without existing vascular disease if the CHD risk level is calculated to be 30% or greater over 10 years. In the near future, risk thresholds for statin treatment are likely to be based on overall vascular risk (CHD, stroke, peripheral vascular disease) and to be revised downwards.

Of course, patients with RA who have prevalent vascular disease (whether CHD, stroke, or indeed peripheral vascular disease) should be considered for statin therapy irrespective of risk factor measures because they fulfill secondary prevention categorisation. It should also be noted that it is the CHD risk level and not the cholesterol level that dictates whether statin treatment is applicable. The above recommendations, although not comprehensive, offer a pragmatic approach to CHD risk management in RA in the context of currently available evidence. Clearly, however, there is a need for better quality data to improve CHD risk assessment in RA.

Conclusion

Evidence for elevated CHD risk in RA is abundant and convincing — best estimates indicate that individuals who have had RA for several years have about a twofold higher risk for CHD compared with non-RA persons independent of most traditional risk factors. Future epidemiologic studies should include HDL cholesterol as a potential explanatory risk parameter. Such excess CHD risk appears to be driven in the main by systemic inflammation both directly by its deleterious effects on blood vessels and indirectly by its accentuation on multiple risk pathways inclusive of lipids, insulin metabolism, clotting, and oxidative parameters. A contribution from existing antirheumatic therapies may also be implicated. Established therapies that lessen disease activity and thus systemic inflammation will likely lessen CHD risk although there remains scope for larger, robust studies. Emerging evidence indicates statins may have dual effects in RA, with a modest disease-modifying effect (requiring confirmation) and a significant lipid-lowering effect, equivalent to the magnitude of lipid reduction in non-RA persons. The latter finding is particularly important because extrapolation of data from statin endpoint trials suggests that the extent of LDL cholesterol reduction accounts for most statin clinical benefit. It would seem sensible to consider statin therapy in RA patients with existing vascular disease and to assess CHD risk in RA patients without prevalent CHD. The latter would involve minimal additional tests (blood pressure and nonfasting lipids in majority) and use of available risk factor charts. Since most RA patients continue to exhibit significant systemic inflammation despite potent therapy, it would seem sensible to multiple risk levels derived from such charts by a factor of around 1.7–2.0 to derive the likely level of risk in RA patients. It should also be noted that it is the CHD risk level and not the cholesterol level that dictates whether statin treatment is applicable.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
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•• of outstanding interest

Rheumatoid arthritis


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Hypoxia and angiogenesis in rheumatoid arthritis
Peter C. Taylor and Bran Sivakumar

Purpose of review
Angiogenesis is a prominent feature of rheumatoid synovitis. Although new blood vessels deliver oxygen to the augmented inflammatory cell mass, the neovascular network is dysfunctional and fails to restore tissue oxygen homeostasis, so that the rheumatoid joint remains a markedly hypoxic environment. The purpose of this review is to discuss the role of hypoxia and angiogenesis in the pathogenesis of rheumatoid arthritis.

Recent findings
Vascular pathologic change, in the form of angiogenesis, is important in the perpetuation of rheumatoid arthritis and, in the form of endothelial dysfunction, contributes to associated cardiovascular comorbidity. Recent data suggest that tumor necrosis factor-α blockade may modify the vascular pathologic changes in rheumatoid arthritis. Angiogenesis is a prominent feature of rheumatoid synovitis. Emerging evidence based on ultrasonographic vascular imaging and angiogenic biomarkers implicates angiogenesis in the active phase of erosive disease. Many factors contribute to the profoundly hypoxic environment that can arise within the joint affected by rheumatoid arthritis. At a cellular level, hypoxia is detected by a mechanism that regulates cytoplasmic concentrations of hypoxia-inducible factor-1α. After translocation to the nucleus, hypoxia-inducible factor-1α binds its partner hypoxia-inducible factor-1β to form a heterodimeric, functional transcription factor, hypoxia-inducible factor-1, which activates a gene program associated with angiogenesis, glycolysis, and adaptation to pH.

Summary
Despite the luxuriant vasculature associated with rheumatoid arthritis synovitis, the joint affected by rheumatoid arthritis is hypoxic. Repetitive cycles of hypoxia and reoxygenation together with oxidants produced by phagocytic cells promote chronic oxidative stress within the microenvironment of the affected joint, leading to the generation of reactive oxygen species with the potential to contribute to tissue damage.

Keywords
angiogenesis, hypoxia, hypoxia-inducible-factor rheumatoid arthritis, vascular endothelial growth factor

Abbreviations
HIF hypoxia-inducible factor
IL interleukin
RA rheumatoid arthritis
TNFα tumor necrosis factor-α
VEGF vascular endothelial growth factor

Introduction
Rheumatoid arthritis (RA) is characterized by polyarticular synovitis with accompanying degradation of cartilage and bone, which often results in loss of structural integrity. This degradation is mediated by several proteolytic enzymes, and current evidence suggests that proinflammatory cytokines are responsible for inducing these catabolic processes. Newly formed blood vessels supply oxygen and nutrients to the proliferating synovial cells, and therefore synovial angiogenesis plays a key role in maintaining the inflammatory and invasive processes [1,2]. The angiogenic phenotype is promoted by several pro-angiogenic molecules, the most potent of which is vascular endothelial growth factor (VEGF). VEGF also induces vascular permeability and endothelial decay-accelerating factor, which is cytoprotective against activated complement and may regulate endothelial proliferation and angiogenesis [3,4].

In-vitro studies indicate that VEGF production can be independently upregulated by pro-inflammatory cytokines and hypoxia, but in vivo, these factors are interdependent (Fig. 1). Despite the angiogenic phenotype, RA joints are hypoxic. In this brief review the roles of hypoxia and angiogenesis in the pathogenesis of RA are discussed.

Hypoxia and rheumatoid arthritis
Synovial fluid samples from patients with RA are hypoxic and acidic, with low glucose and high lactate concentrations indicative of anaerobic metabolism in synovial tissues [5–7]. In the seminal work of Lund-Olesen [6], mean synovial fluid pO2 in RA knee joints was reported to be as low as 27 mm Hg compared with 43 mm Hg in osteoarthritis and 63 mm Hg in traumatic effusions in otherwise healthy control individuals. Subsequent studies supported these findings and even recorded PO2 values below 15 mm Hg [7]. More recently, using silver microelectrodes, our group has recorded mean intra-articular PO2 values of 13 mm Hg in mice with established collagen-induced arthritis and similar levels in patients with inflammatory arthritis [8,9].
Figure 1. Vascular endothelial growth factor (VEGF) is the most potent growth factor characterized to date with specificity for endothelial cells

Production of VEGF production from synoviocytes in vitro can be independently regulated by certain pro-inflammatory cytokines, such as tumor necrosis factor-α, and by hypoxia. But in vivo, these regulatory factors are interdependent. Metabolically active cells involved in sustained synovitis consume oxygen and promote a hypoxic environment. This environment in turn stimulates VEGF production and formation of new blood vessels in an unsuccessful attempt to restore oxygen homeostasis.

Tissue response to hypoxia

The cloning and molecular characterization of the transcription factor hypoxia-inducible factor (HIF)-1 in recent years represents a significant advance in understanding cellular adaptations to hypoxia [10]. HIFs are heterodimeric transcription factors that regulate several adaptive responses to low oxygen tension. HIF-1 comprises HIF-1α (120 kd) and HIF-1β (91–94 kd), subunits that both contain a basic helix–loop–helix domain that permits the recognition and binding to the HIF-1 DNA binding site within regulatory sequences of hypoxia-inducible genes. Peptide and nucleic acid sequence analysis has shown that HIF-1α is identical to the dioxin receptor aryl hydrocarbon nuclear translocator [10]. Intracellular concentrations of HIF-1β are oxygen independent. By contrast, HIF-1α is undetectable in aerobic conditions because of rapid ubiquitination followed by proteosomal degradation that is mediated by von Hippel–Lindau tumor suppressor factor [11]. This interaction requires the oxygen-dependent hydroxylation of three amino acid residues. This critical hydroxylation event becomes rate limiting in hypoxic conditions, so that HIF-1α is no longer degraded [12**]. At oxygen concentrations below 6%, cellular HIF-1α levels rise exponentially to a maximum at approximately 0.5% corresponding to PO2 values of 10 to 15 mm Hg [13]. HIF-1α is then free to bind its constitutively expressed partner, HIF-1β, thus completing the HIF-1 complex that translocates to the nucleus, where it binds hypoxia-responsive elements in the promoters of certain genes, thus upregulating a gene program associated with angiogenesis, glycolysis, and adaptation to pH [14,15].

In-vitro experiments suggest that stabilization of the HIF-1α transcription factor is mediated not solely by hypoxia but also by several growth factors and cytokines important in the pathogenesis of RA, including IL-1β. Furthermore, the addition of interleukin (IL)-1β or tumor necrosis factor (TNF)α to synovial fibroblast cultures up-regulates HIF-1α mRNA [16].

Contributory factors to hypoxia in rheumatoid arthritis

Contributory factors to hypoxia in RA joints include the high metabolic demands of inflamed synovial tissue and the rapid rate of synovial proliferation, so that cells become more distant from the closest blood vessels, compounds the hypoxic state [17]. Other factors promoting raised intra-articular pressures as high as 300 mm Hg include movement and accumulation of synovial fluid in involved joints. This further compromises vasculature and thus exacerbates hypoxia in an already ischemic environment. On completion of movement, intra-articular pressure normalizes and small vessels refill. A high proportion of vessels in rheumatoid synovia express neovascular markers, however, and they lack the accessory cells associated with mature vasculature that in health permit autorregulation of blood flow in response to tissue demand. Our group has demonstrated that in involved joints of mice with collagen-induced arthritis, tissue oxygenation is dysregulated in response to movement in comparison with healthy nonarthritic mice [8]. Similarly, dysfunctional vasculature in the RA joint fails to maintain adequate tissue oxygen homeostasis, and the joint may be susceptible to hypoxic-reperfusion injury, thus favoring a redox environment in which cell systems generate reactive oxygen species by nicotinamide-adenine dinucleotide phosphate—mechanisms [18–20]. If present in high concentrations, reactive oxygen species can lead to tissue damage.

Effects of hypoxia

In vitro, hypoxic culture conditions greatly augment VEGF secretion from synovial fibroblasts after stimulation by IL-1 and TGFβ [21]. Tissue hypoxia in the rheumatoid joint results in increased VEGF mRNA stability and enhanced VEGF gene transcription through the binding of HIF-1 and HIF-2 [22]. These transcription factors are overexpressed in the synovial lining and stromal cells of RA patients relative to synovial tissues from individuals without arthritis [23,24]. One study, however, has reported relatively lower expression of HIF-1α in osteoarthritis synovial tissues compared with RA tissues and determined by immunohistochemistry [23]. By contrast, another study has reported strong expression of HIF-1α and HIF-2α in osteoarthritis [24]. This may reflect differences in tissue processing and antibodies used. Other studies report more limited, patchy expression of nuclear HIF-1α expression in RA synovitis that predominates in the synovial lining layer. Furthermore, exposing fresh synovial tissue explants to hypoxic culture conditions markedly enhances the nuclear expression [25**]. In this way,
the hypoxic environment in the rheumatoid joint promotes transcriptional changes permissive for perpetuation of synovitis. Of note, the synovitis and articular damage associated with adjuvant-induced arthritis is markedly attenuated in mice with a specific deletion of HIF-1α in myeloid lineage cells [26]. In this model system, loss of HIF-1α prevents myeloid cell infiltration and activation through a mechanism independent of the HIF-1 target, VEGF, which is primarily a regulator of inflammation-associated edema. HIF-1α is essential for the normal regulation of glycolytic capacity in myeloid cells. The absence of HIF-1α results in profound reduction of the cellular ATP pool, and this metabolic defect leads to marked impairment of myeloid cell aggregation, motility, and invasiveness [26].

**Angiogenesis in the pathogenesis of rheumatoid arthritis**

Angiogenesis arises when hypoxic, diseased, or injured tissues secrete proangiogenic molecules; it is regulated by a complex set of inducers and inhibitors [1,2]. Angiogenesis is evident on microscopic examination of synovial biopsy tissue from the earliest stages of disease evolution. On arthroscopic inspection of RA joints it is observed as a fine network of vessels over the rheumatoid synovium [27]. The formation of new blood vessels permits a supply of nutrients and oxygen to the augmented inflammatory cell mass and promotes leukocyte ingress, thus contributing to the perpetuation of synovitis.

Studies in experimental models of arthritis suggest that destruction of bone and cartilage may be more closely linked to angiogenesis than to pannus swelling [28,29]. In support of the hypothesis that pannus in the active phase of erosive RA is vascular, our group has recently reported that quantitative power Doppler assessments of synovial vascularity, measured at baseline in all 10 metacarpophalangeal joints, correlate strikingly with the magnitude of radiologic joint damage over the following year [30**]. We have also observed that serum VEGF levels at presentation with early RA correlate highly significantly with the development of radiographic damage over the subsequent year as assessed in radiographs of the hands and feet [31]. Collectively, these observations warrant testing the hypothesis that serum and imaging markers of angiogenesis can be used to identify patients with early RA who are at the highest risk of accelerated joint damage and therefore merit intervention with biologic agents targeting TNFα.

**Vascular endothelial growth factor in rheumatoid arthritis**

In addition to hypoxia, several interdependent processes promote angiogenesis in the RA joint. These include shear stress on the endothelial wall as a result of increased blood flow as well as extravasated plasma proteins such as fibrinogen products. Furthermore, many soluble products of a range of inflammatory cells, including macrophages, lymphocytes, mast cells, and fibroblasts, promote angiogenesis. Among these products are the pro-inflammatory cytokines TNFα, IL-1, and IL-8. In addition, several endothelial growth factors have been demonstrated in RA synovium and tenosynovium, of which VEGF is the most endothelial cell–specific factor characterized to date [2,32–37].

Vascular endothelial growth factor exists as several isoforms generated by alternative splicing of VEGF mRNA [38,39]. There may be a link between VEGF gene polymorphisms and susceptibility to RA [40]. Fibroblast expression of VEGF is up-regulated by IL-1 and TNFα and also by the physical interaction of activated leukocytes and fibroblast-like synoviocytes and engagement of the CD40a ligand [41–44].

Vascular endothelial growth factor is detectable in serum, synovial tissue, and fluids of patients with RA [41,42,45–49]. Neutrophils secrete VEGF, and levels of neutrophil-associated VEGF in RA synovial fluids correlate well with free VEGF in joint effusions and with disease activity in the patient [50,51]. In vitro, human peripheral blood mononuclear cells release VEGF in response to cytokines occurring in RA joints, including TNFα [48]. Release of VEGF from platelets has also been reported [52]. Therefore, VEGF detectable in serum may be derived from several sources.

**Vascular endothelial growth factor, disease activity, and response to therapy**

Serum VEGF concentrations are elevated in patients with RA relative to healthy individuals and those with osteoarthritis. Furthermore, in RA, they correlate with individual and composite measures of disease activity, including acute phase markers and swollen and tender joint counts [31,53,54]. In a cohort of unselected patients attending a rheumatology clinic, we found that serum VEGF concentrations were higher in patients with newly diagnosed RA than in those with long-standing, treated disease [31]. This observation may represent a response to drug treatment, a view supported by other studies demonstrating reduction in serum VEGF concentrations after therapeutic intervention [31,46,52]. In our series, patients with early RA responding to disease-modifying antirheumatic drugs showed significant reductions in serum VEGF concentrations, in contrast to nonresponders to the same treatment, who showed no significant change in serum VEGF [31]. Blockade of TNFα in RA results in rapid marked reduction, but not normalization, of serum VEGF concentrations in a dose-dependent manner [55].

In health, angiogenesis is tightly regulated by a dynamic equilibrium between several inducers and inhibitors [1]. Significant reductions in serum VEGF, a potent inducer of angiogenesis, in response to treatment intervention
in RA suggests that an imbalance between inducers and inhibitors of angiogenesis contributes to persistence of joint inflammation. In support of this hypothesis, concentrations of endostatin, an angiogenesis inhibitor, are not elevated in serum or synovial fluid samples from patients in whom serum VEGF concentrations are raised, and serum endostatin levels are reported to rise after a single infusion with the anti-TNFα agent infliximab [52,56]. Another naturally occurring inhibitor of angiogenesis is soluble Flt-1, an alternatively spliced form of Flt-1, one of the tyrosine kinase receptors that mediate the action of VEGF [37,57,58]. We found serum sFlt-1 to be increased in patients with both early and long-standing RA compared with control individuals. Serum sFlt-1 levels positively correlate with VEGF concentrations in RA patients [31]. Elevated levels of sFlt-1 in RA sera presumably represent an attempted homeostatic mechanism that is inadequate to inhibit VEGF activity.

Diminished vascular permeability accompanying rapid suppression of VEGF levels is likely to be a factor contributing to the early reduction of joint swelling observed in RA patients after anti-TNFα therapy [55]. Furthermore, TNF blockade is followed by reduced synovial angiogenesis and vascular regression in RA and psoriatic arthritis after infliximab therapy [59,60,61,62].

**Endothelial dysfunction in rheumatoid arthritis**

Patients with severe RA die prematurely. The major cause of excessive mortality is cardiovascular disease. Just as the earliest stages of atherosclerosis are characterized by endothelial dysfunction, as demonstrated by abnormal blood flow responses to acetylcholine, recent data indicate that endothelial dysfunction is a feature of the early and established phases of RA [63,64,65]. This dysfunctional state is present independently of conventional cardiovascular risk factors for atherosclerotic disease [63]. This observation implicates other mechanisms associated with chronic inflammation and raises the question of reversibility of endothelial dysfunction with suppression of systemic inflammation in RA [66]. As measured by brachial ultrasonography, it has recently been reported that infliximab has a beneficial, but transient, effect on endothelial-dependent vasodilatation [67,68]. This benefit parallels improvement in disease activity and reduction in acute-phase markers [67]. Endothelial-dependent (post-ischemia), but not endothelial-independent (post-nitroglycerin), vasodilatation is significantly improved 2 and 7 days after infliximab infusion. By 4 weeks after infusion, however, endothelial function returns to baseline values [68]. These studies implicate TNFα as a contributory factor to endothelial dysfunction in RA and suggest that TNFα blockade may potentially retard clinical progression of atherosclerosis and, in particular, reduce the risk of plaque rupture and acute coronary syndrome in these patients.

**Conclusion and future perspective**

Angiogenesis is a prominent feature of rheumatoid synovitis. The formation of new blood vessels permits a supply of nutrients and oxygen to the augmented inflammatory cell mass and so contributes to the perpetuation of joint disease. Emerging evidence based on ultrasonographic vascular imaging and angiogenic biomarkers implicates angiogenesis in the active phase of erosive disease and supports the case for inhibition of synovial angiogenesis as a rational therapeutic strategy [2]. Despite the luxuriant vasculature associated with RA synovitis, the RA joint is hypoxic. Repetitive cycles of hypoxia and reoxygenation, together with oxidants produced by phagocytic cells, promote chronic oxidative stress within the microenvironment of the RA joint, leading to the generation of reactive oxygen species with the potential to cause tissue damage. Changes in cellular oxygenation regulate intracellular concentrations of the transcription factor HIF-1α, which activates a gene program permissive to the perpetuation of synovitis. For this reason, HIF-1α represents a potential molecular therapeutic target in RA. This is a theoretical consideration requiring further experimental substantiation, because expression of HIF-1α may also be of importance for the maintenance of cartilage integrity in a tissue environment that is relatively hypoxic even in health.

Vascular pathologic change, expressed in the form of endothelial dysfunction, also contributes to cardiovascular comorbidity associated with RA. It is possible that optimal and sustained suppression of synovitis in the treatment of RA may have the added benefit of reducing such comorbidities.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- **of outstanding interest**

Hypoxia and angiogenesis in rheumatoid arthritis


This excellent review summarizes current knowledge of the molecular signaling pathways that operate in response to hypoxia. Potential links between HIF-1 signaling pathways and the pathogenesis of RA are discussed.


This excellent comprehensive review discusses the evidence for the role of oxidative stress and hypoxia in the pathogenesis of RA. The potential to exploit aspects of these pathways in novel therapeutic interventions is also discussed.


Han SW, Kim GW, Seo JS, et al. VEGF gene polymorphisms and susceptibility to rheumatoid arthritis. Rheumatology (Oxford) 2004; 43:1173–1177. This paper explores relations between the clinical features of RA and four VEGF polymorphisms. The frequencies of two haplotypes were significantly increased in patients with RA compared with control participants, and carriers of the susceptible haplotypes had a younger age at disease onset but did not show a difference in the progression rate of radiographic joint destruction.


This study reports altered endothelial reactivity, as evidenced by reduced brachial vasodilatation, in patients with RA, including those with low disease activity and with no traditional cardiovascular risk factors and no overt cardiac disease.


The authors demonstrate that TNFα blockade with infliximab has a transient favorable effect on endothelial-dependent, but not endothelial-independent, vasodilatation as assessed by noninvasive brachial ultrasonography. All patients had been treated with infliximab for at least 1 year before the study. It is not yet clear whether such transient restoration of endothelial homeostasis actually retards the clinical progression of atherosclerosis in patients with RA.
Understanding the genetic contribution to rheumatoid arthritis
Annette H. M. van der Helm-van Mil, Joanna Z. Wesoly and Tom W. J. Huizinga

Purpose of review
The identification of the genetic variants that mediate the risk for susceptibility and severity of rheumatoid arthritis will allow the development of new drug targets and also increase the ability to predict disease course. Technical and methodologic progress has fueled the advances in this field.

Recent findings
The second risk factor for rheumatoid arthritis, the PTPN22 polymorphism, has been identified. This genetic variant regulates the threshold of T cell activation. Intriguingly, this variant is a risk factor for diabetes as well. Moreover, it has been shown that multiple genetic variants in one pathway (both in a transcription factor, RUNX-1, as in the transcription factor binding site of RUNX1 in the SLC22A4 gene) can each confer very small risks but by gene–gene interactions can confer a ninefold risk for rheumatoid arthritis. These genetic risk factors have been found to confer risk for multiple autoimmune diseases.

Phenotype–genotype interactions were described by the enhanced prevalence of a rheumatoid arthritis–specific autoantibody (anti–cyclic citrullinated peptide antibodies) in rheumatoid arthritis patients that harbor the rheumatoid arthritis–associated human leukocyte antigen class II genes, the shared epitope alleles. An environmental factor, smoking was demonstrated to confer risk for rheumatoid arthritis, especially in patients positive for both shared epitope and rheumatoid arthritis–specific anti–cyclic citrullinated peptide antibodies.

Summary
Two new pathways, T cell receptor signaling and a hematopoietic-specific signal transduction pathway, have been discovered that allow future pharmacologic interventions. The description of the new genetic risk factors and the interaction with environmental triggers as well as phenotypic features are gradually expanding the ability to predict disease susceptibility and course.

