THE YEAR IN RHEUMATIC DISORDERS

2001

J.S.H. GASTON

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Foreword

The last year has seen a variety of important advances that are chronicled and analysed in *The Year in Rheumatic Disorders 2001*. Professor Hill Gaston and his colleagues have selected reports felt to have current and lasting implications. Focus, as readers will note, is on relatively common and important problems. The huge effort involved should be appreciated by the readers. I am amazed how current and pertinent the material is.

The most dramatic developments in this period have been related to therapy of several of our prominent rheumatic diseases. In an era of almost endless interpretations of this literature by partial and impartial reviewers, it is important that we see analyses as presented in this volume. We need to continue to consider how to put these advances in perspective for ourselves and our patients as we discuss treatment in our journal clubs and conferences.

Basic studies reviewed often suggest ideas for future improved disease characterization and therapy. Although new therapies are helping and in some cases have less toxicity, they are far from the full answer. The new coxibs still provide only partial relief of symptoms in osteoarthritis. Anticytokine therapy, although slowing radiographic progress in rheumatoid arthritis, does not produce remissions and at present only rarely provides the 70–80% or more improvements that patients ultimately need. There is still much to do. We look forward to the *Year in Rheumatic Disorders* 2002, 3, 4 and 5. Can we guess from the basic studies where the next giant steps will come?

Papers reviewed here reflect a wide selection from centres and journals around the world, making ideas and opinions from the leading research scientists from many countries available in this one publication. Although, like most rheumatologists, I am a voracious reader, I found fascinating papers that I had missed. I have enjoyed the thoughtful, scholarly analyses of these papers by the authors. I invite readers to compare them with their own thoughts and implications in their different practices and countries.

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Preface

The function of this volume is to give a fairly concise overview of developments in rheumatology over approximately a 12-month period. Essentially, a rheumatologist held incommunicado for a year in a journal and internet-free zone, and anxious to know what has happened to their subject in the interim, should be able to feel that they are up to speed again after looking through this book. Alternatively, it provides a useful resource for the rest of us who might not quite have found the time to read absolutely all of the relevant journals during the year.

The scope is deliberately wide, reflecting the nature of rheumatology; rheumatologists may genuinely need to know what advantages do the cyclooxygenase-specific non-steroidal anti-inflammatory drugs offer, what's the most reliable way to diagnose carpal tunnel syndrome, and what's the latest cytokine (Answer; inter-leukin-23). For this reason articles cannot be comprehensive in quite the same way as some other current contents volumes. Personal biases of the reviewers in selecting articles has, therefore, been given full rein, the only criterion being whether the reviewer found the article interesting and relevant.

The other point to make as editor is that an entirely rational organization of the selected papers has eluded us; for instance, most papers on anti-tumour necrosis factor therapies are in a single section, but the paediatric chapter includes a paper on the use of etanercept in juvenile idiopathic arthritis. The placing of papers on rarer diseases such as Behçet's is also rather arbitrary. If a paper you expected to see is missing from one of the sections, check whether it might not have been included in another one.

Lastly, my thanks are due to each of my co-authors for timely provision of manuscripts, my long-suffering secretary Hazel Mould for logistic support, and the staff at Clinical Publishing Services, particularly Rosemary Osmond, Penny Bowers and Jonathan Gregory.

Hill Gaston

Part I

Novel therapies

Novel therapies

Introduction

This section is divided into three chapters:

1. Cyclooxygenase (COX) -2-specific non-steroidal anti-inflammatory drugs
2. Inhibition of tumour necrosis factor (TNF-α)
3. Additional targets of anticytokine therapy in rheumatoid arthritis (RA)

Novel therapies are reviewed, starting with papers on the COX-2-specific non-steroidal anti-inflammatory drugs. For completeness, results of some trials of these agents in osteoarthritis (OA) are included, and indeed some trials recruited a mixture of RA and OA patients, as the incidence of side-effects in these large trials are relevant to the use of the same drugs in RA. Several papers on the effects of COX-2-specific drugs on renal function, particularly in the elderly, are included, along with the lack of effect of these drugs on platelet function and the potential disadvantage of losing this action in patients predisposed to thrombotic episodes.

Chapter 2 collects together papers on the efficacy of TNF-α therapies in RA. Again for immediate comparison, the recent, and much more preliminary, trials of anti-TNF in spondyloarthropathies are also included here. The use of anti-TNF therapies was firmly based on a large body experimental work both *in vitro* and in relevant animal models. This work continues and the introduction of the anti-TNF therapies allows additional observations on RA pathogenesis, particularly the importance of recruitment of inflammatory cells to the joint. Preliminary results on the effects of anti-TNF therapies on bony erosion have been very encouraging, so investigations into the role of TNF in bone absorption by osteoclasts are highly relevant. Lastly, some papers on the newly introduced disease modifying drug, leflunomide, are included in this section because of the recent speculations that this drug's activity may be mediated through an effect on TNF signalling.

Finally, in Chapter 3, papers related to the role of other cytokines in RA are reviewed as these provide actual or potential targets of additional novel therapies in RA. Particular attention is paid to the role of interleukin (IL)-1 in RA and, therefore, the efficacy of the natural IL-1 inhibitor, IL-1 receptor antagonist (IL-1ra). A more recently described cytokine, IL-17 has the interesting property of being wholly T cell-derived but sharing many properties with the macrophage-derived cytokines, IL-1 and TNF-α. Thus, a part played by IL-17 in cartilage breakdown and in the formation of erosions would establish a direct link between these aspects of pathology in RA and T lymphocytes. Other T-cell-derived cytokines have been implicated in disease models and by observations with humans; IL-15 plays an important part in both maintaining T lymphocytes and activating them, while IL-4 generally has an anti-inflammatory and anti-joint destructive role in disease models. IL-12 in contrast is derived from non-T cells but influences T-cell differentiation in a proinflammatory direction. Many newly discovered cell surface molecules and cytokines have yet to be integrated into our understanding of RA pathogenesis (e.g. OX40, IL-21, IL-23), but these may also provide targets for disease modulation.

1
Cyclooxygenase-2-specific non-steroidal anti-inflammatory drugs

Overview

A large number of papers have been published in connection with the introduction of the cyclooxygenase (COX) -2-specific non-steroidal anti-inflammatory drugs (NSAIDs). Several very large studies have been conducted with adequate power to detect rates of complications accurately. Although this section of the book is primarily concerned with novel therapies in inflammatory arthritis, papers detailing the use of COX-2-specific drugs are included here for completeness. There is general agreement that the new compounds have similar efficacy to diclofenac, ibuprofen and naproxen, but with a ~10-fold decrease in the incidence of gastric ulceration, down to levels difficult to distinguish from background. As expected, the new drugs do not affect platelets, and this observation can be coupled with a report of thrombotic events in patients with COX-2 inhibition. Such patients seem to have a preexisting pro-thrombotic tendency in whom the loss ,of antiplatelet effects of conventional NSAIDs when changing to a COX-2-specific agent may have been disadvantageous. The large trials suggest that this is not a common situation. Although further work needs to be done, there appear to be no relief from renal side-effects by changing from a conventional NSAID to a COX-2-specific drug.

Celecoxib versus diclofenac in long-term management of rheumatoid arthritis: randomised double-blind comparison. P Emery, H Zeidler, T K Kvien, *et al. Lancet* 1999; **354**:2106–11.

BACKGROUND. NSAIDs inhibit COX, which leads to suppression of COX-1-mediated production of gastrointestinal (GI) protective prostaglandins (PGs). GI injury is a common outcome. The efficacy, safety and tolerability of long-term therapy with celecoxib, a COX-1 sparing inhibitor of COX-2, was compared with diclofenac, a nonspecific COX inhibitor.

INTERPRETATION. Celecoxib showed sustained anti-inflammatory and analgesic activity similar to diclofenac, with a lower frequency of upper GI ulceration or GI adverse events, and tolerability was better.