Keywords
rheumatoid arthritis, signal transduction pathway, T cell receptor

Introduction
The completion of human genome sequencing and the technologic revolution in genotyping is driving projects that aim toward an understanding of the genetic contribution to virtually every common disease by identifying sequence variants associated with these disorders. The motivation to study the genetic contribution is twofold. First, the identification of new critical pathways in disease pathogenesis leads to the identification of new drug targets, leading to more optimal treatments. Second, the increased understanding of pathogenesis will lead to better and more focused treatment protocols. The current clinical prediction models have insufficient power to provide patients with individualized treatments. Given the fact that the efficacy of treatments is proved at the group level by randomized controlled clinical trials, many patients are overtreated or undertreated. It is hoped that prediction of disease outcome by genetic risk factors may lead to more individualized treatment protocols.

For rheumatoid arthritis (RA), historically sound epidemiologic data (the different prevalence of RA in various populations, migration studies, familial clustering and twin studies) demonstrated the genetic contribution to susceptibility to RA [1]. The comparison between the concordance rates in monozygotic twins and the prevalence in the respective populations revealed that approximately 50% of the variation in occurrence of disease is caused by genetic factors [2]. Moreover, large initiatives from Japan, Europe, the United Kingdom, and the United States have been undertaken, in which multicase families are available and genome-wide searches are being performed. Finally, both the resources (large inception cohorts) and the tools (well-developed disease activity measurements and outcome measurements) are available in the field of arthritis to take maximum advantage of the automated genotyping technologies that enable the systematic ascertainment of sequence variants, in the form of single-nucleotide polymorphisms (SNPs), for the genomes of large numbers of individuals. During the past 2 years, this has led to
remarkable progress in understanding the genetic contribution to RA. This review is presented in four sections: progress following linkage data, progress from candidate genes from previously identified linkage regions in RA, progress from candidate genes from previously identified linkage regions in autoimmune disease in general, and progress on phenotypic-genotypic interactions.

**Progress following linkage data**

For the pan-European, the Japanese, the North American Rheumatoid Arthritis, and the United Kingdom consortium, genome scans have previously been performed with an average marker spacing ranging from 10 to 12 centimorgans (cM). The French group published in the past year a scan with a mean marker spacing of 3.3 cM, resulting in an average distance between any RA susceptibility gene and its nearest marker of 0.8 cM [3**]. Nineteen non—human leukocyte antigen (HLA) regions showed suggestive evidence for linkage \((P < 0.05)\). None of these overlapped with regions suggested in other published RA genome scans. To provide an estimate of the error rate of this approach, an assessment of the significance of the number of regions with suggestive evidence for linkage was obtained by using 10,000 computer simulations with the null hypothesis of absence of any true RA gene region. The probability of observing 19 non-HLA peaks by chance was 3.7%, which provided convincing evidence that these peaks contained at least 1 true non-HLA RA gene region. Given that a mean ± SD of approximately 11 ± 4 false-positive peaks were expected, the number of true RA linkage peaks was estimated to be 8 ± 4.

The United Kingdom group explored the utility of SNPs for linkage analysis. A whole-genome screen of 157 families with multiple cases of RA was performed by use of 11,245 genome-wide SNPs [4]. The SNP analysis detected HLA*DRB1, the major RA susceptibility locus \((P = 0.0004)\), with a linkage interval of 31 cM, compared with a 50-cM linkage interval detected by the previously published 10-cM microsatellite scan in the same cohort. Moreover, four additional loci were detected at a nominal significance level \((P < .05)\) in the SNP linkage analysis. The authors concluded that a dense SNP map was very suitable for performing linkage analysis in RA. This approach allowed loci to be defined more precisely.

**Progress from candidate genes from previously identified linkage regions in rheumatoid arthritis**

The Japanese consortium set out by densely mapping candidate regions that were originally defined by whole genome scans [5]. For the previously defined 1p36 region, the Japanese investigators reasoned that the unique specificity of anti—cyclic citrullinated peptide (CCP) antibodies for RA might be caused by differential citrullination caused by mutations in enzymes involved in citrullination. The 1p36 gene region contained all the known genes that encode peptidylarginine deiminases citrullinating (PAD) enzymes, the PAD genes (PAD types 1–4) in a region of 0.3 megabase. A case–control study using 830 RA patients and 736 healthy Japanese control participants identified an association between haplotypes (combinations of SNPs on one chromosome that tend to inherit together) of the gene encoding PAD4 with increased susceptibility to RA. The difference between two haplotypic variants was four SNPs in exons, with three subsequent amino acid substitutions. The RA-susceptible PAD4 variant was shown to produce a more stable transcript than the nonsusceptible variant, implying an increased production of PAD4 by the RA-susceptible variant. Circumstantial evidence for a role of PAD4 in RA was the detection of PAD4 in synovial tissue. Although this observation may provide insight into the generation of anti-CCP antibodies with the (not yet proven suggestion) that enhanced production of citrullinated antigens leads to a higher chance for the development of anti-CCP antibodies, it is not known whether this is specific for the Japanese population. Data from Caucasians in France as well as Caucasians in the United Kingdom showed no association with PAD4 haplotypes and RA [6,7]. More specific data on the PAD4 gene showed that this gene is extremely variable, at least in the white Caucasian population [8]. Therefore, the jury is still out on whether the observed association in the Japanese population is specific for this population or whether associations exist in other populations as well. This last option is important because this may indicate that the amount of citrullinated antigens can be a new target for therapy.

With respect to the progress on the 1p36 region, it is not yet known what proportion of this linkage peak can be explained by PAD4 gene variants. Another candidate gene in this region is the tumor necrosis factor (TNF) receptor type II gene, which has a polymorphism causing the amino acid substitution M to R at position 196. Initially, the United Kingdom group reported an association, but in French families this association between different variants of the TNF-RII gene could be replicated only in a subset of multicase RA families [9,10]. Finally, by taking advantage of the spectrum of phenotypes in a large inception cohort from the Netherlands, it was found that either in RA patients who experienced complete remission or in those with the worst progression to destructive disease and in healthy control participants, the genotype distribution was equal [11*]. Thus, in conclusion it is most likely that variants in the TNF-RII gene are not relevant for the susceptibility to, or the severity of, RA.

The HLA class II molecules are the most powerful recognized genetic factors for RA and contribute at least 30% of the total genetic effect. The HLA-DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408, *1001, and *1402
share a conserved amino acid sequence (QKRAA, QRRAA, or RRRAA) at position 70–74 in the third hypervariable region of the DRB1 chain. These residues constitute an α-helical domain forming one side of the antigen-presenting binding site. The shared epitope hypothesis postulates that the shared epitope motif itself is directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide. Extensive evidence exists showing associations between the shared epitope encoding alleles and susceptibility to RA as well as severity of RA [12–14]. Homozygosity for the shared epitope is associated with a higher risk for the development of RA and with more severe radiologic destruction [13,15]. Regional differences in HLA prevalence and association with RA exist. Associations between HLA-DRB1*0401 and *0404 and RA were first described in western Americans and in the population of northern Europe. HLA-DRB1*1402 was associated with RA in Native Americans, and associations with HLA-DRB1*0101 and *1001 were reported in Indian and Mediterranean patients [16–18]. By contrast, no associations were found in Greeks [19]. Extra-articular manifestations of RA, such as rheumatoid nodules, have been described to occur more often in shared epitope–positive patients [20]. Homozygosity for HLA-DRB1*0401 as well as homozygosity for two different shared epitopes encoding HLA-DRB1 alleles conferred a higher risk for the development of extra-articular manifestations than heterozygosity [20]. A relation of vasculitis with three genotypes containing a double dose of the shared epitope, specifically HLA-DRB1*0401/*0401, *0401/*0404, and *0101/*0401, has been observed as well [21].

Although it is accepted that the shared epitope encoding HLA-DRB1 alleles is associated with RA, a more controversial issue is the question whether predisposition to RA is also conferred by HLA-DQ alleles. Support for a role for HLA-DQ comes from studies in mice and humans [22–25]. The HLA-DQ alleles concerned are the DQ3 and DQ5 heterodimers. Given that they both are in strong linkage with some shared epitope alleles, the individual contribution of the HLA-DQ alleles is difficult to discern. Recently, the HLA region has been fine mapped [4**]. The highest linkage peak was located exactly at the DRB1 locus; however, when the wideness of the linkage peak is considered, haplotype associations cannot be excluded [4*]. Therefore, no definite evidence is available pinpointing RA susceptibility to either HLA-DR or HLA-DQ alleles.

Besides the above-mentioned predisposed effects of HLA-DRB1 alleles, there are also reports on protective effects by certain HLA-DRB1 haplotypes. These haplotypes contain, instead of the shared epitope, another common anchor region consisting of the amino acids DERAA. The HLA-DRB1 alleles that express the DERAA sequence (DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1304) have been shown to protect against RA [24–26]. These studies, however, were performed with relatively few RA patients carrying the DERAA haplotype [24,25]. There is also evidence that patients carrying the DERAA sequence have less erosive disease [27,28]. It is not known whether the effect of the DERAA encoding HLA-DRB1 alleles is truly protective or is due to the effect of the concomitant absence of shared epitope encoding HLA-DRB1 alleles (non-predisposition). More clarity will come from currently performed studies in which many patients with early RA have been monitored for 4 years. Subgroups of patients with the same amount of shared epitope alleles were compared in this study, and the effects of DERAA could therefore be differentiated from non-predisposition. It was observed that the DERAA haplotype conferred a lower risk for the development of RA and was associated with a lower rate of joint destruction (Van der Helm-van Mil, unpublished data).

Progress from candidate genes from previously identified linkage regions in autoimmune disease in general

Linkage data to select candidate genes can be alternatively used by searching for genomic regions that overlap in the scans reported for several diseases such as arthritis, diabetes, asthma, atopic dermatitis, osteoporosis, and inflammatory bowel disease [29]. One of the regions is the 5q31.1–q33.1 region. By very dense SNP mapping using a similar strategy as the PAD4 identification in the Japanese population, the Japanese group performed linkage disequilibrium mapping by the use of SNPs in a case–control approach [30]. In 820 RA patients and 620 control individuals, a risk for RA of 1.3 was identified for a risk allele in the organic cation transporter gene SLC22A4. The expression of SLC22A4 is specific to hematologic and immunologic tissues, and SLC22A4 is highly expressed in the inflammatory joints of mice with collagen-induced arthritis. Intriguingly, the identified SNP affects the transcriptional efficiency of SLC22A4 in vitro by altering the binding affinity of a hematopoietic transcription factor called RUNX1. The next SNPs in this transcription factor were analyzed as well. An association was observed with the minor allele in the RUNX1 gene, conferring a small but significant risk (1.3) in the comparison between 820 RA patients and 620 control individuals. Intriguingly, the biologic data suggest that these two SNPs would have a cumulative effect, given that a transcription factor with less binding capacity has to bind to a disrupted transcription factor binding site, thus resulting in overall loss of function. Indeed, in the analysis of the data from individuals who were genotyped for both SNPs (719 RA patients and 441 control individuals), it was observed that the genotype that was homozygous with respect to the susceptibility alleles of both genes showed a high odds ratio of 9 (95% confidence interval 2–39), whereas the genotype that was homozygous with respect to the susceptible allele
of SLC22A4 and heterozygous with respect to RUNX1 showed a moderately high odds ratio of 2.5 for disease.

The data have been replicated in two other diseases (psoriasis and systemic lupus erythematosus), and studies are under way in cohorts of RA patients of different ethnic backgrounds. This is a nice example of how gene–gene interactions may explain complex traits, while at the same time the crude odds ratio of the gene variant for the disease is quite low.

Progress from candidate genes
The methodology of searching for genes can be divided into the unbiased approach of linkage analysis and subsequent fine mapping. In this method, a linkage hot spot is covered with a grid of markers to search systematically for linkage disequilibrium, the nonrandom association of genes across the genome, and haplotypes. Next, the original region is narrowed, and the disease gene is identified. For multifactorial diseases like RA, the power to detect a risk gene for a common disease with a relative risk of two by linkage disequilibrium mapping necessitates data on transmission in 5000 affected and 5000 nonaffected individuals. Given the fact that these numbers are not available, the choice of a candidate gene has subjective elements because the genes that are intuitively logical will be tested first (see the above-mentioned examples of PAD4 and TNF-RII as candidate genes). A less biased approach is in genome-wide association studies. Patients and control individuals are unrelated and therefore more recombinations have taken place, leading to much smaller regions in which nonrandom association of genes is present. Thus, a positive result is less likely to be caused by linkage of recombining neighboring genes that explain the observed linkage pattern. The obvious curse is that selection of candidate genes is biased by limited knowledge. An interesting alternative was explored by Begovich et al. [31*], who tested the association of SNPs with putative functional consequences in different sets of patients and control individuals. This yielded a large number of putative functional polymorphisms distributed differently in patients and control individuals. In a second set of 463 patients and 926 control individuals, all of Caucasian origin, a risk allele of a hematopoietic-specific protein tyrosine phosphatase, PTPN22, was identified in 17% of the control individuals and 28% of the RA patients. The risk allele changed the function of the protein that functions as a negative regulator of T cell activation, leading to T cells with a lower threshold for T cell activation. This mutation is apparently leading to autoimmune disease in both an American and an Italian population [32*]. These data are a nice example of the power of the technique of whole genome SNP scanning but also emphasize the power of replication to diminish the number of false-positive results [33*]. Although this approach has considerable power to detect risk genes, true positives may be falsely excluded if risk is conferred only in the context of gene–gene interactions such as the RUNX1 pathway genes.

Progress from other candidate genes that were selected by presumed importance is still preliminary. On the teleologic portion of the HLA region is a gene called ‘inhibitor of nuclear factorκB–like gene’ or Iκ-BL. In spite of initial reports of a positive association of a SNP located at –62, a large Spanish study in two cohorts failed to detect any association [34*].

Haplotypes as defined by microsatellites (the IL10R-CA repeat) of the IL10 gene have been previously associated with RA in several ethnic populations with severe RA [35]. Using a SNP –A2849G that tags this haplotype, Lard et al. [36] were not able to find this association in incident cases of RA, but they observed that this haplotype was associated with higher rates of joint destruction in a cohort monitored over time as well as with higher titers of autoantibodies. Recent data indicate the importance of IL10 promoter haplotypes for the outcome of transplantation, indicating its relevance for immune-mediated diseases [37]. Moreover, it has been demonstrated by a robust assay that haplotypes of the IL10 can be transcribed at a different rate, implying a biologic basis for the observed associations [38]. Currently, however, the relation between the different haplotypes and susceptibility to RA is still unclear. For the association of IL10 haplotypes, gene–gene interactions have been suggested by the observation that the association of IL10 genotypes is present only in female patients, not in male patients [39]. Finally, IL10 haplotypes were associated with treatment responses to TNF-blocking agents [40].

For a large number of genes suggested to be relevant in the pathophysiology of RA, association was observed in only one study without replication such as β-adrenergic receptor gene SNPs, RANKL, ICAM-1, VEGF, PDCD-1, and IL1-R gene or data were published describing a lack of association with RA such as CTLA-4, CCR5, mannose-binding-lectin, Toll-like receptor 2 or 4 gene variants [41–50].

Progress from phenotypic-genotypic interactions
Apart from the relevance of gene–gene interactions, progress has been made with respect to identification of gene–environment interactions. The risk conferred by the HLA region has been put in a different perspective, given the significant interaction between smoking and the presence of the shared epitope of HLA-DR as risk factors for seropositive RA, but not at all for seronegative RA [51*]. Thus, the major genetic risk factor for RA is active only in a certain subgroup of RA patients (rheumatic factor positive), and the magnitude of this genetic risk factor is to
a large extent influenced by the presence of an environmental risk factor (here, smoking).

Another clear genotypic–phenotypic association was reported for the presence of CCP antibodies and SE-HLA alleles [52\*]. This was put into functional perspective by the observation that immunity to citrullinated antigens is influenced by the better fit of antigens after citrullination in the HLA-binding groove of the HLA-DR4 allele (one of the shared epitope alleles) [53].

**Conclusion**

The field of genetics has shown remarkable progress. For the coming years it is expected that the technical breakthroughs with respect to rapid and massive genotyping in large cohorts will lead to further elucidation of the risk genes. It has become clear that replication of results, preferably in independent cohorts, is essential for reliable data. Given the extensive collaborations that have been formed, this is not foreseen to be a major problem. The major challenges will be to identify genetic variants that confer small risks by themselves but, by affecting a pathway by several genetic variants, are of great relevance to the elucidation of the genetic causes of RA.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

**• of special interest**

**•• of outstanding interest**


3 Osorio Y, Fortea J, Bukulmez H, et al. Dense genome-wide linkage analysis of rheumatoid arthritis, including covariates. Arthritis Rheum 2004; 50:2757–2765. This is the first publication to provide convincing statistical evidence of the expected number of RA linkage peaks.

4 John S, Shepherd N, Liu G, et al. Whole-genome scan, in a complex disease, using 11,245 single-nucleotide polymorphisms: comparison with microsatellites. Am J Hum Genet 2004; 75:34–84. This is the first use of an array-based SNP typing facility in comparison with conventional microsatellite typing. Moreover fine mapping of HLA class II genes in the linkage peak is performed.


Rheumatoid arthritis

33 Huizinga TW, Patsky DS, Kimberly RP. Associations, populations, and the •

This article gives guidelines for genetic association studies as well as the evidence for these guidelines.

34 Collado L, Rueda B, Calix R, et al. Lack of association between the I kappa BL

This is a nice study using two cohorts to replicate lack of association.


This is the first study to unequivocally demonstrate that IL10 haplotypes can be transcribed at a different rate, thereby providing sound evidence for the observed disease association.


This is the first demonstration of an interaction between an environmental risk factor and a genetic risk factor in RA.

52 Van Gaalen FA, van Aken J, Huizinga TW, et al. Association between HLA •
  class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004; 50:3085–3092.

This study demonstrated that the major genetic risk factor for RA specifically confers risk for the presence of anti-CCP antibodies.

New strategies for immunosuppression: interfering with cytokines by targeting the Jak/Stat pathway
John J. O’Shea, Heiyoung Park, Marko Pesu, Dominic Borie and Paul Changelian

Purpose of review
Numerous immunosuppressants are available, but their adverse effects related to actions on nonlymphoid cells is problematic. Cytokines are key regulators of immune and inflammatory responses, and blocking their actions has become an important modality in treating autoimmune disorders. This review will discuss strategies to develop novel immunosuppressants that arise from advances in the understanding of cytokine signaling.

Recent findings
It is now recognized that large number of cytokines exert their effect by binding to receptors that activate the Janus kinase/signal transducer and activator of transcription pathway, so targeting intracellular signaling pathways is a logical strategy. A selective inhibitor of Janus kinase 3 has now been generated and is effective for transplant rejection in nonhuman primates and other models. Advances have also been made in understanding the functions of Stat family transcription factors, and approaches to interfering with the action of these DNA binding proteins are being devised. In addition, the identification of negative regulators of cytokine signaling offers additional therapeutic opportunities.

Summary
A selective inhibitor of Janus kinase 3 has now been generated and likely represents a new class of effective immunosuppressants. Strategies for targeting signal transducers and activators of transcription pathway are being intensively studied at present and hold potential promise. Multiple endogenous mechanisms exist for negatively regulating cytokine signaling; whether novel therapies can be devised that exploit these mechanisms remains to be determined.

Keywords
cytokines, Janus kinase 3, interleukin, protein inhibitors of activated stats, severe combined immunodeficiency, signal transducer and activator of transcription pathway, suppressors of cytokine signaling

Introduction
There is no shortage of effective immunosuppressive drugs that target a variety of intracellular molecules, but many of the most widely used drugs target ubiquitous molecules. Consequently, these drugs frequently have adverse effects unrelated to their immunosuppressive actions; as a result, a major problem at this time is not the lack of effective immunosuppressive drugs but rather the side effects. It seems logical, therefore, to try to identify agents that target molecules with expression restricted to immune and inflammatory cells. The expectation is that such strategies could generate effective new immunosuppressants with fewer systemic side effects.

Overview of signaling by type I/II cytokine receptors
Because cytokines are key regulators of immunity and inflammation, interfering with these factors has emerged as an effective new strategy for immunosuppression [1,2]. The improved understanding of intracellular cytokine signal transduction affords new opportunities for the development of immunosuppressive drugs. Unfortunately, the term cytokine encompasses a wide range of factors that can bind to a variety of different receptors; this makes it challenging for the nonspecialist to keep track of this expanding array of mediators and to make sense of the molecular basis of their action.

Cytokines that bind so-called type I and II receptors constitute more than fifty factors that regulate processes ranging from body growth and lactation to adiposity. Members of this class of cytokines, however, are especially important for regulating hematopoiesis and host defense. This class of cytokines includes interferons and many interleukins (IL). It is not possible to review all the
actions of these cytokines in this short review, but suffice it to say that they are important in immunoregulation and inflammation [3]. These cytokines control both the innate and adaptive immunity. They are critical for lymphoid development, homeostasis, and differentiation. A word of caution, though: Not all interleukins bind to this class of receptor; in this respect, the term interleukin can lead to confusion. For instance, IL-8 is actually a chemokine, and its receptor is a seven transmembrane G-protein coupled receptor. IL-1, IL-8, IL-17, IL-18, and IL-25 also do not bind to Type I/II cytokine receptors. Additionally, the receptors for tumor necrosis factor and transforming growth factor-β are not included in this family. Signaling by all of these cytokines is distinct from the pathways discussed herein. The known cytokines that bind Type I/II cytokine receptors are summarized in Table 1.

The mechanism involved in signaling by Type I/II cytokine receptors seems to be remarkably straightforward; indeed, the pathway is recognized as a paradigm in signal transduction [4]. These receptors lack intrinsic enzymatic activity, but rather bind to a small family of cytoplasmic protein tyrosine kinases, known as Janus kinases (Jaks) (Fig. 1). There are four mammalian Jaks: Jak1, Jak2, Jak3, and tyrosine kinase 2 (Tyk2) (Table 2). Binding of cytokines to their cognate receptors activates the associated Jak, which in turn autophosphorylates and phosphorylates the receptor. Tyrosine phosphorylation of cytokine receptors provides docking sites for a variety of signaling molecules. The generation of knockout mice and analysis of deficient cell lines have established that Jaks are essential for the initiation of cytokine signaling. By inference, a Jak inhibitor would also block cytokine signaling. As will be discussed, the different Jaks have very distinct functions (Table 2), and this needs to be borne in mind with respect to inhibiting this class of kinases.

One critical family of signaling molecule that binds to phosphorylated cytokine receptors is the group of DNA binding proteins known as the signal transducers and activators of transcription (Stats). These cytosolic proteins bind tyrosine phosphorylated cytokine receptors through their src homology 2 (SH2) domains and then are phosphorylated themselves by Jaks (Fig. 1). The phosphorylated Stats dimerize, translocate to the nucleus, bind DNA at specific elements, and regulate gene expression. There are seven mammalian Stats, which have specific functions (Table 2) [5–7].

### Janus kinase 3, γc, and immune cell function
Cytokines that bind Type I/II cytokine receptors can be subdivided according to their use of shared receptor subunits. One subfamily includes the cytokines IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21; all these cytokines use a common receptor subunit termed the common gamma chain (γc) in conjunction with a ligand-specific subunit [8–10]. Mutations of γc underlie X-linked severe combined immunodeficiency (X-SCID) and account for roughly half of all known cases of SCID (Fig. 2) [11–14]. Deficiency of γc blocks signaling by IL-7, IL-15, IL-4, and IL-21. IL-7 is critical for lymphocyte development and homeostasis of mature peripheral lymphocytes [15,16]. IL-15 is essential for natural killer cell development [17–20]. IL-4 is critical for the differentiation of Th2 cells and works in concert with IL-21 to regulate immunoglobulin class switching in B cells [21–23]. Thus, γc mutations result in a phenotype of SCID designated T−B−NK−, indicative of the fact that T and natural killer cells are absent. Although B cells are present, they are poorly functional, with defective B cell activation and abnormal class switching.

Intracellularly, γc associates with a specific Jak: Jak3. In contrast to other Jaks, which are widely expressed and bind multiple cytokine receptors, Jak3 is predominantly expressed in hematopoietic cells and uniquely binds γc [24–27]. Accordingly, mutations of Jak3 deficiency also result in T−B−NK− SCID (Fig. 2) [28–33].

### The development of a selective Janus kinase 3 antagonist
A corollary of the discovery that Jak3 is required for immune cell development is that purposefully interfering with Jak3 activity or function could be the basis for a novel class of immunosuppressants. Moreover, because Jak3 deficiency results in immunodeficiency and not pleiotropic defects, a highly specific Jak3 inhibitor should also have very limited and precise effects. This contrasts sharply with widely used immunosuppressive drugs, which are directed against ubiquitous targets and have diverse side effects. In principle, the selectivity of a Jak3 inhibitor would have advantages over the current agents.

There has been extensive effort to identify Jak inhibitors, and several inhibitors have been reported to have such activity. They include tryphostin (AG-490), dimethoxyquinazolines (WHI-P154, WHI-P131), undecylprodigiosin

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**Table 1. Cytokines that bind type I/II receptors**

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<thead>
<tr>
<th>Receptors</th>
<th>Cytokines that bind</th>
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<td>Type I cytokine receptors</td>
<td>IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-23, IL-27, IL-31, growth hormone, prolactin, erythropoietin, thrombopoietin, granulocyte colony stimulating factor (CSF), granulocyte-macrophage-CSF, leptin, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, cardiotropin-1</td>
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<tr>
<td>Type II cytokine receptors</td>
<td>IFNα/β, IFNγ, other interferons, IL-10, IL-19, IL-20, IL-21, IL-22, IL-24, IL-26, IL-28A, IL-28B, IL-29, limitin</td>
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</table>
antibiotics (PNU156804), octylaminoundecyldimethylxanthine (CT2576, CT5589), leflunomide, cyclic pyridones, and naphthyl ketones [34, 35]. Some of the selective Jak3 and inhibit other Jaks. Other inhibitors affect disparate pathways, including nuclear factor-kB and T cell receptor signaling or inhibit unrelated tyrosine kinases.

However, an orally available, selective Jak3 antagonist has now been developed (Fig. 3) [36]. The drug, designated CP-690,550, has nanomolar potency against Jak3 and is efficacious in preventing transplant rejection in animal models, including a nonhuman primate renal transplant model; in fact, in the primate model, CP-690,550 was more effective as a single agent than cyclosporine A. One critical issue pertaining to a potential Jak3 antagonist is the extent to which other Jaks are inhibited. Interfering with Jak2 would be particularly problematic because Jak2 is essential for signaling by many hematopoietic cytokines, including erythropoietin, thrombopoietin, and GM-CSF (Table 2). Significant inhibition of Jak2, therefore, could result in anemia, and thrombocytopenia [37]. CP-690,550 is approximately 30 to 100 times less potent for Jak2 and Jak1, respectively, and did not cause granulocytopenia or thrombocytopenia. At the highest doses, mild anemia was noted, indicating that Jak2 antagonism is likely not to be an overwhelming concern for CP-690,550. Animals treated with CP-690,550 did show a modest decline in natural killer cells, presumably because of inhibition of IL-15 signaling; whether this will be clinically relevant with respect to viral infections remains to be determined.

In addition to transplant rejection, clearly CP-690,550 has potential utility in several other clinical settings. The issue of adverse effects is especially important, given that autoimmune disorders occur more frequently in young women in their childbearing years and that treatment is often lifelong. Inhibition of Jak3 might be useful for a range of autoimmune diseases, including psoriasis, psoriatic arthritis, graft-versus-host disease, multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus, and rheumatoid arthritis. The latter disease, rheumatoid arthritis, is of particular interest because of the role of IL-15 in the pathogenesis of this disorder [38]. For example, targeting of the IL-15R using an antagonistic IL-15-Fc fusion protein prevented the development of arthritis and blocked the disease progression [39]. By inference, attenuating IL-15 signaling by inhibiting Jak3 should also be efficacious. IL-4 and IL-9 promote allergic responses, so a Jak3 inhibitor might also be useful in these disorders [40–42].

A theoretic issue with the use of a Jak3 antagonist relates to inhibition of IL-2 signaling. Because IL-2 deficiency is important for maintenance of peripheral tolerance, it is conceivable that inhibition of IL-2 signaling could

Table 2. In vivo function of Jaks and Stats

<table>
<thead>
<tr>
<th>Jak/Stat</th>
<th>Cytokines that activate</th>
<th>Phenotype of knockout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jak1</td>
<td>gp130 cytokines, Type I IFN, IFN-γ, γc cytokines erythropoietin, thrombopoietin, prolactin, growth hormone, βc cytokines, IFN-γ, IL-12</td>
<td>perinatally lethal, neurologic defects, SCID</td>
</tr>
<tr>
<td>Jak2</td>
<td>γc cytokines</td>
<td>embryonically lethal, defective erythropoiesis</td>
</tr>
<tr>
<td>Jak3</td>
<td>γc cytokines</td>
<td>SCID</td>
</tr>
<tr>
<td>Tyk2</td>
<td>gp130 cytokines, Type I IFNs, IL-12, IL-23</td>
<td>modest viral susceptibility, reduced IL-12 response and resistance to arthritis</td>
</tr>
<tr>
<td>Stat1</td>
<td>Type I IFNs IFN-γ</td>
<td>impaired anti-viral response</td>
</tr>
<tr>
<td>Stat2</td>
<td>Type I IFNs</td>
<td>Increased tumors</td>
</tr>
<tr>
<td>Stat3</td>
<td>Many cytokines especially gp130 cytokines</td>
<td>impaired anti-viral response</td>
</tr>
<tr>
<td>Stat4</td>
<td>IL-12</td>
<td>embryonically lethal</td>
</tr>
<tr>
<td>Stat5A</td>
<td>prolactin, other cytokines</td>
<td>defective Th1 differentiation</td>
</tr>
<tr>
<td>Stat5B</td>
<td>growth hormone, other cytokines</td>
<td>defective mammary gland development</td>
</tr>
<tr>
<td>Stat6</td>
<td>IL-4</td>
<td>impaired sexually dimorphic growth</td>
</tr>
</tbody>
</table>

Figure 1. Critical role for Jak and Stats in cytokine signaling

Binding of a cytokine to its cognate receptor activates the associated Janus kinase (Jak). The Jak in turn phosphorylates the receptor, which provides a docking for signal transducers and activators of transcription (Stats) and other signaling molecules to bind the receptor. Stats also become phosphorylated and translocate to the nucleus, where they regulate gene expression.
exacerbate autoimmunity. Monoclonal antibodies against IL-2R-α (CD25, basiliximab, and daclizumab) are used for transplant rejection; however, these agents have not been reported to induce a breakdown in peripheral tolerance and autoimmune disease [43]. A Jak3 inhibitor, which would antagonize all the γc cytokine receptors, would be more immunosuppressive than an IL-2R antagonist. Consequently, the expectation is that such an agent would be even less likely than anti-CD25 antibodies to interfere with tolerance. Obviously though, this possibility will need to be borne in mind in clinical trials.

**Targeting other Janus kinases**

Tyk2−/− mice have impaired IL-12 signaling, and mice with a mutation in Tyk2, have marked resistance to the development of collagen-induced arthritis [44–48]. Therefore, targeting Tyk2 might be a useful strategy for the treatment of TH1-mediated disorders such as arthritis. It should be noted that IL-23 also uses the IL-12Rβ and activates Tyk2, but the effect of Tyk2 deficiency on IL-23 responses has not been examined [49,50]. Deficiency of Jak1 or Jak2 results in perinatal or embryonic lethality, respectively. Therefore, targeting these kinases could have significant toxicities. One could imagine, however, that in the treatment of cancers or leukemia, a greater level of toxicity might be acceptable, assuming that the drug is efficacious.

**Targeting Stats**

Because of their critical and selective functions, Stats are also attractive drug targets. Because they do not have enzymatic activity, one must block Stat expression, recruitment to cytokine receptors, dimerization, or DNA binding. Cytokine recruitment and dimerization are mediated by phosphotyrosine-SH2 interactions, so peptidomimetics have been designed to disrupt these interactions [51,52]. Although phosphotyrosine-SH2 interactions are important for many aspects of intracellular signaling, the generation of phosphopeptidomimetics has previously met with little success. An alternative strategy is the use of decoy oligonucleotides, which would interfere with Stat binding to endogenous DNA [53–55].

Assuming that Stat inhibitors can be successfully devised, which Stats would be useful to target? In terms of immunoregulation, Stat4 and Stat6 might be useful targets [7,21,22,56]. These Stats are critically important for the differentiation of helper T cells. IL-4 activates Stat6, promoting Th2 cell differentiation and allergic response, whereas IL-12 activates Stat4 and drives differentiation of naïve T cells to Th1 cells. These cells produce interferon-γ, which is critical for host defense against intracellular pathogens but also contributes to many autoimmune diseases. In addition, constitutive activation of Stat3 and Stat5
has been noted in a significant proportion of tumors, and increasing attention is being paid to targeting these Stats in cancer [51*,57*,58,59]. Inhibiting Stat3 may be complicated in that the lack of Stat3 in myeloid cells could promote autoimmune disease [60].

**Negative regulators of cytokine signaling**

Cytokine signaling can be attenuated by a variety of including tyrosine phosphatases, protein inhibitors of mechanisms activated stats (PIAS) family members, and suppressors of cytokine signaling (SOCS) (Fig. 4) [61*]. SOCS proteins, classic feedback inhibitors of signaling, bind with their SH2 domains to phosphotyrosine residues in Jaks (SOCS1) or cytokine receptors (SOCS2, SOCS3, and CIS), and block signaling. On the basis of the phenotype of knockout mice, different SOCS family members seem to have distinct functions [61*]. There are four family members in PIAS proteins – PIAS1, PIAS3, PIASx, and PIASy – some of which also seem to have restricted functions. Although all PIAS proteins interact with and inhibit Stat proteins, the mechanisms by which this occurs seems to differ between family members [62**]. PIAS1 and PIAS3 inhibit Stat1, Stat3, and Stat5 activity, respectively, by blocking Stat DNA binding [63,64]. Conversely, the inhibition of Stat4 and Stat1 by PIASx and PIASy does not affect Stat DNA binding and must occur by a distinct mechanism [65]. PIAS proteins have also been shown to have E3 small ubiquitin-like modifier (SUMO) ligase activity. Covalent SUMO modification of target proteins is similar to ubiquitylation, but sumoylation is not considered to target proteins for degradation, and the consequence of Stat sumoylation is unknown. [66–68].

Knockout mice lacking PIAS1 and PIASy have been generated. PIAS1 knockout mice had enhanced interferon responses, whereas PIASy knockout mice demonstrated mild defects in interferon signaling [62**,69,70]. In principle, mimics or inducers of SOCS and PIAS proteins would be immunosuppressive in that such agents would be expected to attenuate the effects of cytokines [71*]. Conversely, activators of the tyrosine phosphatases that regulate Jaks and Stats would also inhibit signaling, but it is not yet clear how drugs can be designed or whether these are truly feasible approaches.

**Conclusion**

In summary, studies in humans with mutations of Jak3 and its associated receptor subunits have predicted that selective Jak3 antagonists could represent a new class of immunosuppressants. In contrast to the targets of existing drugs, Jak3 has limited tissue expression and discrete functions. In principle, a highly selective inhibitor would not be associated with the toxicities seen with existing immunosuppressants. A selective Jak3 antagonist, CP-690,550, has now been developed, and it is not associated with unacceptable toxicities indicative of substantial Jak2 inhibition. The drug is effective in models of transplant rejection, including studies in nonhuman primates. As the drug moves toward clinical trials in humans it will be important to determine other clinical settings ranging from autoimmunity, allergy, and cancer in which this new agent might be useful. The successful generation of a selective Jak inhibitor suggests that targeting other JakS is feasible; targeting Tyk2 might be another strategy for treating immune-mediated disease. In principle, target
Rheumatoid arthritis

Stats and the negative regulators of cytokine signaling could be of use, and these molecules will surely continue to receive considerable attention as therapeutic targets.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest


Phosphopeptidomimetics have long been suggested as possible modes for inhibiting SH2-phosphotyrosine interactions. This study suggests that this strategy may be useful for targeting Stats.


This study provides data indicating that inhibiting Stat3 can promote innate immune responses.


This is an excellent review of the negative regulation of cytokine signaling.


This is a recent review of PIAS proteins.


The first description of an engineered SOCS analog.
Crystal deposition diseases: out of sight, out of mind
Geraldine M. McCarthy

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Current Opinion in Rheumatology 2005, 17:312–313

Abbreviations
BCP basic calcium phosphate
CPPD calcium pyrophosphate dihydrate
DJD degenerative joint disease
MSU monosodium urate
PLM polarised light microscopy
SF synovial fluid

Introduction
Degenerative joint disease (DJD) is the most common form of arthritis that occurs in humans. It is characterised by pain and deformity, and is a cause of substantial morbidity and disability. Concurrent with increased longevity in the 21st century, the prevalence of DJD is steadily rising worldwide. The cause of osteoarthritis is incompletely understood but appears multifactorial. No specific drug has been identified to reverse or prevent the progression of DJD in all cases. However, crystal deposition diseases represent one potentially reversible cause of joint degeneration.

Several different crystal species can be found in synovial fluid (SF). For example, SF monosodium urate (MSU) crystal deposition is associated with the acute inflammatory arthritis of gout, and calcium pyrophosphate dihydrate (CPPD) crystal deposition is associated with a variety of clinical presentations including acute pseudogout. The ultimate consequence of intraarticular MSU and CPPD crystal deposition is aggravated joint degeneration.

Basic calcium phosphate (BCP; hydroxyapatite, octacalcium phosphate, and tricalcium phosphate) crystals are also found in SF and can occur in as much as 60% of samples from osteoarthritic joints [1]. Their presence is associated with more pronounced radiographic joint degeneration, gross synovial hyperplasia, and larger volume joint effusions compared with joints without BCP crystals. BCP crystals have potent biological effects in vitro that have been partly explored, and include their ability to induce mitogenesis and matrix metalloprotease, cytokine and prostaglandin production in articular chondrocytes, and synovial fibroblasts. BCP crystals are also associated with a variety of other clinical presentations, including acute and chronic calcific periartitis and Milwaukee shoulder syndrome [2]. These clinical manifestations correlate well with the potent in-vitro effects of BCP crystals noted earlier.

Treatments for the various types of crystal deposition diseases depend on which crystal types are present. Therefore, accurate identification of crystals in the clinical evaluation of a patient is crucial. For example, urate-lowering therapy is prescribed to promote dissolution of MSU crystals in gout. If the diagnosis is incorrect, inappropriate treatment may be prescribed, leading to prolonged symptoms and permitting irreversible joint damage. MSU and CPPD crystals can be identified clinically by compensated polarised light microscopy (PLM) of SF. However, in many countries, synovial fluid tests have not been subjected to the same rigorous quality control and validation procedures as other forms of laboratory investigation, and only a minority of clinicians undertake PLM themselves [3]. Therefore, one can conclude only that a substantial number of patients with crystal deposition diseases remain undiagnosed.

In contrast to MSU and CPPD, BCP crystals are ultramicroscopic and hence too tiny to be identified by conventional or PLM. Alizarin red S staining has been used to improve SF microscopic examination, but it only detects clumps of BCP crystals, cannot distinguish between BCP and CPPD crystals, and gives many false positives. Previous studies of BCP crystals have required the use of techniques that are expensive, not readily available, or that do not permit high throughput of samples, such as electron microscopy. These problems with BCP crystal identification are impeding clinical study. Although no drug is currently clinically available to inhibit the effects of BCP crystals, some potential candidates, such as phosphocitrate, have been identified and are currently being explored. Unfortunately, without a simple means of detecting BCP crystals, progress on this front has been hindered. The study of BCP crystal deposition disease has advanced only very slowly, at least partly for the same reason. Therefore, it is critical that a rapid, simple, and
accurate diagnostic test be made available to identify BCP crystals specifically and to distinguish them from other clinically relevant crystals.

Unless SF crystals are sought and then seen, one potentially preventable cause of DJD will continue to be ignored. This omission will contribute to the inexorable rise of disability related to joint degeneration worldwide.

References

Inflammation and tissue damage in crystal deposition diseases
Nicola Dalbeth and Dorian O. Haskard

Purpose of review
The crystal-induced arthropathies are characterized by self-limiting episodes of acute inflammation and chronic tissue damage. This review summarizes recent advances in the understanding of the cellular responses to monosodium urate, calcium pyrophosphate dihydrate and basic calcium phosphate crystals.

Recent findings
Factors such as the myeloid related proteins, endothelin-1 and the complement membrane attack complex have been recently identified as mediators of acute crystal-induced inflammation. In addition, signalling pathways involved in both acute inflammation and tissue damage in crystal arthropathies have been further clarified. The potential of macrophage-derived transforming growth factor β1 to play a key role in the resolution phase of acute gout has also been demonstrated.

Summary
Recent work has provided new insights into the regulation of both acute and chronic articular responses to inflammatory microcrystals. Further analysis of these responses may identify potential therapeutic targets for management of the crystal-induced arthropathies.

Keywords
arthritis, basic calcium phosphate, calcium pyrophosphate dihydrate, gout, monosodium urate

Introduction
The crystal arthropathies such as gout and pseudogout are characterized by episodes of acute synovitis with spontaneous resolution of disease. Over time, damage to articular tissue may occur with degeneration of cartilage, bone and surrounding soft tissue structures. This review summarizes recent advances in the understanding of inflammation and tissue damage in the crystal-induced arthropathies, focusing on monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystal related disease.

The acute inflammatory response in crystal-induced arthropathies
Intense infiltration of neutrophils into both synovial fluid and membrane is the hallmark of acute gout, and these cells provide a key cellular mechanism of inflammatory amplification. During the acute gout attack, neutrophils are recruited into the joint through interactions with activated endothelium. Neutrophil recruitment is further enhanced by the local generation of chemotactic factors such as interleukin (IL)-8, monocyte chemoattractant protein-1, C5a, and the myeloid related proteins S100A8 and S100A9. S100A8 and S100A9 are small cytoplasmic proteins that are expressed into the extracellular environment as active heterodimeric complexes of S100A8/A9. Previous work has demonstrated that S100A8 and S100A9 are released following local injection of MSU crystals into a subcutaneous air pouch and that these proteins play an important role in inducing neutrophil migration. Furthermore, high concentrations of S100A8 and S100A9 are present in synovial fluid and plasma of patients with gout [1]. Ryckman et al. have investigated the mechanisms of S100A8/A9 release in gout, and have recently demonstrated that MSU crystals directly induce release of S100A8/A9 from neutrophils in a dose-dependent manner [2*]. Release of S100A8/A9 was inhibited by blockade of CD11b (Mac-1/CR3) and CD16 (FcγRIII), receptors known to modulate neutrophil responses to MSU crystals [3]. The release of S100A8/A9 was also partially suppressed by inhibition of the Src tyrosine kinase, Syk tyrosine kinase or PI3 kinase pathways.

Mononuclear phagocytes also play an important role in the regulation of the acute gout attack. This regulation occurs through the production of soluble mediators such as cytokines and chemokines. The release of macrophage-derived chemokines in response to MSU crystals was examined by Jaramillo et al. [4]. Using the murine macrophage cell line...
B10R, they demonstrated that MSU crystals increased mRNA expression of various chemokines including macrophage inflammatory protein (MIP)-1α, MIP-1β and MIP-2 in a concentration dependent manner. Previous work has demonstrated that these chemokines are expressed by immature monocytes/macrophages during the inflammatory response to MSU crystals. Increased chemokine mRNA expression was noted within 30 minutes of exposure to MSU crystals, peaked at 1–2 hours and decreased after 8 hours. Examination of signalling pathways demonstrated that macrophage exposure to MSU crystals lead to phosphorylation of IkBα and the mitogen-activated protein kinases (MAPK) extracellular signal-regulated kinases 1 and 2 (ERK1/2), as well as the transcription factors activator protein-1 (AP-1) and nuclear factor-κB (NF-κB). Selective blockade of both the ERK1/2 pathway and the NF-κB pathway significantly suppressed chemokine mRNA expression. Overall, these data demonstrated that ERK1/2 dependent signals are required for AP-1 and NF-κB activation and subsequent production of proinflammatory chemokines by MSU crystal-stimulated macrophages. These findings are consistent with previous reports that monocyte IL-8 production in response to MSU crystals is regulated by the ERK1/2 MAPK, as well as binding of the transcription factors NF-κB and AP-1 to the IL-8 promoter [5]. Thus, signalling through the ERK1/2 MAPK provides a common pathway of macrophage activation and cellular recruitment in response to MSU crystals.

The complement pathway may also contribute to recruitment of neutrophils into the synovial cavity during an acute gout attack. Complement activity is usually very low in normal synovial fluid, and is greatly increased in the inflamed synovial fluid of acute gout [6]. A number of complement components, including C1q, C1r and C1s have been eluted from the surface of MSU crystals exposed to plasma [7], and MSU crystals activate the classical and alternative complement pathways in vitro [8,9]. Activation of complement pathways leads to elaboration of C3a and C5a, which modulate leukocyte migration into the joint. In addition to these components, a recent report indicates that the terminal membrane attack complex C5b-9 plays a major role in neutrophil recruitment response to MSU crystals [10••]. Tramontini et al. studied C6-deficient rabbits to determine whether the C5b-9 complex mediates MSU crystal-induced arthritis. These experiments demonstrated that these rabbits had reduced MSU crystal-induced joint swelling, with selective suppression of neutrophil influx and IL-8 release. The membrane attack complex may influence neutrophil ingress through a number of mechanisms, including activation of endothelial cells, resident mast cells or neutrophils.

A further mediator of neutrophil influx in acute gout may be endothelin-1, an endothelial derived peptide that is known to influence neutrophil migration. Getting et al. studied the role of endothelin-1 in acute gout using antagonists of the endothelin-1 receptors ETA and ETB in the murine MSU crystal-induced peritonitis model [11••]. Pre-treatment with the ETA antagonist FR139317 or the ETB antagonist BQ-788 suppressed neutrophil influx in this model. The ETA antagonist inhibited neutrophil accumulation and release of the neutrophil CXC chemokine KC, whereas the ETB antagonist inhibited neutrophil accumulation and endothelial activation, but not KC release. In therapeutic protocols, inhibition of both receptors using the dual antagonist PD145065 was required for suppression of neutrophil influx and KC release.