Table 1.1 Mean (SD) arthritis assessment results at week 24

	Celecoxib		Diclofenac	
	Baseline	**Week 24**	**Baseline**	**Week 24**
Primary assessments				
Physician's assessment*	2.9 (0.7)	2.6 (0.8)	3.0 (0.8)	2.6 (0.8)
Patient's assessment*	3.0 (0.8)	2.7 (0.9)	3.1 (0.8)	2.8 (0.9)
Number of tender or painful joints	20.3 (14.4)	14.5 (14.1)	21.7 (14.4)	16.4 (14.7)
Number of swollen joints	14.9 (10.2)	10.7 (10.1)	14.3 (9.9)	10.4 (10.0)
Secondary assessments				
Pain VAS (mm)	47.4 (21.5)	40.8 (25.5)	51.7 (21.6)	43.1 (25.2)
Duration of morning stiffness (min)	70.0 (71.8)	67.3 (140.6)	98.4 (158.4)	84.5 (189.5)
MHAQ functional disability index	1.2 (0.7)	1.1 (0.7)	1.2 (0.6)	1.1 (0.7)
C-reactive protein (mg/L)	15.1 (32.1)	17.4 (22.9)	18.4 (26.4)	20.5 (27.0)

VAS=visual analogue scale. MHAQ=modified health assessment questionnaire.
*Independent assessments, graded from 1 (very good: symptom-free with no limitation of normal activities) to 5 (very poor: very severe symptoms that are intolerable and inability to carry out all normal activities).
Source: Emery *et al.* (1999).

Table 1.2 GI ulcer frequency

	Celecoxib (n = 212)	Diclofenac (n = 218)	p
Patients with detected erosion, ulcer, or both			
Gastric	38 (18%)	74 (34%)	< 0.001
Duodenal	11 (5%)	23 (11%)	< 0.009
Ulcer incidence by *H. pylori* status*			
Positive serological test	7/93 (8%)	19/87 (22%)	NS
Negative serological test	1/97 (1%)	10/100 (10%)	NS
Ulcer frequency by concomitant corticosteroid use			
Corticosteroid use	2/80 (3%)	12/102 (12%)	NS
No corticosteroid use	6/132 (5%)	21/116 (18%)	NS

*Among patients with known *H. pylori* status only.
Source: Emery *et al.* (1999).

Comment

A double-blind, randomized study of celecoxib 200 mg versus diclofenac slow release (SR) 75 mg twice daily over 24 weeks. All patients had <6 months of rheumatoid arthritis (RA), 326 received celecoxib and 329 received diclofenac.

Patients were assessed for efficacy and tolerability, and two-thirds had upper GI endoscopy, to assess GI safety.

The study patients were representative of the general population of patients with RA, as patients with previous GI complications were included, although those with clinically suspected active peptic ulcer or GI bleeding were excluded. However, no pre-study endoscopies were performed. The two groups were similar, except for a significantly higher mean pain score and longer duration of morning stiffness, implying more severe disease, in the diclofenac group.

Efficacy: Celecoxib and diclofenac showed similar improvements in both primary and secondary outcome measures. Eighty (25%) patients in the celecoxib group and 73 (22%) in the diclofenac group achieved an ACR20 |**1**| response by week 24 (Table 1.1).

Tolerability: GI tolerability was better with celecoxib than diclofenac, with a higher rate of withdrawal by patients on diclofenac (16%) compared with celecoxib (6%) $P<0.001$. The diclofenac group (21% versus 11%, $P<0.05$) also reported abdominal pain more frequently. There were no significant differences in the frequency of other reported adverse events, which were mostly mild to moderate.

Safety: GI symptoms are not an accurate assessment of GI ulceration, and it was important to assess GI safety by endoscopy. More gastroduodenal ulcers were detected on endoscopy in the patients receiving diclofenac than celecoxib (Table 1.2).

The prevalence of ulcers detected in the celecoxib group (4%) is similar to that detected by endoscopy in healthy, symptom-free volunteers or untreated patients. The pathogenesis of these ulcers was not established, but was most likely related to *Helicobacter pylori*.

The prevention of chronic NSAID induced upper gastrointestinal toxicity: a Cochrane Collaboration meta-analysis of randomised controlled trials.
A Rostom, G Wells, P Tugwell, *et al. J Rheumatol* 2000; **27**: 2203–14.

BACKGROUND. Given the number of gastro-protective strategies available, and in the absence of a single large randomized controlled trial (RCT), meta-analysis of available trials is required. Despite the introduction of apparently better tolerated COX-2 selective NSAIDs, issues of cost-effectiveness will make this a valuable study.

INTERPRETATION. A large amount of data is presented. Misoprostil, H_2-receptor antagonists (ranitidine, cimetidine, famotidine, nizatidine all studied) and proton pump inhibitors (omeprazole only) were studied, but without direct comparison—their relative benefits can only be implied from the sizes of the treatment effects compared with placebo. In summary, risk reduction versus placebo was greatest for misoprostil 800 mg in preventing endoscopic gastric ulceration (EGU), and greatest for proton pump inhibitors in preventing endoscopic duodenal ulceration (EDU). Double-doses of H_2-receptor antagonists

were required to protect against both EGU and EDU, and half-doses (400 mg) of misoprostil were considerably less effective. Only misoprostil is reported in a RCT of peptic ulcer complications, with an odds ratio of 0.6 compared with placebo. Tolerability of misoprostil was a problem, however, with more than 40% stopping the drug because of GI upset (particularly diarrhoea).

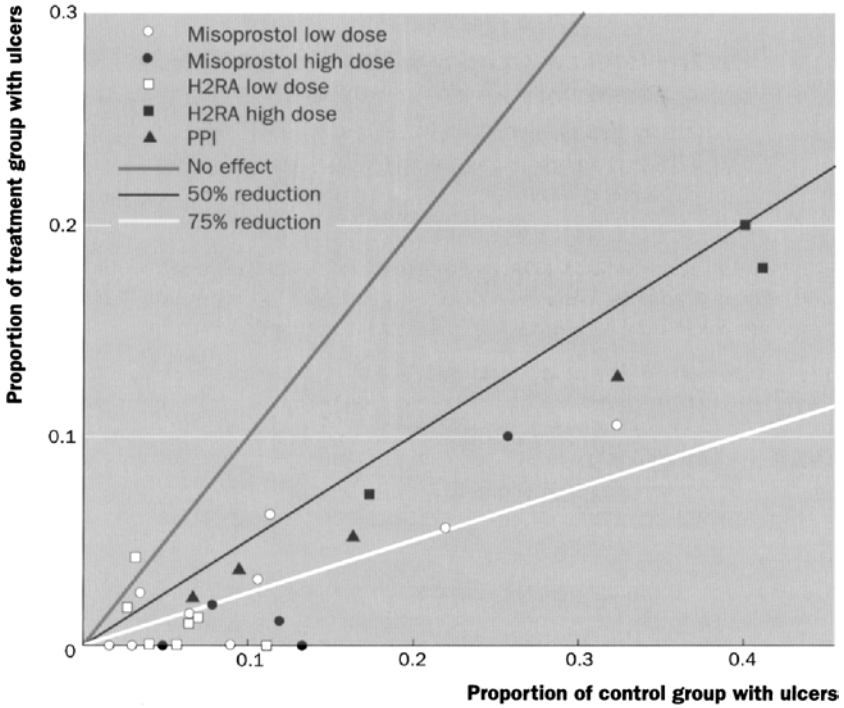

Fig. 1.1 L'Abbé plot. This plot displays the risk reductions observed in the various studies for the three interventions, including those with 1- and ≥3-month outcome measures. Solid lines indicate: no, 50% and 75% relative risk reduction of gastric ulcers. The differences in risk reductions between various studies of a given intervention provide a graphic representation of observed heterogeneity. Source: Rostom *et al.* (2000).