CPPD crystals also induce an acute inflammatory response within the joint during an acute pseudogout attack and, like other microcrystals, are capable of inducing neutrophil degranulation and release of reactive oxygen species. The mechanisms of such neutrophil activation by CPPD crystals have been investigated by Tudan et al. This group has previously reported that full inhibition of the PI3K/Akt pathway or the ERK1/2 MAPK pathway significantly, but not completely, inhibited neutrophil activation in response to CPPD crystals [12,13]. They have recently reported that plasma-opsonized CPPD crystals rapidly induced activity of the p38 MAPK within neutrophils, and that inhibition of p38 suppressed the neutrophil respiratory burst and lysozyme degranulation by approximately 50% [14•]. Thus, it is likely that p38, in parallel with other activating signalling pathways, contributes to neutrophil responses to CPPD crystals.

A hallmark of the acute crystal arthropathies is severe joint pain. This pain may be due to a number of factors, including local production of prostaglandins and bradykinin, and sensitization of nociceptors. Recent reports have provided further insights into the mechanisms of pain in acute gout. When unmyelinated nerve fibers are stimulated, there is release of neuropeptides such as substance P. Substance P results in vasodilatation, plasma extravasation, leukocyte recruitment, mast cell degranulation and release of prostaglandins and cytokines. Lunam and Gentle examined substance P immunoreactive nerve fibers in domestic chick ankle joints, and reported that within 4 hours of intra-articular injection of MSU crystals, there was depletion of substance P from peripheral nerves in the synovial and subsynovial tissue [15••]. These data implicate substance P as a potential mediator of pain and inflammation in acute gout.

Nonsteroidal anti-inflammatory drugs such as indomethacin provide effective analgesia in acute gout. The mechanisms of indomethacin-induced antinociception in experimental urate monoarthritis were studied by Ventura-Martinez et al. [16•]. Using a pain-induced functional impairment model, they demonstrated that the antinociceptive effect of indomethacin was significantly
suppressed by a non-selective inhibitor of nitric oxide synthase (NOS). In addition, local administration of L-arginine (the NOS substrate) or sodium nitroprusside (a nonenzymatic nitric oxide (NO) donor) significantly increased the antinociceptive effect of indomethacin. These findings indicate that in addition to inhibitory actions on prostaglandin synthesis, activation of NO synthesis may play a role in the antinociceptive effect of indomethacin in acute gout.

Tissue damage in crystal-induced arthropathies

The role of NO in the pathogenesis of inflammatory arthritis remains controversial. Although physiologic levels of NO may have protective effects within the joint, high concentrations of NO may contribute to joint damage through degradation of cartilage matrix, enhancement of matrix metalloproteinase (MMP) catabolic activity, suppression of bone production and chondrocyte apoptosis. NO has been detected in air pouch fluid following injection with MSU crystals [17]. Jaramillo et al. studied the capacity of MSU crystals to modulate macrophage NO synthesis [18*]. They have reported that in the murine J774 macrophage cell line and in bone marrow derived macrophages, MSU crystals did not directly induce NO synthesis. However, MSU crystals potentely enhanced inducible NOS (iNOS) mRNA and protein expression and NO production in response to interferon-γ. Consistent with macrophage chemokine production, MSU crystals also exerted their synergestic effects on macrophage NO production by increasing ERK1/2 phosphorylation and NF-κB nuclear translocation in response to interferon-γ.

Chondrocytes also produce NO on exposure to MSU crystals. Liu et al. tested the potential of MSU crystals to directly induce chondrocytes to produce NO and MMP-3 [19**]. In these experiments, MSU crystals strongly induced iNOS gene expression, NO release and MMP-3 release from bovine articular chondrocytes in an IL-1β independent manner. Inhibition of the p38 MAPK pathway, but not the ERK1/2 MAPK pathway, significantly suppressed production of iNOS, NO and MMP-3. Further analysis of signalling pathways demonstrated that MSU crystals induced activation of a signalling cascade typically employed by adhesion receptors, involving an upstream signalling complex of c-Src, the focal adhesion kinase (FAK) family member prolne rich tyrosine kinase (Pyk-2), and the adaptor protein paxillin. In this model, Pyk-2 and c-Src mediated activation of the p38 MAPK pathway, with induction of NO and MMP-3 expression.

Cartilage degeneration is a major feature of BCP crystal-associated arthropathies. BCP crystals induce the production of MMP-1 and MMP-3 by human fibroblasts through the calcium-independent ERK1/2 (p44/p42) MAPK pathway and the calcium dependent PKCα pathway. However, ERK1/2 activation by BCP crystals is independent of PKCα signalling [20]. Reuben et al. have recently demonstrated that BCP crystals activate the ERK1/2 pathway in human fibroblasts through activation of PKCα, a protein kinase that is structurally distinct from other PKC isozymes [21**]. This group reported that inhibition of PKCα synthesis or activity in human fibroblasts resulted in suppression of ERK1/2 phosphorylation in response to BCP crystals. Such inhibition also suppressed the expression of MMP-1 and MMP-3 mRNA and protein. These data indicate that BCP crystals induce MMP production in human fibroblasts through two pathways: activation of the calcium dependent PKC pathway mediated by PKCα, and the calcium independent p44/42 MAPK pathway mediated by PKCβ. These pathways operate independently, but in a complimentary manner for optimal activation of the cellular response to BCP crystals.

Prostaglandin E2 (PGE2) is an important regulator of inflammation, pain and matrix degradation in the crystal-induced arthropathies. PGE2 is capable of inducing cartilage degradation through inhibition of collagen synthesis, induction of MMP production, and increased chondrocyte apoptosis. Inflammatory microcrystals, including BCP crystals, promote production of PGE2 from synovial fibroblasts. Morgan et al. examined the mechanisms of BCP crystal-induced PGE2 release from fibroblasts [22**]. They initially confirmed that BCP crystals induced PGE2 production by human foreskin fibroblasts within 4 hours of culture. PGE2 production was sensitive to inhibition with a selective cyclo-oxygenase (COX)-2 inhibitor at 4 hours, but both COX-1 and COX-2 inhibition was required to suppress PGE2 production at 30 hours. Real-time RTPCR demonstrated that BCP crystals induced a 23-fold increase in COX-2 mRNA that was maximal within 4 hours, whereas mRNA for COX-1 was up-regulated at 24 hours. BCP crystal-induced COX-2 mRNA expression was partially attenuated by selective PKC and PI3K inhibitors, but not by MAPK inhibitors. Furthermore, treatment of fibroblasts with phosphocitrate, a known specific inhibitor of the biologic effects of BCP crystals, suppressed mRNA expression of COX-2. This study also demonstrated that BCP crystals induce IL-1β production in human fibroblasts. Increased IL-1β mRNA expression was detected within 1 hour, was maximal at 8 hours following exposure to BCP crystals, and was abrogated by phosphocitrate. IL-1β has also been implicated in the pathogenesis of articular matrix damage in arthritis, through synthesis of MMPs, release of other proinflammatory mediators, inhibition of chondrocyte and osteoblast function and induction of osteoclast activation.

Resolution of acute crystal-induced arthropathies

Even in the absence of treatment, the acute inflammatory response in gout is typically self-limiting over 7–10 days.
Furthermore, it is well recognized that MSU crystals can be found in the asymptomatic joints of patients with hyperuricaemia. These findings suggest that a balance exists between the factors within the joint that maintain the non-inflamed state in the presence of MSU crystals and the pro-inflammatory response that accompanies an acute gout attack. Factors that may contribute to the non-inflamed state include coating of crystals with protective proteins, and induction of anti-inflammatory transcription regulators, cytokines and hormones. Several observations imply that differentiated macrophages play an important role in the resolution of the acute gout attack. MSU crystals found in asymptomatic joints of patients with hyperuricaemia and interval gout are present within macrophages and almost never within neutrophils [23], indicating that macrophages can interact with MSU crystals within the joint without triggering an inflammatory response. Previous work has demonstrated that mature mouse macrophages and human in-vitro differentiated macrophages ingest MSU crystals without proinflammatory cytokine secretion or endothelial activation [24,25]. Such behavior is in clear contrast to immature monocyte-macrophages, which secrete TNF-α, IL-1β, IL-6 and IL-8 in response to phagocytosis of MSU crystals.

High levels of transforming growth factor β1 (TGFβ1) have been demonstrated in the synovial fluid of patients with acute gout [26], and administration of TGFβ1 significantly inhibited leukocyte infiltration into air pouches injected with MSU crystals [27]. Yagnik et al. have now identified TGFβ1 as a key soluble factor in the suppression of MSU-induced inflammation by differentiated macrophages [28••]. As monocytes differentiated in vitro towards a macrophage endpoint, the loss of the capacity to secrete proinflammatory cytokines in response to MSU crystals was paralleled by a gain in the capacity to release TGFβ1 (Fig. 1). Functional effects of TGFβ1 in this model system included the suppression of (a) monocyte proinflammatory cytokine release in response to MSU crystals, (b) endothelial cell activation in response to monocyte-derived cytokines, (c) macrophage release of TNF-α in response to zymosan. However, not all effects of TGFβ1 are suppressive and this growth factor may contribute to fibroblast proliferation and the physical encasing of crystals away from contact with leukocytes. Taken together, these data support a role for monocyte differentiation in the resolution phase of acute inflammation in gout. A hypothetical model of the role of monocyte–macrophage differentiation in gout is shown in Figure 2. As with any in-vitro model, the interpretation of the data needs to be qualified by the possibility that macrophage differentiation in vitro may not faithfully reproduce the in-vitro situation. In this respect, it is reassuring that monocytes and macrophages derived from human skin blisters showed the same disparity in cytokine secretion in response to MSU crystals as in-vitro differentiated cells, with the macrophage endpoint appearing rather earlier in vivo (40 hours as opposed to 5 days), consistent with the kinetics of a typical gout attack [28••].

Conclusions
Recent reports have further elucidated the pathophysiology of crystal-induced arthritis. Factors such as the myeloid related proteins, endothelin-1 and the complement membrane attack complex have been identified as...
potential mediators of acute inflammatory disease. In addition, signalling pathways involved in both acute inflammation and tissue damage in crystal arthropathies have been further clarified. The key role of macrophage production of TGFβ1 in the resolution phase of acute gout has also been emphasized. Further analysis of pathways that regulate the cellular response to inflammatory microcrystals may identify potential therapeutic targets for management of crystal-induced arthropathies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


This paper extends previous work examining neutrophil signaling pathways, and demonstrates that the p38 MAPK pathway plays a role in neutrophil activation and survival following exposure to CPPD crystals.


This report identifies substance P as a potential modulator of inflammation in the acute gout attack, showing that substance P is rapidly depleted from nerve fibres following intra-articular injection of MSU crystals.


This paper demonstrates that NO may mediate indomethacin-induced antinociception in rats with experimental urate monarthrits.


This paper examines mechanisms of NO release by macrophages in response to MSU crystals. The key findings are that MSU crystals alone do not induce the production of NO, but synergize with interferon-γ to promote NO release. Activation of the ERK1/2 and NF-κB pathways is shown to mediate this effect.


This group reports that MSU crystals induce chondrocyte release of MMP-3 and NO. They also identify a key role for the Pyk-2/Src/paxillin complex in p38 MAPK activation within chondrocytes in response to MSU crystals.


This paper identifies PKCζ as a novel signalling mediator in BCP crystal-induced activation of fibroblasts.


The authors demonstrate that BCP crystals induce early expression of COX-2 with fibroblasts in a manner that is partially dependent on PKC and PI3K. They also show that expression of mRNA for IL-1β is increased in response to BCP crystals. Both IL-1β and COX-2 mRNA expression is suppressed by phosphocholate.


Using both in-vitro and in-vivo models, this report further supports the role of TGFβ1 as a potential anti-inflammatory mediator released by mature human macrophages in response to MSU crystals.
Recent advances in the management of gout and hyperuricemia
Robert L. Wortmann

Purpose of review
To review the recent advances in the management of gout and hyperuricemia.

Recent findings
The first quality indicators for gout management have been proposed. Selective COX II inhibitors, as well as traditional NSAIDs, are effective in acute gout. A new xanthine oxidase inhibitor, febuxostat, and pegylated uricases are in clinical trials.

Summary
The therapeutic aims in gout include termination of the acute attack as promptly and gently as possible, prevention of future attacks, prevention or reversal of complications of the, and prevention or reversal of associated features such as obesity, hypertriglyceridemia, hypertension, or alcoholism.

Keywords
alcoholism, gout, hypertension, hyperuricemia, obesity

Introduction
Gout, or monosodium urate dihydrate crystal deposition disease, is somewhat unique in that its cause and underlying pathophysiology are well understood, it can be diagnosed with absolute certainty, and treatment can be entirely effective. Because of these facts, one could argue that no disease is more readily manageable than gout. However, despite these facts, errors in the management of gout continue to be common [1**••,2**••,3,4•,5].

Although textbook chapters and review articles have provided relatively consistent advice over the last 30 years, there is surprisingly little evidenced-based information in the literature on gout. Previously there have been no published consensus or management standards for gout. This is, at least in part, because there have been no truly new therapeutic agents to treat gout and hyperuricemia since 1964 when allopurinol was released. The clinical trials needed to gain a drugs approval in the 1950s and 60’s were far different than those required today. For example, there were no double-blind, controlled trials published for allopurinol prior to its release. In addition, the endpoint in all of the trials evaluating uricosurics and allopurinol was the percentage of patients who achieved a targeted urate level. No systematic information was assessed concerning elimination of gouty attacks or reduction in tophi size.

Perhaps the most significant new advance in the management of gout during the time frame of this review was the publication of proposed quality indicators for gout management [1**•]. Mikuls, et al. performed a systematic review of the literature and then employed the efforts of a panel of community and academic rheumatologists to develop, what were considered to be, ten valid quality indicators. Rather than list these, they will be highlighted where relevant in this review.

Acute gouty arthritis
Although cold applications are a useful adjunct in treating acute gouty arthritis [6], acute attacks may be successfully terminated by any of several drugs. The time of initiation of therapy is more important then the choice of drug. For any of the agents available, the sooner they are started after the symptoms begin, the more rapidly a complete response will be attained.

If a patient has acute gouty arthritis and lacks both of the relative contraindications to gout treatment — significant renal impairment (a serum creatinine level ≥2 mg/dl or
measured/estimated creatinine clearance \(\leq 50 \text{ mL/min}\) and peptic ulcer disease — then the patient should be treated with an antiinflammatory agent to include one of the following: NSAID, ACTH or glucocorticoid (either systemic or intraarticular administration), or colchicine. This is because antiinflammatory agents have been shown to be both effective and well tolerated for the short-term treatment of acute gout. Patients with renal impairment and a history of peptic ulcer disease may be at a higher risk for gout medication toxicity [1**].

Generally, colchicine is preferred for patients in whom the diagnosis of gout is not confirmed, whereas NSAIDs are preferred when the diagnosis is secure. Colchicine can be administered by oral or intravenous routes. Orally, a dose of 0.5 or 0.6 mg is taken hourly until one of three things occurs: joint symptoms ease; nausea, vomiting, or diarrhea develops; or the patient has taken a maximum of ten doses. If ten doses are taken without benefit, one should question the accuracy of the diagnosis. Colchicine can also be administered intravenously safely [7*], but this is potentially dangerous and rarely necessary.

In the patient with an established diagnosis of uncomplicated gout, the preferred agent of choice is an NSAID. Indomethacin has been the traditional choice of agents in this class. An initial dose of 50 to 75 mg, followed by 50 mg every 6 to 8 hours with a maximum dose of 200 mg in the first 24 hours, has generally been recommended. In reality, all of the NSAIDs can be highly effective in the treatment of acute gouty arthritis including the COX-2 selective agents. Etoricoxib (a COX-2 selective NSAID presently under investigation) was compared with indomethacin in a randomized controlled trial involving 189 patients [8]. Etoricoxib at a dose of 120 mg once daily was shown to be an effective treatment for acute gout, had comparable efficacy to indomethacin at a dosage of 50 mg 3 times daily, and was generally safe and well tolerated. In another randomized, controlled trial, rofecoxib once daily provided more effective treatment for acute gout than did diclofenac sodium SR 150 mg and meloxicam 15 mg administered orally once daily for 7 days [9]. Although rofecoxib is no longer available, these data provide additional evidence that this class of drugs is effective for treating gouty flares.

If a patient cannot take medications by mouth, has active peptic ulcer disease or renal impairment, the choice is among ACTH, intra-articular glucocorticoid, or parenteral glucocorticoids. In some cases, analgesics, including narcotics, may be added as well. The dosages of drugs with recognized effects on serum urate concentrations, including the specific urate-lowering agents, should not be adjusted, initiated or discontinued during an acute attack. Not only do sudden fluctuations in serum urate levels tend to precipitate acute attacks, an inflammatory process already present may be made substantially worse by a fluctuation in the serum urate concentration.

**Prophylaxis**

Small daily doses of colchicine or an NSAID can be used effectively to prevent acute attacks of gout [9-10,11**]. Colchicine in doses of one to three pills a day are usually employed, with indomethacin, or another NSAID, has been substituted (for example, indomethacin 25 mg two times per day) in the colchicine intolerant individual. A program of maintenance colchicine or NSAID may make the difference between frequent episodes of incapacitation and remaining functional. Prophylactic treatment is not recommended unless one also prescribing a urate-lowering agent. Prophylactic use of colchicine or an NSAID may block the acute inflammatory response but will not alter crystals deposition. With continued deposition, but without the warning signs of recurrent bouts of acute arthritis, tophi will develop and joint tissue destruction can advance without notice.

If a patient with tophaceous gout is given an initial prescription for a urate-lowering medication (xanthine oxidase inhibitor, probenecid, or sulfapyrazone) and lacks both significant renal impairment (a serum creatinine level \(\geq 2 \text{ mg/dl or measured/estimated creatinine clearance } \leq 50 \text{ mL/min}\)) and peptic ulcer disease, then a prophylactic antiinflammatory agent (colchicine or NSAID) should be given concomitantly because prophylactic antiinflammatory therapy reduces the risk of rebound gout attacks, which frequently follow the initiation of urate-lowering therapy [1**].

Prophylaxis usually is continued until the serum urate value has been maintained well within the normal range and there have been no acute attacks for a period of 3 to 6 months. It is important to warn patients that discontinuation of the prophylactic medication may be followed by an exacerbation of acute gouty arthritis and advise them what to do should that occur. Prophylactic use of colchicine is not without potential complications.

If a gout patient receives long-term prophylactic oral colchicine (defined as a minimum daily dose of 0.5 mg for a duration of 6 months or longer) and has significant renal insufficiency (a serum creatinine level \(\geq 2 \text{ mg/dl or measured/estimated creatinine clearance } \leq 50 \text{ mL/min}\)), then a complete blood cell count and creatine kinase should be evaluated a minimum of one time for every 6 months of continued use because the risk of colchicine-related myopathy and myelosuppression appears to be substantially increased in the context of reduced renal function [1**].

Neuromuscular toxicity related to colchicine therapy is well recognized. A reversible painful axonal neuromyopathy is more common in individuals who have impaired renal
function and are taking diuretics. Severe respiratory muscle weakness has also been reported [12]. Acute rhabdomyolysis with myoglobinuria and renal failure is most commonly seen in individuals concomitantly taking an HMG-CoA reductase inhibitor (statin) or cyclosporine.

Control of hyperuricemia
Elimination of hyperuricemia with urate-lowering agents can prevent as well as reverse urate deposition. Various opinions exist regarding the most appropriate time to initiate urate-lowering agents. Some physicians regard the first gouty attack as a late event in a disease marked by years of antecedent silent deposition of urate crystals in and around joints and is, therefore, the indication to lower urate levels. Others believe that, because tophi and symptomatic chronic gouty arthritis develop only in a minority of cases and ordinarily very slowly after many years of recurrent acute attacks, initiation of these agents can be delayed until the patient has more attacks. However, some patients never have a second attack, even in the absence of therapy. The probability of such a ‘benign’ course is greatest in patients who have only minimally elevated serum urate concentrations and normal 24-hour urinary uric acid values.

If a patient has hyperuricemia and gouty arthritis characterized by any of the following clinical characteristics, tophaceous deposits, gouty erosive changes on radiographs, or gout attack frequency of ≥2 attacks per year, then the patient should be offered treatment with a urate-lowering drug because urate-lowering drugs have been well-tolerated and effective in decreasing the attack frequency and disease severity for those with severe gout [1**]. Arguably, a case can be made for consideration of urate-lowering therapy after the second attack of gouty arthritis in most patients.

In general, the aim of urate-lowering therapy is to reduce the serum urate concentration to 6.0 mg/dl or less, well below the concentration at which extracellular fluids are saturated with monosodium urate. This value is based on the physiochemical fact that body fluids are saturated with urate when concentrations are greater than 6.8 mg/dl. Thus if levels remain above 6.8 mg/dl, the precipitation and crystal deposition can occur. When levels are maintained below 6.8 mg/dl, deposition will not occur and deposited crystals will dissolve. A recent retrospective examination of 267 patients with gout has reaffirmed that a reduction of serum urate concentrations to 6 mg/dl or lower will eventually result in reduced frequency or prevention of future gouty attacks [13**].

If a gout patient is given a prescription for a xanthine oxidase inhibitor (our uricosuric), then a serum urate level should be checked at least once during the first 6 months of continued use because periodic serum urate measurements are required for appropriate dose adjustments of urate-lowering medications (escalations or reductions) [1**]. In general, the lower the serum urate level achieved with urate-lowering therapy, the more rapid is the reduction in tophaceous deposits.

Target urate levels can be reached pharmacologically by the use of xanthine oxidase inhibitors or uricosuric agents. Xanthine oxidase, the enzyme that catalyzizes the oxidation of hypoxanthine to xanthine and xanthine to urate, is inhibited by agents such as allopurinol, oxyxuprine, and febuxostat. Drugs that reduce serum urate levels by increasing the urinary clearance of uric acid are termed uricosurics and include probenecid and benzbromarone.

Once initiated, the use of a urate-lowering agent is usually continued indefinitely. For those patients with gout who excrete less than 800 mg of uric acid per day and have normal renal function, reduction of serum urate concentration can be achieved equally well with a xanthine oxidase inhibitor or a uricosuric drug. Both classes of drugs are equally effective in preventing deterioration of renal function in patients with primary gout. In most cases, a xanthine oxidase inhibitor is the drug of choice because those agents can be used with fewer restrictions compared with uricosuric agents. In general, the candidate for uricosuric agents is the gouty patient whose hyperuricemia results from uric acid underexcretion (uric acid excretion of less than 800 mg/24 hours on a general diet), who is younger than 60 years of age and has normal renal function (creatinine clearance greater than 80 mL/min), and no history of renal calculi. These agents generally require good renal function to be effective, because they increase the amount of uric acid in the urine, can be associated with nephrolithiasis. In addition, patients prescribed uricosuric agents should be counseled to avoid salicylate use at doses greater than 81 mg per day, because concomitant salicylate use renders the uricosuric agents ineffective.