Comment

The stated aim was to compare all gastro-protective strategies, although the authors later state their main interest was to investigate misoprostil. However, as expected of the Cochrane Collaboration, this is a carefully carried out meta-analysis. The decision to analyse data from studies of endoscopic ulceration was largely in the recognition that few studies of clinical outcomes existed. However, it is important to be aware that of the endoscopic ulcers identified in up to 40% of chronic NSAID users, 85% may never become symptomatic. Furthermore, there is a very poor correlation between upper GI dyspeptic symptoms and the

presence of ulceration. The NSAIDs used in the various primary studies were relevant to everyday practice.

The title of the paper refers to NSAID-induced upper GI toxicity. No justification is given in this paper for the causality implied, and the search strategy used does not clearly indicate that only NSAID-induced ulceration, rather than associated ulceration was studied. The point is not semantic given the greater association of duodenal ulceration with *H. pylori* and the greater efficacy of proton pump inhibitors in *H. pylori* eradication, factors that might therefore influence the tentative comparisons that are made. Number-needed-to-treat can be calculated from the data presented, allowing important cost-effectiveness comparisons to be made with COX-2 drugs, with the *caveat* just mentioned. The study also usefully points out that there is no justification for using a lower dose of misoprostil to reduce adverse effects—if the 800 mg dose is intolerable an alternative should be used.

Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the Celecoxib long-term arthritis safety study (CLASS): a randomized controlled trial.

F E Silverstein, G Faich, J L Goldstein, *et al. JAMA* 2000; **284**(10): 1247–55.

BACKGROUND. Conventional NSAIDs are associated with a spectrum of toxic effects, notably GI effects, because of inhibition of COX-1. Whether COX-2-specific inhibitors are associated with fewer clinical GI toxic effects is unknown.

INTERPRETATION. In this study, celecoxib, at dosages greater than those indicated clinically, was associated with a lower incidence of symptomatic ulcers and ulcer complications combined, as well as other clinically important toxic effects, compared with NSAIDs at standard dosages. The decrease in upper GI toxicity was strongest among patients not taking aspirin concomitantly.

Comment

8059 patients with either RA or osteoarthritis (OA), were randomly assigned to receive celecoxib 400 mg twice daily (two to four times higher than maximum RA or OA doses) (n=3987), ibuprofen 800 mg three times a day (n=1985) or diclofenac 75 mg, twice daily (n=1996) for 6 months.

These patients were representative of the general population with RA or OA, as patients with previous GI bleeding were included, although those with active bleeding within the previous 30 days or previous upper GI surgery were excluded. Usual treatments for RA and OA, including steroids and disease-modifying antirheumatic drugs (DMARDS) and low-dose aspirin (<325 mg/day) for cardiovascular prophylaxis were allowed. All potential upper GI ulcer complications were evaluated according to a predefined protocol. A blinded committee defined these as: upper GI complications, symptomatic ulcers or other diagnoses.

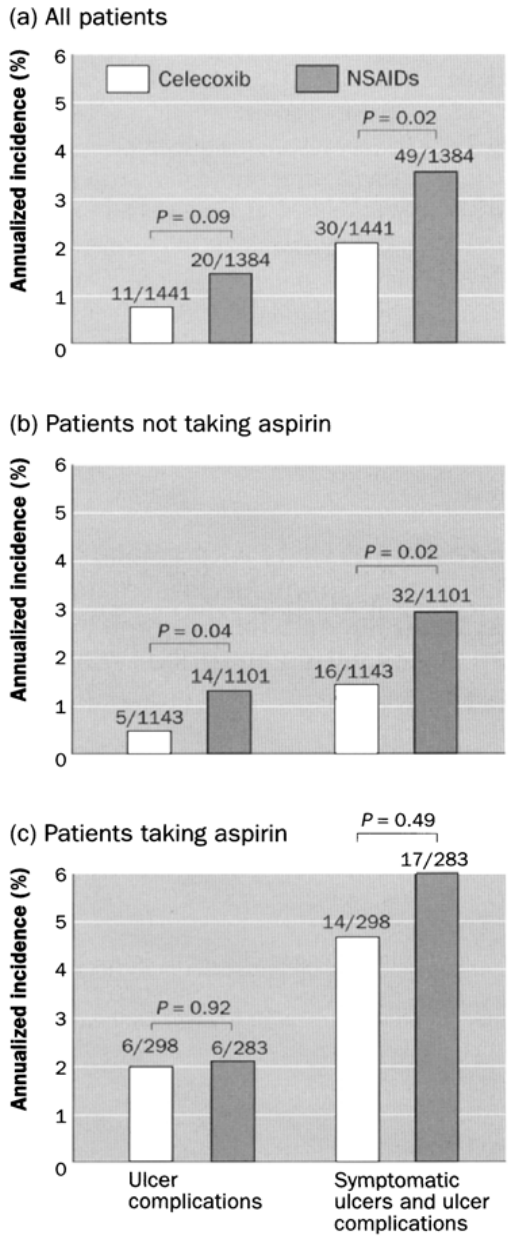

Fig. 1.2 Annualized incidence of upper GI tract ulcer complications alone and with symptomatic gastroduodenal ulcers. Source: Silverstein *et al.* (2000).

Table 1.3 Incidence of GI events in the treatment groups

Type of Event	Rofecoxib Group (N = 4047)	Naproxen Group (N = 4029)	Rofecoxib Group (N = 4047)	Naproxen Group (N = 4029)	Relative Risk (95% CI)*	P Value
	no. with event		rate/100 patient-yr			
Confirmed upper gastrointestinal events	56	121	2.1	4.5	0.5 (0.3–0.6)	< 0.001
Complicated confirmed upper gastrointestinal events	16	37	0.6	1.4	0.4 (0.2–0.8)	0.005
Confirmed and unconfirmed upper gastrointestinal events†	58	132	2.2	4.9	0.4 (0.3–0.6)	< 0.001
Complicated confirmed and unconfirmed upper gastrointestinal events‡	17	42	0.6	1.6	0.4 (0.2–0.7)	0.002
All episodes of gastrointestinal bleeding	31	82	1.1	3.0	0.4 (0.3–0.6)	< 0.001

*CI denotes confidence interval.
†The analysis includes 13 events that were reported by investigators but were considered to be unconfirmed by the end-point committee,
‡ The analysis includes six events that were reported by investigators but that were considered to be unconfirmed by the end-point committee.

822 (20.6%) patients taking NSAIDs withdrew due to adverse effects compared with 732 (18.4%) taking celecoxib P=0.001. Patients taking NSAIDs had higher rates of ulcer complications (0.76% versus 1.45% P=0.09) or symptomatic ulcers (2.08% versus 3.54% P=0.02) than patients taking celecoxib (Fig. 1.2).

The rate of ulcer complications in the celecoxib group was higher than predicted from previous studies, and was related to the use of low-dose aspirin. When the aspirin users were excluded, the incidence of ulcer complications was lower with celecoxib than NSAIDs (0.44% versus 1.27%; P=0.04) and is similar to the incidence in patients not taking NSAID or aspirin in the general population. The ulcer incidences in aspirin users did not significantly differ between the two groups. Bleeding-related events were significantly lower in the celecoxib group (4.0% versus 8.3% P<0.001) as was anaemia (2.6% versus 6.4% P<0.001). Liver transaminases were elevated more frequently in the NSAID group, and 97% of the NSAID group, with abnormal transaminases, were taking diclofenac. Renal events were also more frequent in the NSAID group.

The theoretical increase in thrombotic events by COX-2 inhibitors was not demonstrated in this study. Cardiovascular events were similar in both groups and were also similar in the non-aspirin users.

This is an important large-scale study, but is only of 6 months' duration. Most patients with RA or OA take NSAIDs for long periods, and longer-term data are needed. It might also have been interesting to assess differences between the two disease groups.

Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis.
C Bombardier, L Laine, A Reicin, *et al. N Engl J Med* 2000; **343** (21):1520–8.

BACKGROUND. Each year, clinical upper GI events occur in 2–4% of patients who are taking non-selective NSAIDs. This study assessed whether rofecoxib, a selective inhibitor of COX-2, would be associated with a lower incidence of clinically important upper GI events than the non-selective NSAID naproxen among patients with RA.