Probenecid is the most widely used uricosuric agents available in the United States; Benzbromarone is used for this purpose in other countries as well. Probenecid is metabolized in vivo with less than 5% of the administered dose recovered in the urine. The maintenance dosage of probenecid ranges from 500 mg to 3 g per day and is administered on a twice or three times a day schedule. Benzbromarone is potent uricosuric that is well tolerated and effective in cyclosporine-treated renal transplant patients. In contrast to the other uricosuric agents, benzbromarone can be used with moderate renal dysfunction (creatinine clearance now 25mi/min). However, benzbromarone use may be associated with serious hepatic toxicity. In certain situations, a xanthine oxidase inhibitor is clearly the drug of choice in the gouty patient.
If a gout patient is started on urate-lowering therapy and has either a history of nephrolithiasis or significant renal insufficiency (serum creatinine level ≥2 mg/dl or measured/estimated creatinine clearance ≤50 mL/min), then a xanthine oxidase inhibitor should be started as the initial urate-lowering medication rather than a uricosuric agent (probenecid) because in contrast to xanthine oxidase inhibitors, uricosuric agents increase the renal excretion of urate, enhancing the risk of nephrolithiasis, and may have diminished efficacy in the context of significant renal insufficiency [1**].

A final indication for a xanthine oxidase inhibitor is the failure of uricosuric agents to produce a serum urate concentration lower than 6 mg/dl or patient intolerance to uricosuric agents. A xanthine oxidase inhibitor and a uricosuric drug may be used in combination for the patient with tophaceous gout in whom it is not possible to reduce the serum urate below 6 mg/dl with a single agent. In most settings, if allopurinol does not cause the serum urate to drop below 6 mg/dl, it is the result of insufficient dosing or poor patient compliance.

Allopurinol is presently the only xanthine oxidase inhibitor available by prescription. Allopurinol can be given once a day because its effective half-life is 14 to 18 hours. Dosages required to reach target serum urate levels vary between 200 and 800 mg in patients with normal renal function, with the most common dosage 300 mg per day.

If a gout patient is receiving an initial prescription for allopurinol and has significant renal impairment (defined as a serum creatinine level ≥2 mg/dl or measured/estimated creatinine clearance ≤50 mL/min), then the initial daily allopurinol dose should be <300 mg per day because the risk of allopurinol-related toxicity is increased in the presence of significant renal impairment in gout patients given a daily allopurinol dose equal to or exceeding 500 mg [1**]. Allopurinol is involved in relatively few drug to drug interactions. The most important of these are azathioprine and 6-mercaptopurine.

If a gout patient is given a prescription for xanthine oxidase inhibitor in the setting of required therapy with either azathioprine (Imuran) or 6-MP, then the dose of azathioprine/6-MP should be reduced by a minimum of 50% because concurrent use of a xanthine oxidase inhibitor leads to a substantial increase in serum levels of azathioprine (and 6-MP) and increases the risk for severe drug-related myelosuppression [1**]. In addition, allopurinol can prolong the half-lives of warfarin and theophylline.

About 20% of patients who take allopurinol report side effects with 5% of patients discontinuing the medication. More common side effects include gastrointestinal intolerance and skin rashes. Other adverse reactions include fever, toxic epidermal necrolysis, alopecia, bone marrow suppression with leukopenia or thrombocytopenia, agranulocytosis, aplastic anemia, granulomatous hepatitis, jaundice, sarcoid-like reaction and vasculitis. The most severe reaction is the allopurinol hypersensitivity syndrome that consists of a constellation of findings and may include fever, skin rash, eosinophilia, hepatitis, progressive renal insufficiency, and death. This is most likely to develop in individuals with pre-existing renal dysfunction and those taking diuretics.

Several strategies exist to deal with allopurinol reactions. One is to desensitize the patient to allopurinol. Both oral and intravenous protocols exist [14]. The other has been to use oxypurinol. Oxypurinol is the active metabolite of allopurinol that is excreted in the urine and has a half-life 15 hours. This agent has been available on a compassionate-use basis and is presently in phase III trials [15*]. Unfortunately, 40% of those allergic to allopurinol have similar reactions to oxypurinol. A multicenter, randomized double-blind crossover trial has shown that, using comparable dosages, plasma urate levels are higher with oxypurinol treatment relative to allopurinol, but not by more than 10% [16].

An NDA has recently been submitted to the FDA for febuxostat. Febuxostat is a potent novel selective inhibitor of xanthine oxidase. Phase II and III trials have demonstrated efficacy and safety [17,18,19*]. In addition, it is metabolized in the liver. Therefore dose adjustment does not appear to be necessary in patients with renal dysfunction [20]. A small trial has shown that this agent is tolerated in patients that are sensitive to allopurinol [21*]. This would be predicted because of the differences in chemical structures. Allopurinol is a purine compound and febuxostat is not.

Another agent undergoing clinical trials is pegylated uricase (uricase-PEG-20 and Puricase). Uricase is the enzyme lacking in humans, but present in other animals and bacteria, that converts uric acid to allantoin. A recombinant uricase, rasburicase, has been used in the tumor lysis syndrome but has many limitations that preclude it from being used in gout. The pegylated forms of the enzyme overcome many of the problems that occur with rasburicase and have been used safely in humans with hyperuricemia and gout [22–23]. Although this agent is administered intravenously, it has potential for use in organ transplant patients with gout, patients allergic to allopurinol, patients with renal insufficiency, and those who do not respond to conventional therapies for gout [24*].

Associated conditions

Obesity, heavy alcohol consumption, hyperlipidemia, hypertension are very commonly associated with gout. If a patient is diagnosed with gout and has either obesity
Asymptomatic hyperuricemia

If a patient has asymptomatic hyperuricemia characterized by no prior history of gouty arthritis or tophaceous deposits and no prior history of nephrolithiasis or hyperuricosuria and no ongoing treatment of malignancy, then urate-lowering therapies should not be initiated because there is currently no widely accepted indication for the treatment of asymptomatic hyperuricemia [1**].

The reason there is no widely accepted indication for the treatment of asymptomatic hyperuricemia involves our inability to determine the significance of the relation between hyperuricemia and diseases other than gout. Serum urate levels may not be an independent risk factor for cardiovascular disease, but serum urate levels are a strong predictor of cardiovascular disease and all-cause mortality in healthy middle-aged Finnish men [29] and blood pressure levels are predictive for cardiovascular disease incidence synergistically with serum urate levels [30*]. It still remains to be determined whether these relationships are circumstantial or causal.

Regardless, the finding of hyperuricemia is an indication to determine its cause. Hyperuricemia may be the initial clue to the presence of a previously unsuspected disorder. Identification of the underlying cause may be useful in predicting the potential consequence, if any, of the elevated serum urate concentration and treatment of the underlying cause may reduce the serum urate concentration [31].

In this context, diet is very important. Therefore dietary changes may prove very helpful. Recent evidence indicates that higher levels of meat and seafood consumption are associated with an increased risk of gout, whereas a higher level of dairy product consumption is associated with a decrease risk. Moderate intake of purine-rich vegetables or protein seems not to impose an increased risk of gout [32*]. In addition alcohol intake is strongly associated with an increased risk of gout. This risk varies substantially according to the type of alcoholic beverage, with beer conferring a larger risk than spirits and wine not appearing to increase the risk [33*].

Conclusion

The quality indicators to be used in the management of gout will allow better investigations in this field and help make the management of this disease even better. The addition of new anti-hyperuricemic agents will give clinicians more tools to use to treat gout patients. Despite the fact that there are potent medicines to treat hyperuricemia, attention to diet and treatment of associated, or co-existing, medical problems is essential.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest

First ever published indicators of quality for treatment of gout. While not ideal, this is the best available and an important step in the process of developing quality indicators for this disease.

Thorough review referencing what evidence-based literature that exists in this field.


Study indicating that one reason for poor outcomes in gout is lack of patient compliance.


Although potentially dangerous, intravenous colchicine can be used safely and effectively.


Largest study to date looking at the efficacy of prophylactic colchicine demonstrating its use does decrease acute gouty attacks when patients start urate-lowering therapy. But maintaining urate below 6.0 mg/dl is what ultimately decreases the incidence of gout attacks.
Crystall deposition diseases

30 Lin KC, Tsao HM, Chen CH, Chou P. Hypertension was the major risk factor leading to development of cardiovascular disease among men with hyperuricaemia. J Rheumatol 2004; 31:1152–1158. Emphasizes the importance of blood pressure control for patients with gout.
Extracellular matrix changes regulate calcium crystal formation in articular cartilage
Savitha Kalya and Ann K. Rosenthal

Purpose of review
The pathologic matrix mineralization seen in calcium pyrophosphate dihydrate and basic calcium phosphate deposition diseases identifies a subset of osteoarthritis patients with an unusual joint distribution and rapid progression of disease. Several factors contribute to pathologic matrix mineralization, including changes in the extracellular matrix of articular cartilage. The factors contributing to extracellular matrix changes that promote crystal formation are important and not well understood. Better characterization of these factors will enhance the understanding of the pathogenesis of pathologic matrix mineralization and may identify potential targets for novel therapeutic interventions.

Recent findings
Histologic studies of cartilage from patients affected by calcium crystal arthritis show changes in the pericellular matrix of articular chondrocytes. The amounts and types of collagens, proteoglycans, and calcium-binding proteins are altered. The mechanisms by which these changes occur remain poorly understood. Recent work, however, has implicated alterations in the chondrocyte phenotype and post-translational matrix-modulating enzymes such as the transglutaminases.

Summary
Changes in extracellular matrix are associated with the pathologic matrix mineralization seen in calcium pyrophosphate dihydrate and basic calcium phosphate crystal deposition diseases. The literature on growth plate cartilage provides observations and mechanisms through which extracellular matrix contributes to normal matrix mineralization, and has served as a model on which to base studies in articular cartilage. More studies are warranted to enhance the understanding of how changes in extracellular matrix contribute to crystal deposition diseases.

Keywords
basic calcium phosphates, calcium pyrophosphate deposition, extracellular matrix

Abbreviations
BCP basic calcium phosphate
CPPD calcium pyrophosphate dihydrate
PPI pyrophosphate

Introduction
Osteoarthritis is the most common form of arthritis in adults and presents an increasingly challenging public health problem as the population ages. Pathologic calcium crystals occur commonly in joints affected by osteoarthritis [1]. Of patients undergoing knee replacement for osteoarthritis, for example, 60% have articular calcium pyrophosphate dihydrate (CPPD) or basic calcium phosphate (BCP) crystals [1]. Patients with osteoarthritis with intra-articular calcium crystals have an unusual pattern of joint involvement, greater severity, and more rapid progression than do patients with osteoarthritis without crystals [2–4].

The formation of calcium crystals in articular cartilage is multifactorial and not completely understood. It is tempting to propose that by understanding the early phases of crystal formation, we can identify potential targets for therapy. Here we shall review what is known about CPPD and BCP crystal formation in articular cartilage, briefly review normal articular cartilage matrix composition, and then discuss how extracellular matrix changes contribute to pathologic mineralization in articular cartilage.

Calcium pyrophosphate dihydrate crystal formation
The current paradigm of CPPD crystal formation identifies four participants in this process (Table 1). First, the overproduction of extracellular pyrophosphate (PPi), the anionic component of the crystal, contributes to CPPD crystal deposition. Elegant work has recently been done to show that chondrocytes from CPPD-diseased cartilage produce more extracellular PPi in comparison with normal and osteoarthritic control individuals [5]. Chondrocytes are the primary source of PPi in the joint, although the mechanisms of PPi production remain unclear [6]. PPi may be made de novo by chondrocyte ectoenzymes, such as PC-1, which hydrolyze nucleoside triphosphates. Alternatively or in addition, intracellular PPi may be transported across cell membranes by ANK or other proteins [7]. Second, calcium is necessary for CPPD crystal

Current Opinion in Rheumatology 2005, 17:325–329

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1040-8711
Crystal deposition diseases

Crystal deposition diseases involve the formation of extracellular matrix changes that promote calcium crystal formation in articular cartilage. Calcium concentrations are increased in cartilage from patients with osteoarthritis CPPD [8]. Little is known, however, about the role of calcium in CPPD crystal formation. Third, changes in pericellular matrix, where the smallest and earliest CPPD crystals are formed, also clearly contribute to CPPD crystal formation. Last, matrix vesicles have been implicated in CPPD crystal formation. These are small membrane-bound extracellular organelles that bud off chondrocytes. They are well characterized in growth plate cartilage, where they concentrate ions, enzymes, and substrates necessary for matrix mineralization [9]. Matrix vesicles isolated from articular cartilage can produce CPPD crystals in vitro. Although much recent work on CPPD crystal formation has focused on PPI metabolism, relatively less attention has been paid to the mechanisms by which extracellular matrix and matrix vesicles in articular cartilage contribute to CPPD crystal formation.

Basic calcium phosphate crystal formation

The formation of BCP crystals in articular cartilage is even less well understood than that of CPPD crystal formation. BCP crystals include three types: tricalcium phosphate, octacalcium phosphate, and carbonate-substituted apatite. The prevalence of intra-articular BCP crystals increases with age, and these crystals frequently co-exist with CPPD. The formation of BCP crystals in articular cartilage is even less well understood than that of CPPD crystal formation. The similarities and differences in the factors involved are summarized in Table 1. For example, high levels of extracellular PPI negatively modulate BCP crystal nucleation and growth [12]. Calcium levels may be increased in affected cartilage [8]. Changes in pericellular matrix have also been clearly documented when BCP crystal formation occurs in articular cartilage [13]. Matrix vesicles have been the main focus of studies of BCP crystal formation. Histologic evidence shows matrix vesicles near BCP crystal deposits in articular cartilage [14]. Matrix vesicles isolated from articular cartilage are capable of forming BCP crystals in vitro that are similar those found in osteoarthritic synovial fluid [15].

Normal articular cartilage extracellular matrix

An appreciation of the extracellular matrix changes that promote calcium crystal formation in articular cartilage requires some knowledge of normal pericellular matrix in adult cartilage. Normal adult hyaline cartilage contains collagen types II, VI, IX, X, and XI. Collagen type II is the most abundant of the collagens and is primarily responsible for forming the large fibrils that give cartilage its strength and resilience. Proteoglycans represent the other major component of cartilage extracellular matrix. These are essential for the load-absorbing characteristics of cartilage. They consist of a core protein with one or more glycosaminoglycan side chains. Normal pericellular matrix contains the large cartilage-specific proteoglycan aggregan. It also contains intact type II collagen fibrils as well as unique proteins such as type VI collagen, fibronectin, and osteopontin, rarely seen in other areas of matrix.

Importance of extracellular matrix in pathologic matrix mineralization in articular cartilage

Changes in cartilage extracellular matrix are strongly associated with CPPD deposition disease. The observations that the smallest and earliest crystals form in areas of abnormal pericellular matrix and that CPPD crystals rarely form in tissues other than articular cartilage or areas of chondrocyte metaplasia support an important role for extracellular matrix in crystal formation [16,17]. Histologic evidence further supports a role for extracellular matrix changes in CPPD crystal formation. CPPD crystals are formed in areas of abnormal pericellular matrix and are not seen in areas of normal matrix [18]. Affected cartilage matrix contains damaged type collagen II fibers and increased calcium-binding matricellular proteins. Although small proteoglycans, such as decorin, are increased in the matrix surrounding CPPD crystals, fewer large proteoglycans are present [19].

Similarly, histologic studies of cartilage from joints affected by BCP crystal arthritis show BCP crystals in the pericellular matrix of chondrocytes. Few studies have demonstrated histologic matrix abnormalities during BCP crystal formation, but when similar crystals form in nascent bone, matrix changes often precede mineral formation [20].

Laboratory evidence confirms an important role for extracellular matrix in CPPD crystal formation. CPPD crystals will form in solution but require extremes of temperature, pH, and ion concentration for formation [21]. By contrast, in collagen or agarose matrices, CPPD crystals form spontaneously at much lower concentrations of ions and under physiologic conditions [22].

The rates and quantities of BCP crystals formed in various models are also affected by the presence of matrix [13].

Table 1. Participants in calcium crystal formation in articular tissues

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<th>CPPD</th>
<th>BCP</th>
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<tr>
<td>Pyrophosphate</td>
<td>Promotes</td>
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<td>Calcium</td>
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<tr>
<td>Extracellular matrix</td>
<td>Altered</td>
<td>Altered</td>
</tr>
<tr>
<td>Matrix vesicles</td>
<td>Generate CPPD</td>
<td>Generate BCP</td>
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CPPD, calcium pyrophosphate dihydrate; BCP, basic calcium phosphate.
Laboratory evidence also supports a role for enzymes that post-translationally modify extracellular matrix proteins in CPPD deposition disease. For example, the enzymes called transglutaminases catalyze the formation of ε-(γ-glutamyl) lysine bonds between or within proteins, thereby altering their structure and functions. Two forms of transglutaminases, including tissue (type II) transglutaminase and factor XIII have been described in articular cartilage [23,24]. The protein levels of both transglutaminase enzymes are increased in articular cartilage with aging and osteoarthritis [25,26]. Enzyme activity is concentrated in pericellular matrix in osteoarthritic cartilage [27**]. When extracellular transglutaminase activity is inhibited, CPPD crystal formation is suppressed [27**]. The above evidence implicates a role for transglutaminases in CPPD crystal formation in articular cartilage by changing the pericellular matrix.

Additional evidence supports a potential role for transglutaminases in BCP crystal formation. In bone, these enzymes modify pericellular matrix proteins during matrix mineralization [28]. In osteoblasts, transglutaminases may participate in matrix mineralization [29].

Studies of articular cartilage matrix vesicle behavior also support an important role for extracellular matrix in CPPD crystal formation. There is strong histologic evidence supporting the presence of matrix vesicles in normal and osteoarthritic articular cartilage. The observation that matrix vesicles isolated from osteoarthritic cartilage did not display increased mineralization capacity compared with vesicles from normal cartilage supports the idea that the interaction between matrix vesicles and the pericellular matrix regulates crystal formation in vivo [30].

The mechanisms through which extracellular matrix changes occur in CPPD crystal formation are poorly understood. Age and osteoarthritis are major risk factors for pathologic matrix mineralization. In articular cartilage, chondrocytes regulate matrix production. Chondrocytes in CPPD deposition disease share morphologic and phenotypic features with the hypertrophic chondrocytes responsible for normal matrix mineralization in the growth plate [31,32,36]. This shift toward a hypertrophic phenotype may contribute to the production of abnormal mineral-promoting matrix. Aging and injury may contribute to this altered phenotype.

**Collagen and calcium crystal formation**

Histologic studies show that type II collagen fibrils are fragmented around CPPD crystals, whereas normal matrix contains intact fibrils. Total collagen content as measured by hydroxyproline levels is decreased in cartilage around CPPD crystal deposits [13]. Type I collagen, a collagen not usually present in normal cartilage, is also found in increased quantities in CPPD diseased cartilage. A role for type X collagen in matrix vesicles and as part of the hypertrophic phenotype in articular chondrocytes has also been proposed to play a role in CPPD crystal formation [33,34]. In contrast to our sparse current knowledge of CPPD crystal formation, much is known about matrix mineralization in growth plate cartilage. Thus, growth plate cartilage mineralization has served as a model on which to base hypotheses about articular cartilage matrix mineralization. In the growth plate, there is excellent evidence that type II and type X collagen promote mineral formation. For example, type II collagen increases calcium crystal nucleation and growth [9]. Type II collagen binds to annexin V on the membranes of matrix vesicles and increases the ability of these calcium channels to import calcium into the vesicle [35]. Crystal growth is propagated along type II collagen fibrils [9].

Boskey et al. [13] showed an increased in collagen content in cartilage surrounding BCP deposits. Total hydroxyproline content, which reflects total collagen content, was increased in cartilage containing BCP crystals, in comparison with normal articular cartilage. By contrast, collagen content was decreased in CPPD crystal-containing cartilage, as discussed above. These differences in matrix may influence the kind of calcium crystals that will form: either CPPD or BCP. This study also demonstrated an increase in the total lipids in cartilage affected by both kinds of calcium crystals. This finding may reflect an increased presence of lipid bilayer membrane in the form of matrix vesicles, which have been implicated in both BCP and CPPD crystal formation in articular cartilage.

**Proteoglycans and calcium crystal formation**

Proteoglycans are an important component of the extracellular matrix of articular cartilage. The nature of their core protein allocates them into large proteoglycans like aggrecan and small proteoglycans like fibromodulin, decorin, and biglycan. The role of proteoglycans in CPPD crystal formation remains controversial. Histologic studies show a loss of normal pericellular halo in CPPD disease. There is a decrease in large proteoglycans and an increase in small proteoglycans, such as decorin, in CPPD cartilage [19]. Small proteoglycans may encourage crystal nucleation. The use of growth plate mineralization as a model does little to clarify the role of proteoglycans in pathologic mineralization, because in various models, proteoglycans can either inhibit or promote mineral formation [36,37].

Most studies suggest that large proteoglycan aggregates inhibit hydroxyapatite growth in a variety of models [36,38–40]. Matrix vesicles are also enriched in metalloproteinases that degrade proteoglycans [41]. Logically, large, highly charged proteoglycans like aggrecan could interfere with mineral formation by chelating calcium or sterically hindering calcium crystal growth. Histologic studies suggest that a reduction in proteoglycan hydrophobicity
Crystal deposition diseases

Small dermatan sulfate proteoglycans, such as decorin and biglycan, may also play important roles in growth plate mineralization. Decorin aids in type II collagen fibril formation, and biglycan is associated with type VI collagen fibrils. These fibrils can interact with type II collagen fibrils or aggrecan by way of matrilins [46]. Both bind calcium and calcium crystals [47]. They are often found at sites of mineralization [42,48]. In one model, these small proteoglycans encouraged crystal nucleation but inhibited crystal growth [47].

Calcium-binding proteins

Calcium-binding proteins, including S-100, osteopontin, and osteonecstin (secreted protein acid-rich and rich in cysteine [SPARC]), are increased in the extracellular matrix of CPPD-diseased cartilage. Osteopontin is a phosphoglycoprotein with a complex structure that participates in multiple physiologic and pathologic processes [49]. It is present in osteoarthritic cartilage, especially near hypertrophic chondrocytes. It participates in normal and pathologic matrix mineralization. Osteopontin may have an important role in CPPD crystal formation for the following reasons: (1) It has a large calcium-binding capacity. (2) It is a transglutaminase substrate [50]. (3) It is seen in pathologic calcification at other sites such as atherosclerotic plaques and benign breast microcalcifications. (4) It is increased in the pericellular areas of hypertrophic chondrocytes where crystals are formed, and is coordinately regulated with PPi in osteoblasts [51].

Osteonecstin, or SPARC, like osteopontin, is a transglutaminase substrate present in articular cartilage [52]. Osteonecstin binds calcium and participates in normal and pathologic matrix mineralization [53].

Another calcium-binding protein, S-100, is increased in hypertrophic chondrocytes around CPPD crystals and may contribute to the increased accumulation of intracellular calcium seen in hypertrophic chondrocytes [31].

Although one could hypothesize the mechanisms by which these calcium-binding proteins contribute to matrix mineralization, they remain unclear and need to be further explored.

Conclusion

Extracellular matrix changes have been well documented in calcium crystal deposition diseases such as CPPD and BCP. These changes play an important role in the pathogenesis of crystal formation in articular cartilage. The mechanisms by which these changes contribute to pathologic matrix mineralization are poorly understood. Further studies are warranted for a better understanding of the pathogenesis of crystal deposition diseases.