INTERPRETATION. In patients with RA, treatment with rofecoxib, a selective inhibitor of COX-2, is associated with significantly fewer clinically important upper GI events than treatment with naproxen, a nonselective inhibitor.

Comment

A randomized study of 8076 patients with RA aged >50 years (or >40 years taking long-term steroids) receiving either rofecoxib 50 mg daily (twice usual dose) or naproxen 500 mg twice daily. The primary end-point was confirmed clinical upper GI events (gastroduodenal perforation or obstruction, upper GI

bleeding and symptomatic gastroduodenal ulcers). A blinded committee assessed these, according to prespecified criteria. There was a possibility of increased thrombotic events in the patients receiving rofecoxib, and therefore cardiovascular events were also assessed. Rofecoxib and naproxen had similar efficacy in RA, assessed by standard questionnaires. The median follow up was 9 months in both groups (range 0.5–13) and significantly lower rates of confirmed upper GI events and complications were found in the patients taking rofecoxib compared with naproxen (Table 1.3).

The other most common adverse events leading to discontinuation of treatment were upper GI symptoms; lower in the rofecoxib group (3.5% versus 4. 9%). Routine endoscopies were not performed, as other studies had previously shown reduced ulcer rates with rofecoxib, and it was unclear whether this correlated with a reduction in clinical upper GI events. The results of this study correspond to the endoscopic studies and suggest that rofecoxib reduces both ulcer rates and clinically important upper GI events. These are also similar to results from comparable studies with celecoxib.

Renal side-effect rates were low and as expected, similar for both groups (1. 2% rofecoxib, 0.9% naproxen).

The rate of myocardial infarction (MI) was lower in the naproxen group than rofecoxib (0.1% versus 0.4%). This high rate of MI occurred in 4% of the patients who were at high risk of MI, and required, but were not taking, aspirin prophylaxis. These patients accounted for 38% of the patients who had MIs; the rate of MI in patients not at high risk, was not significantly different between the two treatment groups (rofecoxib 0.2% versus 0.1% naproxen). When these data became available (after the end of the trial) the manufacturer notified all ongoing studies using rofecoxib, to change exclusion criteria, and allow use of low-dose aspirin.

COX-2 inhibitors in osteoarthritis

The potentially improved tolerability and safety of the COX-2-selective NSAIDs is one of the most important therapeutic advances in OA. In addition to the potential benefit of reduced GI risk, reduced renal toxicity might also be anticipated with the COX-2-specific NSAIDs.

Treatment of osteoarthritis with celecoxib, a cyclooxygenase-2 inhibitor: a randomized controlled trial. W Bensen, J J Fiechtner, J I McMillen, *et al. Mayo Clin Proc* 1999; **74**:095–105.

BACKGROUND. Celecoxib is the second COX-2 selective NSAID to reach the market. This study evaluates its efficacy and safety in knee OA compared with a well-established treatment.

INTERPRETATION. Patients had treatment-responsive OA of the knee. Twice daily celecoxib 100 mg or 200 mg were equally efficacious and

comparable with naproxen. Both were superior to placebo in global assessments by physician and patient, and in specific, validated OA scales (WOMAC, OA severity scale) over a 12-week period.

Comment

The study period was quite short in the context of a chronic condition such as OA. However, selective inhibition of COX-2 appears to be sufficient to achieve the benefits associated with NSAID treatment. Tolerability and safety data are provided without statistical analysis, the authors referring to their other published studies in RA patients, which showed no significant increase in risk. A wide age range was included, excluding only those with recent (within 30 days) peptic ulcer disease or 'active concomitant GI or renal disorders' (not specified). While this suggests celecoxib is a safe alternative in these patients, it must be remembered that patients were randomly allocated to naproxen, which did not have any significant increase in adverse effects compared with celecoxib suggesting this, too, is a safe option.

Effects of celecoxib and naproxen on renal function in the elderly.
A Whelton, G Schulman, C Wallermark, *et al. Arch Intern Med* 2000; **160**:1465–70.

BACKGROUND. This study evaluates the anticipated reduction in renal toxicity to which the elderly are particularly vulnerable, being frequent NSAID users and often having renovascular co-morbidity.

INTERPRETATION. After 6 days of treatment, a statistically significant difference appeared between naproxen and celecoxib in the reduction in glomerular filtration rate (GFR). This was consistent in the crossover phase of the study. There was no statistically significant difference in the reduction in PGE_2 and 6-keto-$PGF_{1\alpha}$ seen with both agents, and the effects on renal sodium excretion were transient in all patients.

Comment

The absence of a statistically significant difference in the reduction of urinary PG excretion is interesting, in the context of the proposed reason for a COX-2-selective NSAID being favourable in the kidney. However, as the reductions were small and below the sensitivity limit of the assay, the trend towards a greater reduction with naproxen may, in fact, be clinically significant (but note the results of Swan *et al,* who also examined an elderly population, discussed elsewhere in this chapter). In applying these results to prescription practice, it is important to bear in mind that the participants were in good health, and in particular were not taking ACE inhibitors and were normotensive. The dose of celecoxib was escalated on the fifth day from 200 mg twice daily dose to 400 mg

twice daily without adverse effect on GFR which is reassuring (though the higher dose is not indicated in OA).

A randomised trial of the efficacy and tolerability of the COX-2 inhibitor rofecoxib *vs* ibuprofen in patients with osteoarthritis

R Day, B Morrison, A Luza, *et al. Arch Intern Med* 2000; **160**: 1781–7.

BACKGROUND. While the efficacy of COX-2 selective NSAIDS has been established compared with placebo, their place relative to treatments in current guidelines remains unclear.

INTERPRETATION. Treatment: placebo, ibuprofen 800 mg three times a day, rofecoxib 12.5 mg once a day or 25 mg once a day; acetominophen (paracetemol) as required was allowed. At 6-week follow-up, treatments were equally effective across a range of parameters in patients with OA of hip or knee. Adverse effects were less frequent with the low dose rofecoxib, and led to discontinuation of treatment more frequently in the ibuprofen group (largely due to GI intolerance).

Comment

This study selected patients whose OA symptoms were responsive to NSAID (by worsening during the wash-out period), or who required regular paracetemol and were judged on a physician assessment to be fair, poor or very poor. There was a considerable list of exclusions, particularly with respect to renal function, cardiac status, and concomitant medication increasing GI risk (warfarin, ticlopidine, steroid). The primary outcome measures included a recognized range of general OA measures (WOMAC, physician overall assessment, patient assessment of treatment utility) and those specific to the joint in question (tenderness, pain on walking). While the authors planned to include paracetemol use as a secondary outcome, this is not reported in their analysis. The equivalence demonstrated was consistent across these parameters, which supports the validity of their conclusion. Ibuprofen at the study dose might well be expected to cause a significant rate of GI intolerance and discontinuation. Therefore, the comparison perhaps does not reflect normal clinical practice, where an alternative NSAID might be prescribed in preference to the higher dose of ibuprofen, or a gastro-protective agent might be added.

It is worth emphasizing the authors' remark that rofecoxib should be first prescribed at the lower dose in view of the comparable efficacy and lower adverse effect rate. The study does not, however, appear to support using the higher dose if 12.5 mg is ineffective.

Reduced risk of upper gastrointestinal ulcer complications with celecoxib, a novel COX-2 inhibitor.
J L Goldstein, F E Silverstein, N M Agrawal, *et al. Am J Gastroenterol* 2000; **95**(7):1681–90.

BACKGROUND. This study aims to assess the rate of upper GI ulcer complications (bleeding, perforation, or gastric outlet obstruction) associated with celecoxib, a specific COX-2 inhibitor, compared with the rate associated with non-specific NSAIDs.

INTERPRETATION. The incidence of upper GI ulcer complications associated with celecoxib was eightfold lower than with non-specific NSAIDs. The incidence of ulcer complications in celecoxib-treated patients was similar to that in patients receiving placebo in the RCTs, and to that in non-NSAID users reported in the literature.

Comment

This is the pooled analysis of 14 multicentre RCTs and separate analysis of one long-term, open-label trial to assess efficacy and safety of celecoxib in arthritis. RCTs enrolled 11008 patients with OA or RA treated for 2–24 weeks and compared celecoxib with placebo, a non-specific NSAID or both.

The open-label study assessed the long-term safety of celecoxib and recruited 5155 patients from the RCTs. All patients had endoscopies at the end of their previous RCT, and no further endoscopies were performed routinely. All OA patients were treated with 100–200 mg celecoxib twice daily and RA patients 200–400 mg twice daily. All potential upper GI ulcer complications were investigated according to protocol and predefined as bleeding, perforation, or gastric outlet obstruction. A blinded committee adjudicated this.

RCTs: Eleven patients had upper GI ulcer complications—nine bleeding and two obstructions. Rates of upper GI ulcer complications were 0.03% with celecoxib and 0.33% with NSAIDs. The annualized incidence of upper GI ulcer complications was eightfold lower for celecoxib than NSAIDs (0.2% versus 1. 68% P=0.002). The rate found with NSAIDs corresponds to the results of previous studies and, therefore, the adjudication of the upper GI ulcer complications appears appropriate and the routine endoscopies performed in five of the RCTs do not appear to have detected a higher rate of upper GI ulcer complication and skewed the results.

Open-label study: nine patients developed upper GI ulcer complications; all were bleeding ulcers. The annualized incidence rate for upper GI ulcer complications was 0.18%.

The annualized incidence rates of upper GI ulcer complications with celecoxib was low in both the RCTs (0.2%) and the open-label study (0.18%) and similar to incidence rates reported in patients not taking NSAIDs.

This paper presents data from a large number of patients taking celecoxib: from RCTs with appropriate comparators and the open-label study that reflects

clinical practice. As these were initially designed as efficacy studies the power was insufficient to detect large enough numbers of upper GI ulcer complications to allow subgroup analysis of other risk factors (e.g. aspirin use, *H. pylori* status).

The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis.
G Steinbach, P M Lynch, R K Phillips, *et al. N Engl J Med* 2000; **342**(26):1946–52.

BACKGROUND. Patients with familial adenomatous polyposis (FAP) have a nearly 100% risk of colorectal cancer. In this disease, the chemopreventive effects of NSAIDs may be related to their inhibition of COX-2.

INTERPRETATION. In patients with FAP, 6 months of twice-daily treatment with 400 mg of celecoxib, a cyclooxygenase-2 inhibitor, leads to a significant reduction in the number of colorectal polyps.

Comment

Patients with FAP have a mutation of the adenomatous polyposis coli (APC) gene and develop multiple adenomatous polyps, which evolve into colonic carcinoma. Sporadic adenomas also develop mutations of this gene and progress to carcinoma and, therefore, studies in FAP may increase the understanding of sporadic adenomas and colon cancer.

Epidemiological studies have shown reduced rates of adenomas and colon cancer among NSAID users and small trials with NSAIDs have shown regression of colorectal adenomas in patients with FAP. The chemopreventive effects of NSAIDs may be partly due to COX-2 inhibition, as it is expressed in inflammatory disease, premalignant and malignant lesions. Further research implicates COX-2 in mitogenic growth factor signalling and the downregulation of apoptosis, thus promoting tumour growth.

The GI toxicity of NSAIDs may limit their long-term use for cancer prevention, and this study uses celecoxib to determine whether COX-2 inhibition could reduce the extent of polyposis in patients with FAP.

Seventy-seven patients with FAP were randomized to receive celecoxib (100 or 400 mg twice daily) or placebo for 6 months. Previous NSAID trials had shown regression of polyps within 6 months. All patients were assessed endoscopically and to be included had >5 polyps of >2 mm.

Treatment with 400 mg celecoxib twice daily resulted in a significant reduction in the mean number of polyps and the polyp burden (sum of polyp diameters). A non-significant improvement was seen with 100 mg twice daily (Table 1.4).

A reduction of >25% in the mean number of polyps was seen in 53% of the high-dose group P=0.003, 31% of the low-dose group P=0.08 and 7% of the placebo group. The regression of polyps occurred throughout the rectum and

Table 1.4 Per cent change from baseline in the mean number of polyps and colorectal polyp burden in patients with FAP treated with placebo or celecoxib for 6 months*

Variable	Placebo (N = 15)	100 mg of Celecoxib Twice Daily (N = 32)	400 mg of Celecoxib Twice Daily (N = 30)
Per cent change in no. of colorectal polyps	−4.5 ± 16.4	−11.9 ± 30.3	−28.0 ± 24.0
P value		0.33	0.003
Per cent change in colorectal polyp burden†	−4.9 ± 17.3	−14.6 ± 31.7	−30.7 ± 25.7
P value		0.09	0.001
Per cent change in no. of rectal polyps‡	−3.1 ± 31.1	−3.4 ± 35.0	−22.5 ± 26.0
P value		0.52	0.01

*Plus-minus values are means ±SD. P values are based on the two-sample Wilcoxon statistic for the comparison of celecoxib with placebo, in the intention-to-treat analysis. Negative numbers indicate decreases, and positive numbers increases.
†The colorectal polyp burden was calculated as the sum of the polyp diameters.
‡ Seven subjects had no rectal polyps at base line or on final evaluation. These subjects are considered to have had 0% change.
Source: Steinbach *et al.* (2000).

colon with high-dose celecoxib, and this was not related to age or previous colectomy. Both doses of celecoxib were well tolerated, and there were no significant differences in the incidence of adverse events between the three groups. Previous NSAID studies showed recurrence of adenomas after discontinuing the drug, which was not assessed by this study.

It is uncertain whether long-term treatment with COX-2 inhibitors would prevent carcinoma in FAP, as other non-COX mechanisms are probably involved in colon carcinogenesis. COX-2 inhibitors may play a part in treatment as an adjunct to colectomy, by suppressing progression of polyps, while waiting for colectomy or suppressing polyp formation in patients with residual rectum.

Effects of celecoxib, a novel cyclooxygenase-2 inhibitor, on platelet function in healthy adults: a randomized, controlled trial.

P T Leese, R C Hubbard, A.Karim, *et al. J Clin Pharmacol* 2000; **40**(2): 124–32.

BACKGROUND. Non-specific NSAIDs inhibit both COX-1, involved in platelet function and COX-2, which mediates the inflammatory response. Specific COX-2 inhibitors would, therefore, be expected to have minimal effects on platelet function.

INTERPRETATION. Supratherapeutic doses of celecoxib did not reduce platelet aggregation or serum thromboxane (TX) levels or increase bleeding time and, therefore, appear to be COX-1 sparing, relative to conventional NSAIDs.

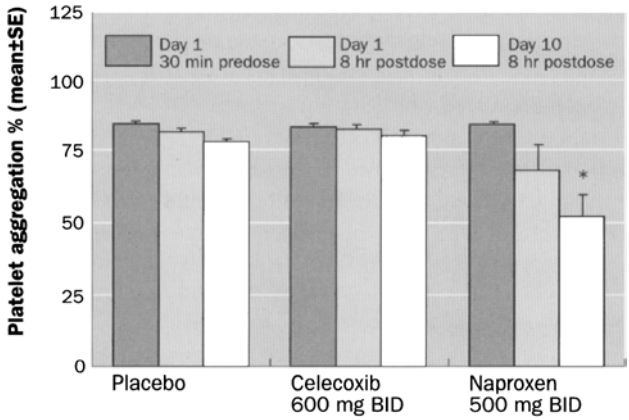

Fig. 1.3 Platelet aggregation responses to arachidonate before dosing and at 8 h postdose on days 1 and 10. Statistical comparisons are based on changes from baseline. Asterisk indicates $P<0.05$ for change from baseline versus celecoxib and placebo. None of the differences between celecoxib and placebo was statistically significant at any postdose time point. Source: Leese *et al.* (2000).