Acknowledgements

The authors thank Claudia Gohr for her generous assistance.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

** of outstanding interest

Calcium crystal formation in articular cartilage  Kalya and Rosenthal 329


Genetic studies of chondrocalcinosis
Yun Zhang and Matthew A. Brown

Purpose of review
Our understanding of the causation of the chondrocalcinosis and other disorders characterized by ectopic mineralization is rapidly increasing, and genetic studies have contributed substantially to recent major advances in the field. This review will discuss what is known about the genetics of chondrocalcinosis and what we have learned from genetic studies to date.

Recent findings
Chondrocalcinosis is one of a family of conditions associated with ectopic mineralization. This family also includes disorders of mineralization of bone and spinal and other ligaments, and vascular calcification. There has been increasing evidence of the key role of transport and metabolism of inorganic pyrophosphate (PPi) in control of mineralization, and as the likely explanation for the association of a variety of genetic variants with chondrocalcinosis and ectopic mineralization elsewhere. This may be an overly simplistic view of this family of conditions, with recent evidence suggesting that, for example, ANKH variants may not all predispose to chondrocalcinosis by effects on PPi transport, but may also influence chondrocyte maturation.

Summary
Understanding the control of the process of mineralization and its tissue specificity are important steps in the search for rational therapies for these conditions.

Keywords
chondrocalcinosis, genetics, inorganic pyrophosphate

Introduction
Disordered calcification of cartilage and other skeletal tissues occurs commonly among the elderly, yet the reasons for this are very poorly understood. Up to 5% of the human population shows radiographic evidence of chondrocalcinosis, with the incidence rising with age to 15–40% over 60 years of age [1–4]. The proportion of asymptomatic chondrocalcinosis in the general population is unknown but clearly substantial. Symptomatic presentations of chondrocalcinosis include acute arthritis termed ‘pseudogout’ and various forms of chronic arthropathy. Chronic arthropathy is most common in elderly women, and while usually mild arthropathy can lead to quite severe and rapidly destructive arthritis. There is currently no specific treatment to slow or prevent the gradual joint deterioration due to chondrocalcinosis or the progression of the crystal deposition itself, other than treatment of any underlying biochemical or metabolic disorders. Clearly improved understanding of the aetiopathogenesis of chondrocalcinosis is required if rational therapies are to be developed.

Although most cases of chondrocalcinosis are nonfamilial, there is considerable evidence that genetic factors are involved. This evidence includes

• the existence of known monogenic causes of chondrocalcinosis,
• reports of families with monogenic segregation of chondrocalcinosis, and
• mouse models with genetic mutations inducing chondrocalcinosis.

The elucidation of these genetic factors has contributed greatly to our understanding of the causation of chondrocalcinosis and other disorders of ectopic mineralization.

Genetic epidemiology of chondrocalcinosis
Many multicase families with chondrocalcinosis have been reported in the literature [5–24]. Most familial cases appear to be inherited in an autosomal dominant manner, with early onset and varying severity even within families. The existence of such families confirms that chondrocalcinosis can be caused by high penetrance single gene disorders in humans. Very few of these families have ever been studied genetically, although the potential benefits of identifying the genes involved are considerable. Even if more common polymorphic variants of these genes are not associated with chondrocalcinosis, the fact that mutations of the genes do cause the disease gives incontrovertible...
evidence that the gene concerned is involved in the aetiopathogenesis of the condition at least in some cases.

Whether genetic risk factors are involved in later life chondrocalcinosis has not been well established. Until recently, only small studies of the recurrence risk had been reported for the general community. These suggested recurrence risk rates in first-degree relatives of 11–28% [12,17]. These recurrence risk rates appear quite high and are likely to be higher than the prevalence of the disease in appropriately matched unrelated individuals, but that was not formally established by these investigators.

A recent study has compared the recurrence rate of chondrocalcinosis of the knee in siblings of index cases and unrelated community members [25*]. They found a significantly increased familiarity of chondrocalcinosis (sibling recurrence risk ratio 2.0, \( P = 0.015 \)) and for pyrophosphate arthropathy (chondrocalcinosis and osteoarthritis combined) (sibling recurrence risk ratio 2.3, \( P = 0.024 \)). Following adjustment of the data to account for the association of the condition with age, gender and knee pain, these findings were no longer significant. While adjustment for established disease-causing covariates such as age and gender would clearly be appropriate, the reasons for the adjustment for knee pain are unexplained. Although it is widely believed that chondrocalcinosis may cause and accelerate osteoarthritic changes, this has not yet been established in longitudinal studies. It is possible that the familiarity observed in this study for chondrocalcinosis may reflect genetic causes of osteoarthritis rather than primary chondrocalcinosis. Nonetheless, this study shows that the familiarity of knee chondrocalcinosis in elderly patients is low, as would be expected for a common disease. Future genetic studies investigating ‘sporadic’ chondrocalcinosis should therefore focus on younger, more severely affected cases.

**Monogenic forms of human chondrocalcinosis**

The genetic disorders known to be associated with chondrocalcinosis can be divided into conditions where chondrocalcinosis is the principal disease manifestation, and those where it is secondary to disease elsewhere. With regard to the former category, two chromosomal regions, lying on chromosomes 5 and 8, have been linked with human conditions characterized by CPPD chondrocalcinosis. In one North American family with early onset osteoarthritis and chondrocalcinosis, linkage was established with chromosome 8q (CCAL1, MIM 600668, [26]). The association with early onset osteoarthritis raises the possibility that the chondrocalcinosis was secondary to this, rather than being the primary cause of the arthropathy. The gene underlying this linkage has yet to be mapped.

**The ANKH gene**

The chondrocalcinosis-gene located at the CCAL2 locus, lying on chromosome 5p, has been demonstrated to be the *ANKH* gene. The identification of a loss of function mutation in the *ANKH* gene as the cause of profound ectopic calcium hydroxyapatite deposition in a mouse model originally thought to resemble ankylosing spondylitis, the *ank/ank* mouse, has given important insights into the biochemical control of calcium crystal deposition. The defective gene involved in this phenotype is thought to be a membrane pyrophosphate transporter, and dysfunction of the gene causes elevation of intracellular pyrophosphate and reduction in extracellular pyrophosphate [27]. Our group and others have demonstrated that mutations of the human homologue of this gene, *ANKH*, causes familial autosomal dominant CPPD chondrocalcinosis [28,29]. Several variants have now been reported to be associated with this condition, and with the condition autosomal dominant craniometaphyseal dysplasia (OMIM 123000) (disease-associated variants listed in Table 1). We have also demonstrated that a common promoter region polymorphism is associated with ‘sporadic’ chondrocalcinosis, albeit in cases with severe, widespread, early-onset disease [30]. The low or absent familiality of mild disease in later life suggests that genetic studies in such populations are not likely to be fruitful.

The functional effects of chondrocalcinosis-associated *ANKH* variants are still not completely understood. Preliminary studies in COS7 cells of some *ANKH* variants associated with chondrocalcinosis did not show significant effects on PPi levels [29]. Potential explanations for this include: the cell type that was studied was not representative of those involved in chondrocalcinosis; the effect of the mutation may only be apparent under particular conditions; the studies did not investigate effects on *ANKH* transcription/translation; or that *ANKH* mutations cause chondrocalcinosis through effects other than on PPi transport. Recent evidence demonstrates that *ANKH* variants associated with familial and sporadic chondrocalcinosis cause increased transcription/translation, but have varying effects when expressed by transient transfection in a chondrocyte cell line [30**]. While some variants increased extracellular PPi, others affected chondrocyte maturation, reflected by expression of type X collagen. Ank expression in the developing mouse skeleton has been found to be high in hypertrophic ossification centers consistent with a role in regulating chondrocyte maturation, as well as in nonmineralized skeletal tissues such as tendons and the superficial layer or articular cartilage, consistent with a role in inhibition of mineralization [31]. These preliminary studies show that there is much yet to be learned about ANKH function, and that although it is likely to be involved in PPi transport, other functional effects may be important. If this protein is to be a therapeutic target in CPPD
Table 1. ANKH variants associated with human diseases

<table>
<thead>
<tr>
<th>Location</th>
<th>Site (bp)a</th>
<th>Variantsb</th>
<th>Amino acid</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>In ankylosing spondylitis</td>
<td>–378</td>
<td>GGC ins</td>
<td>–</td>
<td>Canadian sporadic [35]</td>
</tr>
<tr>
<td></td>
<td>–75</td>
<td>CCCGTCGC ins</td>
<td>–</td>
<td>Canadian sporadic [35]</td>
</tr>
<tr>
<td>In chondrocalcinosis</td>
<td>5′-UTR</td>
<td>–11</td>
<td>C→T</td>
<td>1 large British familial CPPD [29]</td>
</tr>
<tr>
<td></td>
<td>–4</td>
<td>G→A</td>
<td>–</td>
<td>British sporadic [30]</td>
</tr>
<tr>
<td></td>
<td>+13</td>
<td>C→A</td>
<td>PST</td>
<td>2 American pedigrees</td>
</tr>
<tr>
<td></td>
<td>+14</td>
<td>C→T</td>
<td>PSL</td>
<td>1 large Argentinean familial CPPD [28]</td>
</tr>
<tr>
<td></td>
<td>143</td>
<td>T→C</td>
<td>T48M</td>
<td>1 large French familial CPPD6 [29]</td>
</tr>
<tr>
<td>Exon 12</td>
<td></td>
<td>GAG del</td>
<td>E490del</td>
<td>1 British pedigree [29]</td>
</tr>
<tr>
<td>In craniometaphyseal dysplasia</td>
<td>875</td>
<td>T→C</td>
<td>W292R</td>
<td>Russian sporadic [32]</td>
</tr>
<tr>
<td></td>
<td>992</td>
<td>T→C</td>
<td>C331R</td>
<td>1 German pedigree [32]</td>
</tr>
<tr>
<td></td>
<td>1122</td>
<td>CTC del</td>
<td>S375del</td>
<td>2 American pedigrees [33]</td>
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<td>S375del</td>
<td>1 American pedigree [32]</td>
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<td>TCT del</td>
<td>F376del</td>
<td>2 American pedigrees [33]</td>
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<td></td>
<td>1128</td>
<td>CTT del</td>
<td>F377del</td>
<td>2 German pedigrees [32]</td>
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<tr>
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<td>1166</td>
<td>A→G</td>
<td>P380insA</td>
<td>Israeli sporadic [32], 1 American pedigree [33]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G→A</td>
<td>G389R</td>
<td>1 Australian, 1 Swiss pedigree [32]</td>
</tr>
</tbody>
</table>

*aPosition numbered from A of ATG start codon; amino acid position numbered from Met = 1.
*bComparing with the public sequence and at the same strand as ATG.
*cThe sites of all the variants are not the same as published in the references, as they have been recalculated from ATG start codon.
*dPosition from the beginning of exon 10.

chondrocalcinosis, then it may not be adequate to block its role in PPI transport alone. Elucidating the structure and protein interactions would very helpful in determining the functional effects of ANKH, and for rational therapy design.

As mentioned above, mutations of the ANKH gene have also been implicated in autosomal dominant craniometaphyseal dysplasia (MIM 123000). This rare condition is characterized by abnormal mineralization of membranous and enchondral bone, causing thickening of craniofacial bones, widened long-bone metaphyses and increased cortical thickness [32,33]. Not all families with the condition and established linkage to the chromosome 5 region encoding ank were found to have any coding region polymorphisms, raising the possibility that these families have mutations elsewhere in the promoter or 3′ UTR of the gene. Identifying such mutations could be very informative regarding the determinants of expression and RNA stability of the gene products. Ank expression has been demonstrated to be increased by TGF-beta1 in cultured chondrocytes, but little else is known about the control of expression of this gene [31]. The functional effects of the coding region variants have not been reported, although they are predicted to have a dominant negative effect. This is in contrast to the loss of ank function in the ank/ank mouse, where the heterozygotes are phenotypically normal. Autosomal recessive craniometaphyseal dysplasia has also been reported, and maps to 6q21–22 [34]. This 13-megabase region encompasses some enticing candidate genes such as COL10A1, the prototypic marker of the chondrocyte differentiation associated with the onset of mineralization.

In addition to the demonstrated role polymorphisms of ANKH in ‘sporadic’ chondrocalcinosis, it has been suggested that common ANKH variants may play a role in other conditions such as ankylosing spondylitis and epilepsy. Ankylosing spondylitis and the spondyloarthropathies are characterized by joint inflammation, characteristically of the axial skeleton. In contrast to the situation in rheumatoid arthritis, this inflammation leads to new bone formation, a process that is very poorly understood. Two studies have investigated the possible involvement of ANKH variants in ankylosing spondylitis. Tsui et al. demonstrated weak linkage and association of microsatellites close to the gene with susceptibility to the disease [35]. Our own study showed no evidence of linkage to the chromosome 5p ANKH locus, or of association of ANKH polymorphisms with either susceptibility to or severity of disease [36]. It is our belief that if ANKH is involved in this condition, it is more likely to be involved in influencing the rate of spinal ankylosis, rather than being a triggering factor for disease itself. Longitudinal studies of radiographic progression may be required to address this hypothesis. Johnson et al. have recently demonstrated that the proinflammatory cytokine IL-1β, polymorphisms of which are strongly associated with susceptibility to ankylosing spondylitis [37], can promote chondrocyte calcification through effects on transglutaminase-2 activity [38,39]. This exciting finding suggests a potential therapeutic route for preventing the progressing ectopic ossification which is the major cause of disability in this condition.

The identification of an ANKH mutation associated with seizures and chondrocalcinosis raises the possibility that
ANKH variation may play a role in some seizure disorders [29,40]. ANKH is widely expressed in neural tissue, and it will be interesting to determine why only one specific ANKH variant should cause seizures.

**Nucleotide pyrophosphate synthetase**

A genetic link between chondrocalcinosis and some forms of spinal ossification is suggested by the reported co-occurrence of the conditions in both humans [41,42], and in the tip-toe walking mouse (‘ttw’), a model of the human condition ossification of the posterior longitudinal ligament (OPLL), which develops spinal ossification and hydroxyapatite arthropathy [43]. The mutant gene causing this mouse phenotype encodes the enzyme plasma cell membrane glycoprotein-1 (PC-1), which is a nucleotide pyrophosphate synthetase enzyme (NPPS). PiP is hydrolyzed from ATP by the nucleoside triphosphate pyrophosphohydrolase activity of a family of three NPPS ectoenzymes [44]. PC-1 is expressed in a variety of cells and tissues including osteoblasts and chondrocytes, and is the only NPPS present in membrane-limited matrix vesicles [45].

The human homologue of the PC-1 gene (also known as NPPS1, ENPP1) is encoded at chromosome 6q23.2 and has 25 exons. Two variants of NPPS1, IVS20-11delT and IVS15-14C→T, are associated with OPLL susceptibility [46]. It has not been reported whether these variants have functional effects. These findings raise the possibility that variants of this gene may also be associated with chondrocalcinosis. Further support is lent to this hypothesis by the recent report of a child affected by severe calcium calcinosi [47].

**Other genetic conditions complicated by chondrocalcinosis**

A wide variety of other genetic conditions are associated with chondrocalcinosis. Gitelman [57] and Bartter disease are both associated with chondrocalcinosis, possibly due to their association with chronic hypomagnesaemia. Magnesium is a cofactor of alkaline phosphatase, and it is postulated that these conditions lead to mild functional hypophosphatasia. Iron and copper overload, associated with haemochromatosis and Wilson's disease, respectively, are thought to favor calcium crystal nucleation as well as inhibiting alkaline phosphatase activity. Mild iron overload associated with heterozygosity for the C282Y HFE variant, homozygosity for which is the major cause of inherited haemochromatosis, does not substantially influence the risk of CPPD chondrocalcinosis [58].

Chondrocalcinosis and ossification of ligaments of the spine and elsewhere are common complications of X-linked dominant hypophosphataemic rickets (OMIM 307800). This condition is caused by mutations in the PHEX gene [59], which is thought to be involved in breakdown of a phosphaturic factor, possibly FGF23. Cases have high renal phosphate clearance and chronic hypophosphataemia. Phosphate replacement improves bone mineralization and growth and reduces deformity if given prior to skeletal maturity. This therapy is difficult because of the short half-life of phosphate requiring multiple daily doses, and because it can stimulate secondary hyperparathyroidism, and is therefore not usually prescribed once skeletal maturity is achieved. In later life, affected individuals frequently develop florid osseous enthesopathy often leading to spinal canal stenosis, and vascular calcification due to ectopic mineralization. It is likely that the chronic hypophosphataemia characteristic of this condition is associated with low inorganic pyrophosphate levels; this may explain the ectopic hydroxyapatite deposition. Certainly as these complications represent major causes of morbidity for affected individuals, further research is warranted.

Ectopic mineralization also occurs in osteoprotgerin (OPG) knockout mice [60]. In these mice, OPG deficiency leads to unopposed osteoclast activation by RANKL. Severe osteoporosis ensues, complicated by ectopic mineralization in the vascular tree and costal cartilages, without marked disturbance of serum calcium or phosphate levels. Whether these effects are caused by direct effects of OPG...
deficiency, or by subtle disturbance of serum calcium and phosphate levels, has also not been established. This triad of features is commonly found in elderly osteoporosis patients; the role of OPG deficiency in such cases is unknown but with the development of OPG and anti-RANKL therapies for osteoporosis could be quite relevant.

**Conclusion**

There is much yet to be learned about the causes of chondrocalcinosis and other disorders of ectopic mineralization. There is a mounting body of evidence that disordered PPI metabolism and transport are key to this process, but this view may be overly simplistic and further research is clearly required. We have learned much from studies of genetic causes of chondrocalcinosis, but for most families reported with monogenic forms of the condition, the genes have not been mapped. Even for mapped chondrocalcinosis genes such as ANKH, our understanding of its functional role is preliminary. The value of further research in this field is clear given the high prevalence of the involved conditions, limited current understanding of the aetiopathogenesis, and lack of effective treatments.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Further detail provided in addition to that already reported in [29]. Mutation in ANKH causes chondrocalcinosis and seizures. Question its relevance to other epilepsy cases.


Elegant study demonstrates the central role of inorganic pyrophosphate in the balance between tissue mineralization with hydroxyapatite and CPPD.

Purpose of review
Calcium-containing crystals can cause the degeneration of articular tissues in two separate pathways. In the direct pathway, crystals directly induce synoviocytes to proliferate and produce metalloproteinases and prostaglandins. The other pathway, the paracrine pathway, involves the interaction between crystals and macrophages/monocytes, which leads to the synthesis and release of cytokines, which can reinforce the action of crystals on synoviocytes and/or induce chondrocytes to secrete enzymes and which eventually causes the degeneration of articular tissues. The purpose of this review is to highlight the recent findings of the biologic effect of these crystals.

Recent findings
In the past few years, major advances in the understanding of the biologic effect of crystals and the signal transduction pathway of crystal-induced cell activation offer a unique opportunity to examine the role of crystal in osteoarthritis and cartilage degeneration.

Summary
Evidence for a causal role of crystals in cartilage degeneration in osteoarthritis is primarily inferential and is based on correlative data. Clinical observations indicate that exaggerated and uniquely distributed cartilage degeneration is associated with these deposits. Measurements of putative markers of cartilage breakdown suggest that these crystals magnify the degenerative process. Studies have shown two potential mechanisms by which crystals cause degeneration. These involve the stimulation of mitogenesis in synovial fibroblasts and the secretion of metalloproteinases by cells that subject these crystals to phagocytosis. New information on how crystals form and how they exert their biologic effects will help in the design of an effective therapeutic approach.

Keywords
biologic effects, calcium-containing crystals, osteoarthritis, signal transduction

Introduction
Calcium-containing crystals such as basic calcium phosphate (BCP) and calcium pyrophosphate dihydrate (CPPD) are two of the most common forms of pathologic articular materials that are associated with destructive arthropathies involving cartilage degeneration [1,2]. At concentrations found in pathologic human joint fluids, these crystals exert biologic effects on cultured cells in a manner similar to growth factors like platelet-derived growth factor, epidermal growth factor (EGF), and serum. It has been demonstrated that both crystals stimulate fibroblast, synoviocyte, and chondrocyte mitogenesis in vitro; stimulate the production of prostaglandin E2 (PGE2) via the phospholipase A2/cyclooxygenase (COX) pathway; activate phospholipase C and inositol phospholipid hydrolysis; induce the expression of the proto-oncogenes, c-fos and c-myc; induce the synthesis and secretion of matrix metalloproteinases (MMPs) 1, 3, 8, 9, and 13; and downregulate tissue inhibitors of metalloproteinase [3,4,5,6–13]. By contrast to other mitogenic and growth factors, BCP crystal–elicited signal transduction pathways have not been completely studied. Now, however, some of the component molecules involved in calcium-containing crystal signal transduction mechanisms have been identified.

The ultimate biologic effects of calcium-containing crystals on cells in vivo are an increase in MMP synthesis and secretion and increased mitogenesis. These effects seemed to correlate with calcium deposition disease in vivo. The increased production of matrix-degrading MMPs by synoviocytes results in articular damage and degeneration and the release of additional crystals from the surrounding tissue, and mitogenesis leads to an increase in synoviocytes that generate more MMPs [3]. Of great interest are the signal transduction mechanisms by which crystal-induced upregulation of MMP synthesis and secretion and increased mitogenesis are mediated.
Mechanism of crystal-induced cell activation

Role of Ca²⁺ influx

Treatment of human fibroblasts with BCP crystals induces a rapid transient rise of intracellular Ca²⁺ levels within seconds, followed by a slow, sustained increase within 60 minutes after stimulation. Experiments involving crystal stimulation of cells in Ca²⁺-free media and pretreatment of cells to prevent intracellular crystal dissolution suggested that the initial transient rise in intracellular Ca²⁺ is due to Ca²⁺ influx from outside the cell, and the second sustained rise is due to crystal dissolution [14].

One possible result of the influx of Ca²⁺ from outside to inside the cell is the activation of the protein kinase Pyk2, followed by signaling through the Ras cascade to induce the p42/44 mitogen-activated protein kinase (MAPK) pathway [15–18]. Another possibility is that the rise in intracellular Ca²⁺ that occurs upon crystal treatment of cells is responsible for the activation of cAMP response element binding protein (CREB), a key transcriptional regulator of the c-fos gene that is important for mediating c-fos activation in response to elevated levels of intracellular calcium. CREB binds to the CRE element in the c-fos promoter, a DNA binding site with similarity to the 12-o-tetradecanoyl-phorbol 13-acetate (TPA) response element activating protein-1 (AP-1) binding site [21].

Role of P42 and p44 mitogen-activated protein kinase pathway

Members of the extracellular signal-regulate kinase (ERK) family of Ser/Thr kinases are key regulators of a variety of signal transduction cascades that play a central role in mediating cellular responses elicited by many different environmental agents. Three distinct ERK-dependent signaling cascades have been identified in mammalian cells. These can be distinguished on the basis of the particular ERK members activated: p42/p44 MAPKs (ERK2/ERK1), p38 MAPK, or p46/p54 stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNKs). P38 MAPK and the SAPK/JNKs mediate signals in response to cytokines and environmental stress. The p42/p44 MAPK pathway was the first identified and is the best understood ERK-based signaling pathway [17,18]. This pathway is required for extracellular stimulation of growth and Ras transformation of cells. The p42/p44 MAPKs are believed to regulate proliferation by a mechanism that involves activation of genes associated with cell proliferation, including primary response genes such as c-fos and c-jun.