Comment

Previous studies have shown increased postoperative blood loss and higher rates of epistaxis among NSAID users. NSAIDs can cause prolonged bleeding via inhibition of platelet COX-1 activity. Celecoxib, a specific COX-2 inhibitor, does not affect platelet function in normal therapeutic doses (400 mg twice a day).

This double-blind, randomized study compared platelet responses with naproxen (standard dose, 500 mg twice a day), celecoxib (three to six times higher than usual dose, 600 mg twice a day) and placebo in three groups of healthy subjects, over 10 days. Platelet function was assessed by: *ex vivo* platelet aggregation in response to standard agonists (arachidonate, collagen and U46619 —a TXA_2 agonist), bleeding time and serum TXB_2 levels.

Naproxen produced a reduction in platelet function after the first dose, which continued during the study. Platelet aggregation with arachidonate was most impaired, with less effect on collagen-induced aggregation and no significant reduction of U46619-induced aggregation. The variation in results is compatible with the different mechanisms for these agonists. Arachidonate-induced platelet aggregation is wholly dependent on COX-1-mediated conversion to TXA_2, while collagen-induced aggregation is only partially dependent on platelet TXA_2 production and U46619 stimulates platelet aggregation via a non-COX pathway.

Celecoxib did not reduce platelet aggregation in response to the three agonists, and the results were not statistically different from placebo. Bleeding time results showed the same trends as platelet aggregation.

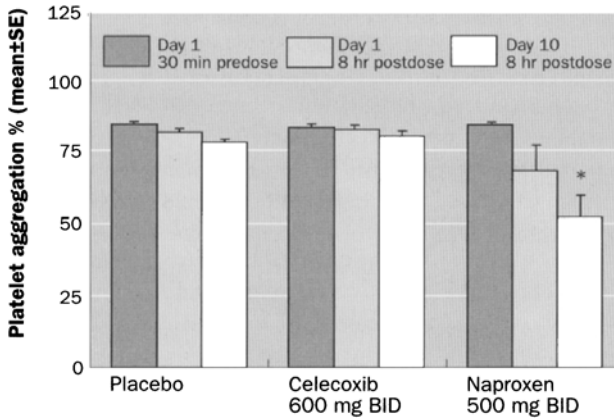

Fig. 1.4 Platelet aggregation responses to collagen before dosing and at 8 h postdose on days 1 and 10. Statistical comparisons are based on changes from baseline. Asterisk indicates $P<0.05$ for change from baseline versus celecoxib and placebo. None of the differences between celecoxib and placebo was statistically significant at any postdose time point. Source: Leese *et al.* (2000).

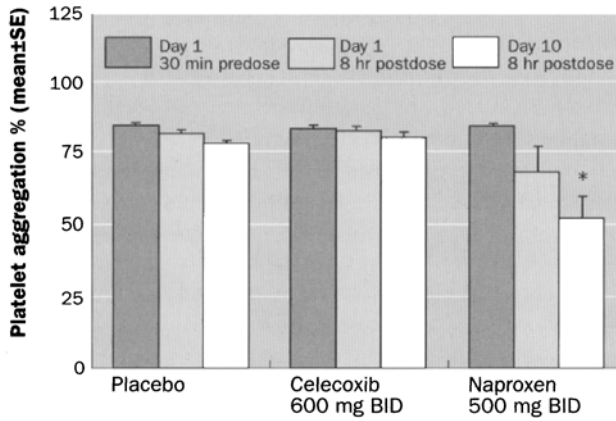

Fig. 1.5 Platelet aggregation responses to U46619 (a TXA_2 receptor agonist) before dosing and at 8 h postdose on days 1 and 10. Statistical comparisons are based on changes from baseline. None of the differences between any of the three groups was statistically significant at any postdose time point. Source: Leese *et al.* (2000).

TXB_2 is a stableTXA_2 metabolite, and marker for COX-1-mediated platelet PG synthesis. Naproxen produced a large fall in serum TXB_2 level, but there was only a small non-significant reduction with celecoxib.

Celecoxib, even at supratherapeutic doses, did not inhibit COX-1 and did not impair platelet aggregation and haemostatic mechanisms. COX-2 inhibitors should, therefore, have advantages for pain treatment over conventional NSAIDs in patients with platelet dysfunction and bleeding tendencies.

Thrombosis in patients with connective tissue diseases treated with specific cyclooxygenase 2 inhibitors. A report of four cases.

L J Crofford, J C Oates, W J McCune, *et al. Arthritis Rheum* 2000; **43**(8):1891–6.

BACKGROUND. Specific COX-2 inhibitors, unlike non-selective NSAIDs, reduce systemic production of prostacyclin, but do not inhibit platelet activation; therefore, there are theoretical concerns that COX-2 inhibitors could shift the haemostatic balance towards a prothrombotic state. Four patients, with connective tissue diseases, developed ischaemic complications after receiving celecoxib.

INTERPRETATION. Patients with diseases that predispose to thrombosis should be monitored carefully if they are treated with COX-2 inhibitor drugs.

Table 1.5 Temporal relationship between initiation of COX-2 inhibition treatment and development of thrombotic symptoms

	Patient 1	Patient 2	Patient 3	Patient 4
Duration of treatment prior to symptoms	2 weeks (2 doses)	1 week	2 days (3 doses)	2–5 months*
Prescribed dosage	200 mg twice daily as needed	100 mg twice daily	200 mg once or twice daily	200 mg twice daily

*Symptoms attributed to vasculitis 2 months after initiation of treatment in this patient may, in retrospect, have been due to thrombosis.
Source: Crofford *et al.* (2000).

Comment

TX and PGs are regulators of platelet and endothelial cell function. TXA_2 from activated platelets is a platelet aggregant and vasoconstrictor, prostacyclin (PGI_2) from endothelial cells, inhibits platelet activation and induces vasodilation. Inhibition of COX-1 and COX-2 by non-selective NSAIDs, results in the reduction of both platelet TXA_2 and endothelial PGI_2. COX-1 is the only COX isoform found in platelets, therefore, COX-2 inhibitors have no effect on platelet TXA_2, but decrease PGI_2 and may shift the haemostatic balance towards a prothrombotic state. Four patients with connective tissue diseases, characterized by Raynaud's phenomenon and anticardiolipin antibodies (ACA), lupus anticoagulant (LAC) or antiphospholipid syndrome (APS), developed ischaemic complications after treatment with celecoxib.

The mechanism for thrombosis in APS is complex, but patients with anti-phospholipid antibodies (aPL) have elevated TXA_2, suggesting that platelet activation may be important in thrombosis. A previous study demonstrated elevated urinary TXA_2 metabolites with aPL, and showed a correlation with the future development of vascular complications (myocardial infarction, stroke or

deep venous thrombosis). Urinary TXA_2 metabolites were measured in two of these four patients and were also elevated.

This is the first report of thrombosis in association with a specific COX-2 inhibitor and further studies are needed to assess their thrombotic risk. These patients were already predisposed to thromboses, and the risk may have been increased by treatment with a COX-2 inhibitor.

Effect of cyclooxygenase-2 inhibition on renal function in elderly persons receiving a low-salt diet. A randomized, controlled trial.

S K Swan, D W Rudy, K C Lasseter, *et al. Ann Intern Med* 2000; **133**:1–9.

BACKGROUND. NSAIDs inhibit both COX-1, whose inhibition is associated with GI ulceration, and COX-2, whose inhibition is associated with therapeutic benefits. Although agents that do not produce COX-1 activity may have fewer adverse effects, targeted disruption of the COX-2 allele in mice has resulted in severe renal problems, suggesting that COX-2 inhibition may also produce adverse effects. This study determines the effect of rofecoxib (a specific COX-2 inhibitor) on renal function in elderly patients.

INTERPRETATION. The effects of COX-2 inhibition on renal function are similar to those observed with non-selective NSAIDs. Thus, COX-2 seems to play an important part in human renal function.

Comment

COX-2 is constitutively expressed in the kidney and appears to be important for renal haemodynamics and electrolyte homeostasis. This study had two parts: a single-dose study to observe maximum effects on the kidney (single doses of NSAIDs have greater effects on renal function than long-term administration), and a multiple-dose study to reflect clinical practice. All patients received a low sodium diet throughout the study period, which increases renal dependence on PG production.

Single-dose study: 15 elderly patients received single doses of rofecoxib 250 mg (five to 20 times the usual dose), indomethacin 75 mg or placebo in randomized three-period crossover study.

Multiple-dose study: 60 elderly patients were randomized to receive either rofecoxib 12.5 or 25 mg daily, indomethacin 50 mg three times daily or placebo for 6 days.

Single-dose study: compared with placebo, rofecoxib and indomethacin both produced significant reductions in GFR of 0.23 and 0.18 ml/s. Differences between the two drugs were not significant (Table 1.6).

Both rofecoxib and indomethacin reduced urinary sodium excretion significantly, compared with placebo (68.35% and 48.95%, $P<0.001$ and $P=0$.

Table 1.6 Short-term effects of single doses of rofecoxib, indomethacin and placebo on GFR*

Treatment	Patients	Predose Inulin Clearance†	Postdose Minimum Inulin Clearance†	Peak Reduction in Glomerular Filtration Rate†	Difference in Peak Reduction (95% CI)	*P* value
	n	← mL/s →				
Rofecoxib, 250 mg	15	1.59 ± 0.05	0.90 ± 0.04	0.61 ± 0.04‡		
Indomethacin, 75 mg	15	1.46 ± 0.05	0.96 ± 0.04	0.56 ± 0.04‡		
Placebo	15	1.49 ± 0.05	1.14 ± 0.04	0.38 ± 0.04‡		
Rofecoxib vs. indomethacin					0.05 (−0.04 to 0.15)	> 0.2
Rofecoxib vs. placebo					0.23 (0.14 to 0.33)	< 0.001
Indomethacin vs. placebo					0.18 (0.09 to 0.28)	0.003

*Values expressed with the plus/minus sign are the mean ±SE.
†Least-squares mean calculated by using analysis of variance.
‡Within-treatment comparison, $P \leq 0.05$.
Source: Swan *et al.* (2000).

Table 1.7 Effect of rofecoxib or indomethacin therapy on GFR, as determined by iothalamate clearance*

Treatment	Patients	Minimum Iothalamate Clearance on Day −1†	Minimum Iothalamate Clearance on Day 6†	Reduction in Iothalamate Clearance‡	Difference in Reduction (90% CI)	*P* value
	n	←		*mL/s*	→	
Placebo	15	1.28 ± 0.07	1.29 ± 0.07	0.03 ± 0.04		
Rofecoxib, 12.5 mg	15	1.37 ± 0.07	1.20 ± 0.07	0.17 ± 0.04		
Rofecoxib, 25 mg	15	1.24 ± 0.06	1.12 ± 0.06	0.16 ± 0.04		
Indomethacin, 50 mg three times daily	15	1.23 ± 0.06	1.14 ± 0.06	0.13 ± 0.04		
Rofecoxib, 12.5 mg, vs. placebo					0.14 (0.05 to 0.25)	0.019
Rofecoxib, 25 mg, vs. placebo					0.13 (0.03 to 0.23)	0.029
Indomethacin vs. placebo					0.10 (0.00 to 0.21)	0.086
Rofecoxib, 12.5 mg, vs. indomethacin					0.04 (−0.06 to 0.14)	> 0.2
Rofecoxib, 25 mg, vs. indomethacin					0.03 (−0.07 to 0.13)	> 0.2
Rofecoxib, 25 mg, vs. rofecoxib, 12.5 mg					−0.01 (−0.11 to 0.09)	> 0.2

*Values expressed with the plus/minus sign are the mean ±SE.
†Observed values,
‡Values (least-squares means) calculated by using an analysis of covariance model that adjusted for treatment, site, and baseline glomerular filtration rate. Because values are adjusted, they differ slightly from the differences between the unadjusted observed values.
Source: Swan *et al.* (2000).

005), while only rofecoxib reduced urinary potassium excretion significantly (11.28%, P=0.012). The drugs also resulted in a significant increase in serum potassium concentration, by 2.3% for rofecoxib and 4.19% for indomethacin (P=0.04 and 0.001) and a reduction in serum sodium, although this was only significant with indomethacin.

Multiple-dose study: there were more modest reductions in GFR seen with 12.5 or 25 mg rofecoxib daily or indomethacin 50 mg three times daily compared with placebo (0.14, 0.13 and 0.10 ml/s), and again there were no significant differences between the two doses of rofecoxib or indomethacin (Table 1.7).

There was no consistent reduction in either urinary sodium or potassium excretion with rofecoxib or indomethacin and only indomethacin caused a significant decrease in serum sodium (3.75%, P=0.003) and increase in serum potassium (6.16%, P=0.045).

Other studies have shown similar effects on renal function with celecoxib and, therefore, these appear to be class effects. This was a very short study and all results returned to normal within 2 weeks. It can be inferred that similar effects would be seen in the longer term.

As with non-selective NSAIDs, patients with low effective circulating fluid volume (e.g. cardiac failure, diuretic users or cirrhosis) are also at risk of reduced GFR and renal impairment with COX-2-specific inhibitors.

Conclusion

The advent of the COX-2-specific NSAIDs is clearly a major development in rheumatology, not least because of the very large numbers of patients who take NSAIDs and the substantial morbidity and even mortality associated with the use of non-selective agents. Trials certainly reassure us that the likelihood of patients suffering gastric ulceration leading to perforation or serious haemorrhage is very low in patients treated with COX-2-specific compounds—perhaps similar to the background risk in these patient populations. This means that it is difficult to justify prescription of a non-selective drug in patients who have adverse risk factors for peptic ulceration—but this is in fact a sizeable proportion of the whole population requiring NSAIDs, particularly if factors such as age are given due weight. This conclusion has economic implications for health-care providers, but in the past substantial sums were being spent on various forms of gastric protection, not all of them effective.

Nevertheless, COX-2-selective drugs do still have some of the disadvantages of their non-selective cousins, particularly their effects on the kidney. The incidence of GI symptoms (even though these are not due to peptic ulceration) is also significant. Clinicians are used to having to try a whole range of conventional NSAIDs before finding the drug that suits a particular patient in terms of efficacy and lack of side-effects. A similar process may well be required for COX-2-specific drugs, but currently there are only two to choose from—but several in the pipeline. It seems safe to predict that in 10 years' time use of

conventional non-selective NSAIDs will have decreased substantially and been replaced by a range of COX-2-specific drugs of varying potency and with idiosyncratic side-effects. The only threat to this prediction would be the emergence of some major as yet unanticipated side-effect of the COX-2-specific drugs.

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1. American College of Rheumatology 20% responder index. Felson DT, Anderson JJ, Boers M, *et al.* American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* 1995; **38**:727–35.

2
Inhibition of tumour necrosis factor-α

Overview

Tumour necrosis factor-α (TNF-α or TNF) is a member of a growing family of cytokines, with a characteristic structural homology, that includes Fas ligand, CD40 ligand, TNF-related, apoptosis inducing, ligand (TRAIL), osteoprotegrin ligand and several others less well characterized (the TNF ligand superfamily). Originally identified as a factor produced in response to preparations of bacteria or bacterial lipopolysaccharide that caused haemorrhagic necrosis of tumours, it has subsequently been shown to have a variety of pro-inflammatory effects, particularly activating the innate immune system. It induces fibroblast proliferation and up-regulates matrix metalloproteinases (MMPs). It also induces vascular endothelial cells to express appropriate adhesion molecules, facilitating cellular migration to sites of inflammation, such as the joint. The effects of TNF on cellular migration and activation of the immune system are aided by its induction of other pro-inflammatory mediators, including interleukin (IL)-1, nitric oxide and prostaglandins (PG). TNF itself is produced by a large variety of immune cells, although it is the production, by macrophages, that appears to be the dominant source in rheumatoid arthritis (RA). The involvement of TNF in arthritis was initially suggested by finding upregulated expression of the cytokine, and its receptor (TNFR), in the joints of rheumatoid patients. The pivotal role of TNF in inflammatory arthritis has also been confirmed in a number of experimental animal models. This increasing evidence supporting a central role for TNF in the pathogenesis of RA has led to interest in targeting this molecule with specific inhibitors. A study of one of these inhibitors (etanercept—a soluble TNF receptor) was published last year. This year also saw the publication of a large, multicentre randomized control trial of another of these inhibitors (infliximab—an anti-TNF antibody) (the ATTRACT study).