The BCP and CPPD crystals activate MAPK p42/p44 but not the p38 protein kinase cascade pathway [22]. Both crystals also cause phosphorylation of a nuclear transcription factor, CREB, on serine 133, a residue essential for the ability of CREB to transactivate. Treatment of cells with PD98059, an inhibitor of mitogen-activated extra cellular signal-related protein kinase (MEK)1, an upstream activator of MAPKs, U0126 (a novel inhibitor of MEK1 and MEK 2), or phosphocitrate (PC) significantly inhibited the crystal activation of p42/p44 MAP kinases, CREB serine 133-phosphorylation, c-fos, and cell proliferation in a dose-dependent fashion. This indicates that the MAPK pathway is the mediator of crystal-induced signals to the nucleus [22].

On the basis of these findings, Brogley et al. [23] attempted to determine the role of the p42/44 MAPK signal transduction pathway in crystal-induced expression of MMPs. Treatment of fibroblasts with PD98059 blocks the induction of crystal-stimulated MMP-1 and MMP-3 expression. PD98059 and phosphocitrate reduced the level of crystal-induced MMP-1 and MMP-3 mRNA expression to that observed in nonstimulated cells, in which EGF and TPA were used as positive controls for stimulation of MMP mRNA expression, respectively. Likewise, PD98059 treatment of cells blocked the EGF-induced and crystal-induced increase in MMP-1 and -3 protein expression and secretion, as demonstrated by Western blotting and zymography. These results suggest that the p42/44 MAPK pathway is the important signal transduction pathway of crystal-induced and EGF-induced MMP-1 and MMP-3 expression.

Role of protein kinase C isozymes

Crystal treatment of cells results in translocation of the PKC enzyme from the cytosolic to the membrane fraction of the cell, an indicator of PKC activation [7,8,24,25]. Downregulation of PKC activity using the TPA inhibits crystal-induced c-fos and c-myc expression and mitogenesis in fibroblasts, indicating that PKC activity is essential for these crystal-induced effects to occur [7,8].

To explore the link of activation of PKC and p42/44 MAPK in crystal induction of MMP, it was shown that treatment of fibroblasts with the PKC inhibitors staurosporine or Bis-I dose-dependently suppresses BCP crystal-induced MMP-1 and MMP-3 transcripts and protein expression [23]. Crystal-induced PKC activation requires an influx of extracellular Ca²⁺, because crystal stimulation in a Ca²⁺-free environment inhibits PKC translocation. PKC-α is the only Ca²⁺-dependent isozyme activated. A preferential inhibitor of Ca²⁺-dependent PKC isozymes, Gö 6976, is most effective at blocking crystal-induced PKC translocation and MMP-1 expression. Crystal-induced activation of p44/42 MAPK was independent of PKC-α because the PKC inhibitors, Bis I and Gö6976, had exerted no effect on p44/42 MAPK; conversely, the p44/42 MAPK inhibitors, PD098058 and U0126, had no effect on PKC. It was concluded that crystal stimulation of MMP-1 and MMP-3 mRNA and protein expression is dependent on the Ca²⁺-dependent PKC signal transduction pathway and that the PKC-α isozyme is specifically involved in the pathway [24].
The possibility, however, that another PKC isozyme, which was not sensitive to the Ca2+-dependent PKC inhibitors, might be required for the crystal-induced activation of the p44/42 signal transduction pathway could not be ruled out [25*]. Evaluation of the PKC isozymes from all the PKC sub-families shows that only PKCα and PKCμ are expressed in human fibroblasts. The fact that PKCα is Ca2+-dependent and PKCμ is Ca2+-independent and that they belong to two different PKC subfamilies suggests different roles for these isozymes in crystal-induced cell activation [26]. Inhibition of PKCμ synthesis and activity by antisense oligodeoxynucleotides and H-89, respectively, results in the inhibition of p44/42 MAPK activation, thus demonstrating that p44/42 MAPK activity is dependent on PKCμ. Inhibition of PKCμ also results in the inhibition of MMP-1 and MMP-3 mRNA and protein expression as a result of p44/42 MAPK inhibition [25*].

These data lead to the latest hypothesis that BCP crystals activate cells via two independent pathways. One pathway is the Ca2+-dependent PKC pathway characterized by PKCα and modulated by mobilized intracellular Ca2+ generated by the sequential hydrolysis of PLC-PIP2-IP3, by transient opening of the Ca2+ channel, and by crystal endocytosis and dissolution [14–16,27–31]. The mobilized Ca2+ in the cytosol then modifies the activation of PKCα by diacylglycerol (DAG) and induces its translocation to the plasma membrane, where it becomes physiologically active [32]. Some of the mobilized Ca2+ diffuses through the nuclear pores into the nucleus, where it enhances the crystal induction of c-fos mRNA [14,33,34]. It has been shown that either PKC isozymes can act in the cytoplasm and cause nuclear effects indirectly by triggering signaling pathways directed towards the cell nucleus or PKC itself can act in the cell nucleus [35]. The other pathway is the Ca2+-independent p44/42 MAPK pathway, which is mediated by the Ca2+-independent PKCμ. Crystals activate the sequential hydrolysis of PLC-PIP2, producing DAG, which activates PKCμ, which in turn activates p44/42 MAPK generated from the Ras-Raf-MEK-P44/42 MAPK signaling cascade [22–24]. The activated and phosphorylated p44/42 MAPK then migrates to the nucleus to mediate crystal-induced cellular responses. It was concluded that these two pathways, although independent, complement each other for the efficient regulation of cellular responses to crystal stimulation.

**Mechanism of crystal-induced metalloproteinase synthesis**

Transcriptional regulation of MMPs is dependent on multiple DNA binding elements located in their promoters. The promoters of the MMP-1 and MMP-3 genes contain a previously mentioned binding site for AP-1 known as the TPA response binding element, named for its ability to mediate induction of transcription in response to TPA and other PKC activating agents [36]. Transcriptional activation of MMP genes by growth factors and cytokines is preceded by a rapid transient increase in AP-1 protein expression [30,31]. It has been determined that an AP-1 site is located in the promoters of all the MMPs except for MMP-2, and MMP-1 and MMP-3 each have a second AP-1 site.

Transfections with hMMP1 luciferase reporter plasmids in synoviocytes revealed that the induction of hMMP1 promoter by BCP crystals was mainly mediated through the -72AP-1 element. Elimination of the -72AP-1 element either by mutagenesis or by deletion abolished the induction of hMMP1 promoter activity by BCP crystals almost completely. Interestingly, a mutation at the -88PEA-3 site also abolished the induction of hMMP1 promoter. Further mutation at the -181AP-1 site resumed the induction, which indicated that the -181AP-1 element had an effect opposite that of the -72AP-1 element. The effect of -181AP-1 could be inactivated either by a mutation at this -181AP-1 site or by the -88PEA-3 element. In addition, dominant-negative Ras, Raf, and MEK1/2 could block the induction of hMMP1, and a MEK1/2-specific inhibitor (U0126) could block the induction of hMMP1 and c-fos by BCP crystals [37]. McCarthy et al. [7] also reported that crystal treatment of fibroblasts resulted in the activation of both AP-1 and NFκB. These data indicate that multiple elements, including at least AP-1 and PEA-3, are involved in the induction of the hMMP1 gene expression by BCP crystals and that the induction follows the Ras/MAPK/c-fos/AP-1/MMP1 signaling pathway [37].

**Role of early growth response genes**

Early growth response (EGR) genes were originally identified on the basis of their rapid induction of gene expression in quiescent fibroblasts stimulated by serum [38]. Although the amino acid sequences of four family members are distinct, they all interact with the Sp1-type of DNA target element. EGR protein alters gene transcription through mechanisms dependent on both co-activators and co-repressors. Transcriptional co-activators such as CREB-binding protein and p300 can interact directly with the activation domain of EGR-1 and increase its transactivating activity [39]. EGR proteins serve as sensors of extracellular signaling pathways that play key roles in regulating cell proliferation, differentiation, and function. Recently, Zeng et al. [40] showed that BCP crystals could induce the message levels of EGR-1 and EGR-3, which peaked at approximately 1 hour. By contrast, the message level of EGR-2 increased steadily and peaked 24 hours after BCP crystal stimulation. Using an EGR-2 promoter–driven luciferase reporter gene expression system, it was confirmed that BCP crystals could induce expression by 13-fold in comparison with a negative control. This induction is BCP crystal concentration–dependent and can be abolished by either NaPC, p44/42 MAPK inhibitor U0126, or calcium chelators EGTA and TMB-8, but not by
SAPK2/p38 and the PKC inhibitor Bis-I. The induction of EGR-2 expression significantly enhanced the binding of transcription factors such as c-fos, SRF, and c-myc to enhancer elements and activated the p44/p42 MAPK signaling pathway. This study demonstrates that crystals induce EGR-2 transcription through a PKC-α-independent p44/p42 MAPK pathway, and that induction of EGR-2 may subsequently activate genes regulated by SRF, c-myc, and c-fos, which may play key roles in regulating fibroblast proliferation [40].

Crystal-induced inflammatory response

Spontaneous apoptosis of neutrophils may be inhibited by various proinflammatory stimuli, which may result in prolonged lifetimes and responses by these phagocytic cells with the potential for extended inflammation. Tidan et al. [41,42] reported that both CPPD and MSU crystals inhibited spontaneous and TNF-α-associated apoptosis of human neutrophils via the ERK1/ERK-2 and PI3K/Akt-mediated pathways. This strongly suggested that CPPD crystals function to induce acute inflammatory response by stimulation of neutrophil activation and repression of apoptosis. More recently, a follow-up study by the same authors indicated that CPPD crystals repressed the TNF-α-induced neutrophil apoptosis via the repression of caspase 3–mediated apoptosis-associated p38 kinase activity [43*].

Both BCP and CPPD crystals exert several biologic responses that may injure articular structures. Crystals provoke the generation of prostaglandins, especially PGE2 [4,5*,44]. Phosphatidylcholine and phosphatidylethanolamine are the major sources of arachidonic acid for PGE2 synthesis, confirming that the phospholipase A2/COX pathway is the predominant route for PGE2 production [45]. Recently, Morgan et al. [5*] confirmed that BCP crystals upregulate COX enzymes, in particular COX 2, which in turn induces PGE2 production and interleukin-β expression in fibroblasts. This suggests that BCP crystal might be an important amplifier of PGE2 production through the induction of the COX enzymes and the pro-inflammatory cytokine interleukin-1β.

Both CPPD and BCP crystals can be phlogistic and membranolytic, causing inflammatory attack. So far, the molecular mechanism of crystal-induced membranolysis is still unclear. Using the molecular dynamics simulations of the phospholipid bilayer–CPPD crystal to determine how the interactions between the bilayer and the crystal affect the dynamics and stability of the phospholipid bilayer, Wierzbicki et al. [46] reported that the interactions between the surface of CPPD and the extracellular layer of the hydrated dimyristoyl phosphatidylcholine phospholipid bilayer may lead to decoupling of the external layer from the intracellular side of the membrane. In turn, a local thinning of the layer on the intracellular side of the membrane occurs, which favors water penetration leading to membranolysis. They identified that the [010] crystal surface of CPPD is responsible for the interaction with the phospholipid bilayer, which leads to crystal-induced membranolysis. The process is very similar to the lysis induced by melittin in bee and snake venom [46]. It is interesting to note that when the same [010] CPPD crystal surface binds to an anticalcification agent such as phosphocitrate, it leads to the retardation of crystal growth and cessation of crystal growth [47]. This finding suggests the potential future direction for the design of the next generation of therapeutic agents for calcium crystal arthropathy.

The BCP crystals also stimulate the endocytotic activity of cells. Because calcium-containing crystals are associated with many different macromolecules, including DNA fragment cytokines, these particulates may be undergo endocytosis together with crystals disturbing the homeostasis of normal molecular signaling. This finding could be important for our understanding of the potential pathologic role of crystals in crystal arthropathy [48].

Conclusion

In summary, evidence for a causal role of crystals in cartilage degeneration in osteoarthritis is primarily inferential and is based on correlative data. Clinical observations indicate that exaggerated and uniquely distributed cartilage degeneration is associated with these deposits. Measurements of putative markers of cartilage breakdown suggest that these crystals magnify the degenerative process. Studies show that calcium-containing crystals can cause the degeneration of articular tissues in two separate pathways. In the direct pathway, crystals directly induce fibroblast-like synoviocytes to proliferate and produce metalloproteinasises and prostaglandins. The other pathway, the paracrine pathway, involves the interaction between crystals and macrophages/monocytes, which leads to the synthesis and release of cytokines that can reinforce the action of crystals on synoviocytes and/or induce chondrocytes to secrete enzymes and that eventually cause the degeneration of articular tissues. New information about how crystals form and how they exert their biologic effects will help us design an effective therapeutic approach.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest
Crystal deposition diseases


This report presents evidence that CPPD crystal-associated induction of neutrophil activation and regression of TNF-a-induced apoptosis is mediated by the p38 MAPK signal transduction pathway. This strongly suggests that CPPD crystals function to induce an acute inflammatory response by stimulation of neutrophil activa- tion and repression of apoptosis.


Gout: epidemiology and lifestyle choices
Hyon K. Choi and Gary Curhan

Purpose of review
Recent scientific data serve to illuminate the links between dietary and other factors and the incidence of gout. This review summarizes recent literature about the prevalence and incidence of gout as well as risk factors for gout.

Recent findings
Epidemiologic studies suggest that the overall disease burden of gout is substantial and growing. Gout seems to be relatively common not only in men but also in older women. A recent large prospective study investigated several purported dietary factors for gout and confirmed some of the long-standing suspicions (red meats, seafood, beer, and liquor), exonerated others (total protein, wine, and purine-rich vegetables), and also identified potentially new protective factors (dairy products). A study based on the Third National Health and Nutrition Examination Survey suggested that these factors affect serum uric acid levels parallel to the direction of risk of gout. In addition, adiposity, weight gain, hypertension, and diuretics were all found to be independent risk factors for incident gout, whereas weight loss was found to be protective.

Summary
The disease burden of gout remains substantial and may be increasing. Some of the recently confirmed lifestyle factors may explain the increasing incidence of gout. The public health implications of dietary and lifestyle recommendations should take into account other associated health benefits and risks, because many of these factors have health effects beyond their influence on gout.

Keywords
epidemiology, gout, lifestyle factors

Introduction
Gout is an inflammatory arthritis mediated by the crystallization of uric acid within the joints and is often associated with hyperuricemia. Epidemiologic studies suggest that the overall disease burden of gout is substantial and may be increasing. Recently, the prevalence of self-reported physician-diagnosed gout in the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) was found to be greater than 2% in men aged over 30 years and in women aged over 50 years [1]. The prevalence increased with increasing age and reached 9% in men and 6% in women aged over 80 years [1]. Previously, series of the National Health Interview Survey showed that the prevalence of self-reported gout doubled between 1969 and 1985, but the increasing trend seemed to substantially slow between 1988 and 1996 [2,3]. A multicenter study of general practices in the United Kingdom reported that the prevalence of gout in 1991 had increased threefold compared with the estimates from the 1970s [4]. Similar trends between the 1960s and 1992 were observed in Maori Indians as well as European descendants in New Zealand [5]. More recently, a study based on a managed care population in the United States suggested that the overall prevalence of gout or hyperuricemia in 1999 increased by 80% compared with that in 1990 [6]. A similar increasing trend was observed in Chinese population surveys performed in the 1990s [7]. Furthermore, the incidence of primary gout (i.e., without diuretic exposure) doubled over the past 20 years, according to the Rochester Epidemiology project, whereas the proportion of gout associated with diuretic use decreased during the period [8].

Gout seems to pose a substantial disease burden among older women, whose proportion is growing in the general population because of their increased longevity. Although gout occurs more often in men than in women before menopause, the disease burden in women after menopause approaches that in men. The prevalence of gout estimated in the NHANES III was 3.5% in women aged 60 to 69 years and increased to 4.6% in women aged 70 to 79 years and 5.6% in those aged 80 years and older [1]. These may be overestimates, given that they were based on self-reports of physician-diagnosed gout; however, even if the true age-specific prevalences were 50% lower, they would still be substantial with the prevalence of gout in this population approaching that of rheumatoid arthritis (2% in the NHANES III [9]). Furthermore, the Rochester Epidemiology project data indicate that the incidence of primary gout has doubled among women over the past 20 years [8].
**Dietary risk factors and the risk of gout**

Purine-rich foods and high protein intake had long been thought to be risk factors for gout, but the associations had not been prospectively confirmed [10,11]. Metabolic experiments in animals and humans demonstrated the urate-raising effect of artificial short-term loading of purified purine [12–15]. By contrast, small-scale case–control studies that retrospectively assessed dietary intake in patients with confirmed gout and control individuals failed to find an association between purine intake and gout [16,17]. In addition, the possibility that the consumption of dairy products has a role in protecting against gout has been raised by previous studies [18,19]. In a recent study, the relation between these purported dietary risk factors and incident gout was prospectively examined over a 12-year period in 47,150 male participants (the Health Professionals Follow-up Study [HPFS], 730 incident gout cases) with no history of gout at baseline [20**]. Men in the highest quintile of meat intake had a 41% higher risk of gout than did those in the lowest quintile, and men in the highest quintile of seafood intake had a 51% higher risk than did those in the lowest quintile (Table 1), but purine-rich vegetable consumption was not associated with an increased risk of gout. Furthermore, men in the highest quintile of dairy intake had a 44% lower risk of gout than did those in the lowest quintile, and the inverse association was limited to low-fat dairy consumption. Although total protein intake and animal protein intake were not significantly associated with the risk of gout, men in the highest quintile of vegetable protein intake had a 27% lower risk of gout than did those in the lowest quintile, and men in the highest quintile of dairy protein intake had a 48% lower risk of gout than did those in the lowest quintile (Table 2). Dairy protein may exert its urate-lowering effect without the concomitant purine load contained in other animal protein sources such as meat and seafood, given that dairy products have a low purine content [19,21]. It is also possible, however, that other factors in dairy products may be responsible for the inverse association. The absence of the inverse association with high-fat dairy products could result from the counteracting effect of saturated fats contained in high-fat dairy products. These fats are positively associated with insulin resistance, which reduce the renal excretion of urate [22–24,27]. A recent Taiwanese case–control study (91 patients with gout and 91 control individuals) suggested a protective effect of folate and dietary fiber against gout (odds ratios, 0.43 and 0.37 between the extreme tertiles, respectively) [17]. These interesting findings call for prospective confirmation.

### Alcoholic beverages and the risk of gout

The association between alcohol consumption and risk of gout has been suspected since ancient times, but the association had not been prospectively confirmed. In the HPFS, increasing alcohol intake was associated with increasing risk of gout (a dose–response relation) [28**]. In comparison with men who did not drink alcohol, the multivariate relative risk of gout increased from 1.25 (95% CI 0.95–1.64) for alcohol consumption of 5 to 9.9 grams per day to 2.53 (1.73–3.70) for 50 grams per day or more (P for trend < 0.001). This risk varied substantially according to the type of alcoholic beverage: beer conferred a larger risk than liquor, whereas moderate wine drinking did not increase the risk (Table 3) [28**]. These findings confirmed the long-held belief of relation between alcohol intake and gout. In addition, they suggested that certain nonalcoholic components that vary among these alcoholic beverages play an important role in the incidence of gout. Beer is the only alcoholic beverage acknowledged to have a large purine content, which is predominantly guanosine, a readily absorbable nucleoside.  

### Table 1. Food groups and multivariate relative risks (RRs) of incident gout in the Health Professionals Follow-up Study (HPFS) in the highest quintile of intake compared with the lowest quintile

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total meats</td>
<td>1.41</td>
<td>1.17–1.94</td>
</tr>
<tr>
<td>Seafood</td>
<td>1.51</td>
<td>1.17–1.95</td>
</tr>
<tr>
<td>Purine-rich vegetables</td>
<td>0.96</td>
<td>0.74–1.24</td>
</tr>
<tr>
<td>Total dairy foods</td>
<td>0.56</td>
<td>0.42–0.74</td>
</tr>
<tr>
<td>Low-fat dairy foods</td>
<td>0.58</td>
<td>0.45–0.76</td>
</tr>
<tr>
<td>High-fat dairy foods</td>
<td>1.00</td>
<td>0.77–1.29</td>
</tr>
</tbody>
</table>

*Published with permission [20**].

### Table 2. Protein consumption and multivariate relative risks of incident gout in Health Professionals Follow-up Study (HPFS) in the highest quintile of intake compared with the lowest quintile

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>1.07</td>
<td>0.84–1.36</td>
</tr>
<tr>
<td>Animal protein</td>
<td>0.96</td>
<td>0.74–1.23</td>
</tr>
<tr>
<td>Vegetable protein</td>
<td>0.73</td>
<td>0.56–0.96</td>
</tr>
<tr>
<td>Dairy protein</td>
<td>0.52</td>
<td>0.40–0.68</td>
</tr>
<tr>
<td>Non-dairy animal protein</td>
<td>1.18</td>
<td>0.90–1.53</td>
</tr>
</tbody>
</table>

*Published with permission [20**].

### Table 3. Alcoholic beverage use and multivariate relative risk for incident gout in the Health Professionals Follow-up Study (HPFS)

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total alcohol intake (per 10 g daily)</td>
<td>1.17</td>
<td>1.11–1.22</td>
</tr>
<tr>
<td>Beer (per 12-oz glass, bottle, or can daily)</td>
<td>1.49</td>
<td>1.32–1.70</td>
</tr>
<tr>
<td>Liquor (per shot daily)*</td>
<td>1.15</td>
<td>1.04–1.28</td>
</tr>
<tr>
<td>Wine (per 4-oz glass daily)*</td>
<td>1.04</td>
<td>0.88–1.22</td>
</tr>
</tbody>
</table>

*The RR for individual alcoholic beverages are presented as the increase in risk of gout associated with an increment of one daily serving of the standard portion size. Total alcohol intake was determined by multiplying the consumption of each beverage by the ethanol content in its standard portion (12.8 g for beer, 11.0 g for wine, and 14.0 g for liquor). Published with permission [28**].
Adiposity and the risk of gout
Adiposity has been positively associated with serum uric acid levels and has been proposed to increase the risk of gout. Although several prospective cohort studies have evaluated the association between obesity and gout, the lack of data and the small number of gout cases limited the comprehensive adjustment of relevant covariates [30–33]. Specifically, no prospective information had been available about the relation between obesity and incident gout after adjustment for dietary factors, which themselves may be risk factors for gout and vary with adiposity. In the prospective analysis in the HPFS, body mass index (BMI) and waist-to-hip ratio were both strongly and positively independently associated with the risk of incident gout in men [34••]. In comparison with men with BMI 21 to 22.9 kg/m², the multivariate relative risks of gout were 1.95 (1.44–2.65) for men with BMI 25 to 29.9 kg/m², 2.33 (1.62–3.36) for BMI 30 to 34.9 kg/m², and 2.97 (1.73–5.10) for BMI greater than 35 kg/m² (P for trend < 0.001). The multivariate relative risk for gout among men in the highest waist-to-hip ratio quintile (0.98–1.39) compared with those in the lowest quintile (0.70–0.88) was 1.82 (95% CI 1.39–2.39; P for trend < 0.001). Furthermore, in comparison with men who maintained their weight (−4 to +4 pounds) since age 21, the multivariate relative risk of gout for men who gained 30 pounds or more was 1.99 (1.49–2.66). By contrast, the multivariate relative risk for men who lost 10 pounds or more since the study baseline was 0.61 (95% CI 0.40–0.92) [34••]. Increased adiposity may lead to hyperuricemia by way of both increased production and decreased renal excretion of urate [10,11]. Factors not related to uric acid, such as chronic joint trauma due to excess weight, have been proposed as an additional explanation for the association between obesity and gout [11,35].

Hypertension, diuretics, and the risk of gout
Hypertension has been recognized as a risk factor for gout, and diuretic use has been shown to elevate serum uric acid levels [32,35,36]. No prospective study, however, has demonstrated their independent contributions to the risk of incident gout, primarily given their tight association (i.e., diuretics are often used to treat hypertension) and a small study sample size. Additionally, because hypertension is associated with other risk factors for gout, such as adiposity, dietary and nutritional factors, alcohol, and chronic renal failure, it is important to adjust for these factors to examine hypertension as an independent risk factor for gout and determine the magnitude of the association. During 12 years of follow-up among men with no previous history of gout in the HPFS, hypertension (RR 2.31; 95% CI 1.96–2.72) and diuretic use (1.77; 1.42–2.20), both were independently associated with an increased risk of incident gout [34••].

Diet and uric acid level
The relations between different dietary factors and incident gout among men observed in the HPFS led to a hypothesis that these factors affect serum uric acid levels parallel to the direction of risk of gout [20••]. Furthermore, various commonly consumed foods have long been suspected of affecting the serum uric acid level, but few data have been available. To address these study questions, uric acid levels (as a surrogate outcome) measured in the NHANES III (1988–1994) were investigated [37•]. By use of data from 14,809 participants (6,932 men and 7,877 women) aged 20 years and older, the relations between intake of purine-rich foods, protein, and dairy foods and serum uric acid levels were studied. Serum uric acid levels increased with increasing total meat or seafood intake and decreased with increasing dairy intake. After adjustment for age, the uric acid level differences between the extreme quintiles of intake were 0.48 mg/dl for total meat (95% CI 0.34–0.61; P for trend < 0.001), 0.16 mg/dl for seafood (95% CI 0.06–0.27; P for trend 0.005), and −0.21 mg/dl for total dairy intake (95% CI −0.37 to −0.04; P for trend 0.02). After adjustment for other covariates such as age, sex, BMI, serum creatinine, hypertension, alcohol use, and diuretic use, the differences between the extreme quintiles were slightly attenuated but remained significant (all P values for trend < 0.05). Total protein intake was not associated with serum uric acid level in multivariate analyses (P for trend 0.74). Those who consumed milk one or more times per day had a lower serum uric acid level than those who did not drink milk (multivariate difference, −0.25; 95% CI −0.40 to −0.09; P for trend < 0.0001). Similarly, those who consumed yogurt at least once every other day had a lower serum uric acid level than those who did not consume yogurt (multivariate difference, −0.26; 95% CI −0.41 to −0.12; P for trend < 0.0001).

Alcoholic beverages and uric acid level
Many studies have shown that increased alcohol intake is associated with hyperuricemia [10,11,30,38–43], but few data are available about the effect of individual alcoholic beverages (i.e., beer, liquor, wine) on serum uric acid levels. The relations between different alcoholic beverages and incident gout among men observed in the HPFS also led to the hypothesis that these alcoholic beverages affect serum uric acid levels parallel to the direction of risk of gout [28••]. By use of the same NHANES data set as described above, the relations among intake of beer, liquor, and wine and serum uric acid levels were examined
The mean serum uric acid level increased with increasing total alcohol intake (P value for trend < 0.001). After adjustment for age, the difference in serum uric acid levels compared with no intake increased with increasing beer or liquor intake (P values for trend < 0.001), but the association was inverse with increasing wine intake (P for trend < 0.001). After adjustment mutually for the individual alcoholic beverages in addition to other risk factors, the differences remained significant for beer (0.46 mg/dl for each additional serving; 95% CI 0.32–0.60; P for trend < 0.01) and for liquor (0.29 mg/dl; 0.14–0.45; P for trend < 0.01). For wine, however, such adjustment eliminated the inverse association (0.04 mg/dl for each additional serving; 95% CI –0.20 to 0.11; P for trend 0.6). These findings closely agree with the observed associations between these alcoholic beverages and incident gout observed among men in HPFS and suggest that moderate wine drinking may not increase serum uric acid levels as do other alcoholic beverages [28**].

Conclusion
The disease burden of gout is substantial and may be increasing. Some of the recently confirmed lifestyle risk factors may explain the increasing incidence of gout. For example, it is conceivable that the dietary changes and obesity epidemic over last few decades may explain part of the increasing incidence of gout. Other potential lifestyle risk factors not yet investigated, such as saturated fats or fructose, deserve future investigations. Finally, it is important to note that the public health implication of dietary and lifestyle recommendations should take into account other associated health benefits and risks, because many of these factors have health effects beyond their influence on gout.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest


This National Survey—based study addressed the relation between different alcoholic beverages and serum uric acid levels.
Eicosanoids, osteoarthritis, and crystal deposition diseases
Eamonn S. Molloya and Geraldine M. McCarthya,b

Purpose of review
Eicosanoids are produced by chondrocytes, synoviocytes, and subchondral osteoblasts within the osteoarthritic joint and are involved in normal joint physiology as well as in the pathogenesis of joint disorders such as osteoarthritis. Calcium-containing crystals are found in most osteoarthritic joints and have been implicated in osteoarthritis. Recent advances in the understanding of the potential role of eicosanoids in the pathogenesis of osteoarthritis and in potential therapeutic targeting of eicosanoid pathways are reviewed.

Recent findings
The ability of interleukin-1β to upregulate microsomal prostaglandin E2 synthase-1 in synovial fibroblasts and chondrocytes of patients with osteoarthritis has been demonstrated. A potential role for prostaglandin E2 in downregulating interleukin-1β–induced inflammatory responses has also been described. Basic calcium phosphate crystals can upregulate cyclooxygenase-1 and cyclooxygenase-2 expression, both of which contributed to the observed increase in prostaglandin E2 production in human fibroblasts. Novel potential mechanisms of inhibition of eicosanoid synthesis are also discussed. Last, further evidence of amelioration of osteoarthritis in animal models by the dual 5-lipoxygenase/cyclooxygenase inhibitor licofelone has been reported.

Summary
The inhibition of prostaglandin synthesis has long been a cornerstone of the pharmacologic treatment of osteoarthritis. Nevertheless, prostaglandins may have potentially beneficial as well as deleterious effects in osteoarthritis. In addition, other eicosanoids such as leukotrienes have also been implicated in the pathogenesis of osteoarthritis. Therefore, more selective inhibition of prostaglandin pathways and/or inhibition of leukotriene activity may prove to be effective therapeutic strategies in osteoarthritis.

Keywords
basic calcium phosphate crystals, cyclooxygenase, lipoxygenase, osteoarthritis, prostaglandins

Abbreviations
BCP basic calcium phosphate
COX cyclooxygenase
EGR-1 early growth response 1
ERK extracellular regulated kinase
IL1β interleukin-1β
MAPK mitogen-activated protein kinase
MCP-1 monocyte chemoattractant protein-1
MMP matrix metalloproteinase
OA osteoarthritis
PCR polymerase chain reaction
PGE2 prostaglandin E2
PGES prostaglandin E synthase
PPAR peroxisome proliferator-activated receptor

Introduction
Osteoarthritis (OA) is the most common form of arthritis, and its prevalence is expected to rise considerably as the population ages. It is the foremost cause of disability in the elderly population, disabling approximately 10% of those over the age of 60 years [1]. Estimates of the annual cost of OA to the United States economy exceed $60 billion [1]. Much work remains to be done to unravel the complex pathophysiology of OA and to translate this knowledge into effective curative and/or preventative strategies.

Eicosanoids are arachidonate-derived lipid mediators found in virtually all tissues in the body. They are involved in the control of many physiologic processes and are also implicated in the modulation of inflammatory responses. The key eicosanoids are prostanooids (prostaglandins and thromboxanes) and leukotrienes, which are produced by the action of cyclooxygenase (COX) and 5-lipoxygenase, respectively. Nonsteroidal anti-inflammatory drugs, inhibitors of COX, have long been used in the treatment of OA and can provide symptomatic benefit in OA. The exact role of prostaglandins in the pathogenesis of OA and thus the consequences of inhibiting their synthesis have not been fully elucidated, however. It has been demonstrated that PGE2 of synovial origin mediates the interleukin-1β (IL1β)–induced cartilage degradation in a synovial membrane–cartilage co-culture model [2]. PGE2, however, may also be involved in the response to mechanical stress and can antagonize the effects of IL1β on cartilage matrix [3,4]. The role of eicosanoids in OA has been recently reviewed, in particular in relation to osteoblast function [5].

Crystals of basic calcium phosphate (BCP) and calcium pyrophosphate dihydrate (CPPD) have been implicated in the pathogenesis of OA [6]. One or both of these
calcium-containing crystal species are identified in 52 to 92% of OA synovial fluids [7,8]. Both of these crystal types can modulate eicosanoid production. CPPD crystals have previously been reported to increase prostaglandin production in mammalian cells [9]. Given that there has been little further work in this area recently, however, this review will focus on the relation between eicosanoids and osteoarthritis and BCP crystal deposition.

**Basic calcium phosphate crystals activate prostaglandin synthetic pathways**

Crystals of BCP may contribute to OA pathogenesis by stimulating synovial fibroblast mitogenesis, upregulating IL1β expression, and increasing matrix metalloproteinase (MMP) and nitric oxide production [6]. It has been known for some time that BCP crystals can increase PGE2 production in mammalian cells [9–11]. More recently, however, it was demonstrated that this induction of PGE2 is associated with induction of both COX isozymes in human foreskin fibroblasts [12*]. Real-time polymerase chain reaction (PCR) demonstrated a 23-fold upregulation of COX-2 mRNA by BCP crystals, maximal at 4 hours. BCP crystals also upregulated IL1β mRNA expression, peaking at 8 hours. Thus, although IL1β may contribute to the observed COX-2 upregulation, the different time courses of induction suggest that BCP crystals directly induce COX-2. Maximal (1.75-fold) COX-1 mRNA induction was seen at 24 hours. Inhibition of protein kinase C and phosphatidylinositol 3-kinase diminished BCP crystal-induced COX-2 mRNA expression. BCP crystal–induced COX-2 mRNA expression was also abrogated by phosphocitrate, which seems to inhibit all biologic activities of BCP crystals. PGE2 production at 4 hours was abolished by pretreatment with NS398, a selective COX-2 inhibitor. NS398 only partially inhibited PGE2 production at 30 hours, however, which suggests that COX-1 also contributes to PGE2 production in human fibroblasts.

Our more recent work has evaluated the effect of BCP crystals in human OA synovial fibroblasts [13]. Although an early COX-2 upregulation was again apparent, COX-1 upregulation was much more pronounced than in the human foreskin fibroblast model. Maximal (19-fold) induction of COX-1 mRNA expression was detected by real-time PCR 32 hours after BCP crystal stimulation. Increased COX-1 protein was also found on Western blotting of cell lysates. Increased cellular production of PGE2 and prostacyclin was detected by enzyme-linked immunosorbent assay. By use of, SC-560 (a COX-1 selective inhibitor), and SC-236 (a COX-2 selective inhibitor), production of both prostaglandins was found to be COX-2 mediated at 4 hours and COX-1 mediated at 32 hours.

In fact, the role of COX-1 in OA is increasingly being recognized. Knorth et al. [14*] examined the relative contributions of COX-1 and -2 to PGE2 release from OA synovial tissue. The effects of SC-560; SC-58125, a COX-2 selective inhibitor; and diclofenac, a nonselective COX inhibitor on PGE2 production were measured. Inhibition of PGE2 production at 83%, 62.8%, and 30.6% was seen for diclofenac, SC-58125, and SC-560, respectively. This suggests that COX-1 may contribute to the pool of prostaglandins in OA synovium. It remains to be established, however, whether COX-1–derived prostaglandins play a pathologic role in OA.

**Involvement of microsomal prostaglandin E2 synthase 1 in osteoarthritis**

Prostaglandin E2 synthases (PGES) convert PGH2 to PGE2. Two of these, cytosolic PGE2 synthase (cPGES) and microsomal PGE2 synthase-2 (mPGES2) are generally constitutively expressed [15]. The third synthase, microsomal PGE2 synthase-1 (mPGES1), is generally expressed at low levels at baseline and is inducible by pro-inflammatory stimuli [16]. mPGES1 and cPGES are generally functionally coupled with COX-2 and COX-1, respectively [17,18]. Kojima et al. [19*] recently examined the expression of PGE2 synthases in chondrocytes from patients with OA. The expression of mPGES1 (and COX-2) mRNA was significantly upregulated by IL1β and tumor necrosis factor-α. Positive immunostaining for mPGES1 was also detected in chondrocytes in sections of articular cartilage from patients with OA. Lysates of IL1β-stimulated chondrocytes demonstrated increased PGES activity, as measured by the conversion of PGH2 to PGE2. This activity was inhibited by MK-886, which can inhibit mPGES1 activity. The expression of cPGES, mPGES2, and COX-1 was not altered by stimulation by pro-inflammatory cytokines. These results suggest that mPGES1 may be upregulated by pro-inflammatory cytokines in OA chondrocytes and play a role in the generation of PGE2. Selective mPGES1 inhibition could reduce PGE2 production while allowing ongoing basal production of PGE2 for homeostatic purposes through activity of the other PGES, making it an attractive therapeutic strategy in OA and other forms of arthritis. In this regard, the findings in the mPGES1 knockout mouse are interesting [20,21]. There were no apparent adverse consequences of the lack of mPGES1, but there was significant amelioration of the clinical and histologic features in a model of inflammatory arthritis.

Other recent work has elucidated the mechanisms involved in upregulation of mPGES1 in chondrocytes by IL1β. Masuko-Hongo et al. [22*] demonstrated, by quantitative real-time PCR in articular chondrocytes from patients with OA, that IL1β led to an almost threefold upregulation of mPGES1 mRNA expression. Increased mPGES1 protein was also detected by Western blotting. The signal transduction pathways involved were assessed by use of specific inhibitors of mitogen-activated protein kinases (MAPKs): PD98059, an inhibitor of the extracellular regulated kinase 1/2 (ERK-1/2); SB203580, an inhibitor...
of the p38α and β MAPK; and SC906, a selective inhibitor of the p38α isoform. IL1β–induced mPGES1 (and COX-2) protein synthesis and PGE₂ production was diminished by PD98059 and SB203580. Whereas SC906 reduced COX-2 protein synthesis and PGE₂ production, it did not affect IL1β-induced mPGES1 protein synthesis. These results suggest that IL1β induction of mPGES1 proceeds via the ERK1/2 and p38β MAPK signal transduction pathways in osteoarthritic chondrocytes.

Prostaglandin E₂ as a modulator of inflammation
Prostaglandin E₂ has been implicated as a modulator of chemokine production in monocytes, an effect mediated through the EP4 receptor [23]. Largo et al. [24*] examined the effect of PGE₂ on IL1β-induced monocyte chemotactic protein-1 (MCP-1) production from OA synovial fibroblasts. PGE₂ inhibited IL1β-induced MCP-1 expression in a dose-dependent manner. Pretreatment with diclofenac or meloxicam led to an augmentation of IL1β-induced MCP-1 expression that was overcome by the administration of exogenous PGE₂. 11-deoxy-PGE₁, a dual agonist at the EP2 and EP4 receptors, and to a lesser extent butaprost, a selective EP2 receptor agonist, also diminished IL1β-induced MCP-1 expression. Sulprostone, a dual agonist at the EP1 and EP3 receptors, had no effect. These data suggest that PGE₂, acting through the EP2 and EP4 receptors, downregulates MCP-1 expression, thereby potentially interfering with inflammatory cell recruitment into the osteoarthritic synovium. Thus, by inhibiting this regulatory mechanism, nonsteroidal anti-inflammatory drugs may actually aggravate the inflammatory process in OA.

Novel mechanisms of prostaglandin synthetic pathway inhibition
Cheng et al. [25**] described the regulation of IL1β-induced mPGES1 expression in OA synovial fibroblasts by peroxisome proliferator-activated receptor (PPAR)-γ ligands. 15-deoxy-Δ12,14 prostaglandin J₂ (15d-PGJ₂) and the thiazolidinedione troglitazone, both PPARγ agonists, dose-dependently inhibited IL1β-induced mPGES1 mRNA expression and protein synthesis and PGE₂ production. These PPARγ ligands also abolished IL1β-induced activation of the mPGES1 promoter. Treatment with the PPARγ ligand Wy14643 had no effect on these responses. GW9662, a PPARγ antagonist, diminishes the effect of the PPARγ agonists on mPGES1 expression, further supporting the involvement of PPARγ in the control of IL1β-induced mPGES1 expression. Furthermore, the suppressive effect of the PPARγ ligands was exaggerated if wild-type PPARγ was overexpressed and was reduced after overexpression of a dominant negative PPARγ. The upregulation of mPGES1 in response to IL1β has previously been shown to involve the transcription factor early growth response 1 (Egr-1). Cheng et al. [25**] also demonstrated that 15d-PGJ₂ and troglitazone prevented Egr-1 activation of the mPGES1 promoter as assessed by transient transfection studies and interfered with the IL1β-induced DNA binding activity of Egr-1 on electrophoretic mobility shift and supershift assay. These data demonstrate for the first time that PPARγ can modulate mPGES1 and Egr-1, thereby providing another potential mechanism of the anti-inflammatory effects of PPARγ.

Other recent data suggest that prostaglandin pathways may be targeted by therapies other than traditional nonsteroidal anti-inflammatory drugs and COX-2 selective inhibitors. Largo et al. [26] have demonstrated that glucosamine sulfate inhibited IL1β-induced nuclear factor-κB activity, COX-2 mRNA expression, and protein synthesis and PGE₂ production in human OA chondrocytes. Nakamura et al. [27] found that among other effects, glucosamine hydrochloride inhibited PGE₂ production in OA chondrocytes and synoviocytes and normal chondrocytes. Gouze et al. [28] examined the effect of overexpression of glutamine fructose-6-phosphate in IL1β-induced responses in rat chondrocytes. Glutamine fructose-6-phosphate is the rate-limiting step in the synthesis of intracellular glucosamine derivatives. Adenovirus-mediated gene transfer of glutamine fructose-6-phosphate prevented both the decrease in chondrocyte proteoglycan synthesis and the increase in chondrocyte NO and PGE₂ production stimulated by IL1β [28]. Manceiro et al. [29] investigated the effects of high- and low–molecular-weight hyaluronan preparations in cultured OA chondrocytes. Neither hyaluronan preparation affected basal PGE₂ production, whereas the low–molecular-weight preparation, but not the high–molecular-weight preparation, reduced IL1β-induced PGE₂ production by 70%.

Dual lipooxygenase/cyclooxygenase inhibition
Although prostaglandins have long been implicated in the pathogenesis of OA, the role of other eicosanoids has received relatively little attention until recently. Leukotrienes are products of 5-lipoxygenase that act as chemoattractants and amplify the inflammatory process. Leukotrienes are elevated in OA joints [30–32]. In addition, LTB4 has been shown to upregulate IL1β and tumor necrosis factor-α synthesis in OA synovial explants [33]. Licoferone, a pyrrolizine derivative that competitively inhibits 5-lipoxygenase and cyclooxygenase activity, is under clinical investigation as a treatment for OA. The available clinical data suggest that licoferone may be as effective as and safer than current anti-inflammatory therapy in OA [34]. Recent data from animal models support the hypothesis that licoferone may be an effective therapeutic strategy in OA. Lajeunesse et al. [35*] studied the effect of licoferone in an anterior cruciate ligament transection model of OA in a dog. Licoferone reduced levels of PGE₂,
insulin-like growth factor 1, and urokinase plasminogen activator, and it was associated with a reduction in the size of cartilage lesions in this model. The same group also examined the effect of licofelone on morphologic changes in subchondral bone [36]. Increased production of MMP-13 was noted in bone cells from anterior cruciate ligament—deficient knees, and there were a greater number of osteoclasts staining strongly positive for MMP-13 and cathepsin K. Subchondral bone resorption occurred in anterior cruciate ligament—deficient knees, as indicated by a decrease in bone surface and trabecular thickness. Licofelone inhibited these morphologic and biochemical changes in a dose-dependent manner. Boileau et al. [37] also investigated the effects of licofelone on MMP13 in human OA chondrocytes. They demonstrated by real-time PCR and specific enzyme-linked immunosorbent assay that licofelone dose-dependently suppressed IL1β-stimulated MMP13 mRNA expression and protein synthesis. This was mediated via the inhibition of the p38 MAPK/AP1 pathway and the transcription factor CREB. These observations suggest that licofelone might retard OA disease progression in addition to providing symptomatic relief for patients with OA. Clearly, this hypothesis requires further evaluation in a randomized controlled trial.

Conclusion
Eicosanoids such as prostaglandins and leukotrienes are implicated in the pathogenesis of OA. The production of prostaglandins in OA is likely to be driven by IL1β, but potentially also by calcium-containing crystals in a significant number of cases. Inhibition of prostaglandin synthesis has been a key element in the pharmacologic therapy of osteoarthritis, even though the consequences of this therapy on osteoarthritis disease progression have not been fully elucidated. Because prostaglandins may have potentially beneficial as well as deleterious effects in OA and leukotrienes have also been implicated in OA pathogenesis, more selective inhibition of prostaglandin pathways and/or inhibition of leukotriene synthesis may prove to be effective therapeutic strategies in OA.

References and recommended reading


The beneficial effects of licofelone on cartilage changes in a canine model of OA are reported.

Prevention of resorption of subchondral bone by licofelone in a canine model of OA is described.

This paper delineates the mechanism of MMP-13 inhibition by licofelone.
This bibliography is compiled by clinicians from the journals listed at the end of this publication. It is based on literature entered into our database between 1 January 2004 and 31 December 2004 (articles are generally added to the database about two and a half months after publication). In addition, the bibliography contains every paper annotated by reviewers; these references were obtained from a variety of bibliographic databases and published between the beginning of the review period and the time of going to press. The bibliography has been grouped into topics that relate to the reviews in this issue.

- Papers considered by the reviewers to be of special interest.
- Papers considered by the reviewers to be of outstanding interest.

The number in square brackets following a selected paper, for example [7], refers to its number in the annotated references of the corresponding review.

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2005, 17:351–391
© 2005 Lippincott Williams & Wilkins.
ISSN 1040–8711

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