The place of anti-TNF therapies has been discussed in detail by John Klippel in a useful editorial in the *New England Journal of Medicine* |**1**|.

Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial (ATTRACT study).

R Maini, E W St Clair, F Breedveld, *et al. Lancet* 1999; **354**:1932–9.

BACKGROUND. Two types of specific TNF inhibitor have been recently developed: a chimeric antibody (infliximab—the focus of this study) and a soluble receptor (etanercept). Infliximab has the critical binding region of a mouse antibody bound to the body of a human antibody (to reduce potential immunogenicity). Previous studies have established the efficacy of this antibody in RA. This large, placebo controlled, double-blind randomized trial was undertaken in patients who had an inadequate response to methotrexate; this was done in order to determine whether infliximab, added to methotrexate, was a safe and effective therapy.

INTERPRETATION. During 30 weeks, treatment with infliximab plus methotrexate was more efficacious than methotrexate alone in patients with active RA not previously responsive to methotrexate.

Comment

Four hundred and twenty-eight patients with active RA, defined as six or more swollen and tender joints plus two of: (a) morning stiffness greater than or equal to 45 minutes; (b) erythrocyte sedimentation rate (ESR) >28 mm/h; and (c) C-reactive protein (CRP) >2 mg/dl; who had received continuous methotrexate for at least 3 months (and on a stable dose for at least 4 weeks) were recruited. They were randomized to placebo (88 patients) or one of four dose regimens of infliximab (81–87 per group) in addition to their methotrexate, which, despite its lack of efficacy in this cohort of patients, was continued at the same dose. No significant differences were noted in the disease characteristics of the different groups at baseline. Patients randomized to active treatment received either 3 mg/kg or 10 mg/kg of infliximab at weeks 0, 2 and 6. They then continued on the same dose at either 4-weekly or 8-weekly intervals. Patients were assessed every 4 weeks and followed for 30 weeks. The primary end-point was the American College of Rheumatology criteria for 20% global improvement on a validated composite scale (ACR20) at week 30.

Significantly more of the infliximab-treated patients achieved an ACR20 (P <0.001) compared with placebo, irrespective of the dose and timing regimen (Fig. 2.1). The response was rapid with >90% of the eventual responders achieving the ACR20 response within 6 weeks of commencing therapy. Significantly more patients in all the treatment groups also achieved ACR50 and ACR70 responses (50% and 70% improvement in the composite scores).

In addition to the clinical improvements, laboratory measures also improved. A significant reduction in rheumatoid factor and CRP was also seen in all treated groups.

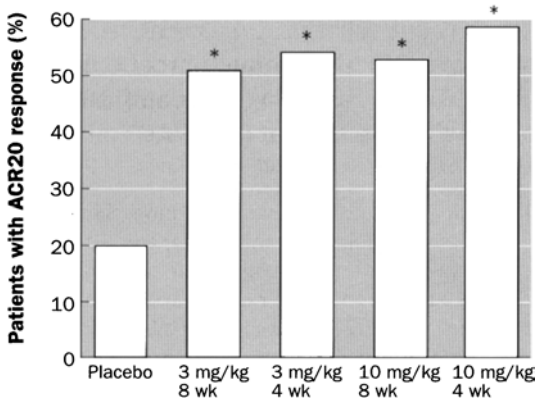

Fig. 2.1 Proportion of patients with ACR 20% response at week 30 by treatment group. Number of patients and those responding in each group are also shown.

*P<0.001 versus placebo. Source: Maini *et al.* (1999).

All the dose regimens of infliximab were well tolerated, although mild infusion reactions (most commonly headache and nausea) were noted, particularly within 1 h of the first infusion. Thirty-five (36%) of the placebo group discontinued treatment (22 due to lack of efficacy) compared with eight to 16 (9–18%) in the infliximab groups (5–11 due to lack of efficacy). Serious adverse events, and specifically serious infections, were no different in the treatment and placebo groups. The critical role of TNF in the immune response to intracellular pathogens would imply an increased risk of certain infections (e.g. tuberculosis and *Listeria*). Longer follow-up will be required, on many more patients, to see if this theoretical risk translates into a practical concern. It was noted that antibodies to double-stranded DNA were seen in 16% of the treatment group compared with 0% in the placebo arm. A further patient developed a drug-induced lupus syndrome with treatment (but did not develop double-stranded DNA antibodies) and was withdrawn from treatment. Antibodies to double-stranded DNA have been reported in previous anti-TNF studies, but whether this finding predicts the later development of a lupus syndrome is unknown and will again require further long-term monitoring.

Conclusion

This trial indicates that infliximab is both efficacious and relatively safe in RA but long-term surveillance is still required. The results are similar to those seen with etanercept and confirm the pivotal role of TNF in rheumatoid disease.

Table 2.1 Individual clinical and laboratory assessments of disease activity at baseline (week 0) and 30 weeks* and per cent change from baseline†

	Placebo		Infliximab								% change from baseline at 30 weeks†				
			3 mg/kg every 8 wk		3 mg/kg every 4 wk		10 mg/kg every 8 wk		10 mg/kg every 4 wk		Placebo	3 mg/kg every 8 wk	10 mg/kg every 4 wk	10 mg/kg every 8 wk	10 mg/kg every 4 wk
Week	0	30	0	30	0	30	0	30	0	30					
Swollen joint count (0–66)	19	13	19	9	20	9	20	7	23	6	–20	–52	–50	–60	–64
IQR	13, 28	8, 26	13, 30	4, 18	12, 29	4, 15	13, 28	4, 15	15, 31	3, 12					
p value	–		<0.001		<0.001		<0.001		<0.001			<0.001	<0.001	<0.001	<0.001
Tender joint count range (0–68)	24	16	32	12	31	11	30	12	35	9	–26	–59	–65	–58	–65
IQR	16, 48	7, 33	16, 46	3, 21	20, 39	4, 25	20, 44	4, 22	22, 44	2, 25					
p value	–		0.032		0.031		0.012		0.002			0.006	0.003	<0.001	<0.001
Pain score range (VAS 0–10 cm)§	6.7	5.9	7.0	3.8	6.9	3.5	6.7	3.1	6.6	3.7	–6	–33	–43	–50	–35
IQR	5.0, 8.0	3.3, 7.4	5.6, 8.1	2.3, 6.9	4.8, 7.8	1.2, 6.2	5.8, 7.8	1.2, 5.7	5.0, 7.7	1.1, 5.8					
p value	–		0.075		<0.001		<0.001		<0.001			0.026	<0.001	<0.001	<0.001
Evaluation's global score range (VAS 0–10 cm)§	6.5	5.0	6.1	2.6	6.2	2.6	6.4	2.6	6.0	2.5	–13	–53	–59	–58	–59
IQR	5.2, 7.4	3.0, 7.0	4.8, 7.1	1.5, 5.2	4.6, 7.3	0.9, 4.6	5.0, 7.1	1.3, 4.3	4.9, 7.0	1.2, 4.2					
p value	–		<0.001		<0.001		<0.001		<0.001			<0.001	<0.001	<0.001	<0.001
Patient's global score range (VAS 0–19 cm)§	6.2	5.5	6.6	3.6	5.7	3.0	6.4	3.7	6.0	3.3	–7	–23	–30	–40	–47
IQR	4.3, 8.1	3.1, 7.5	4.9, 7.8	1.8, 6.7	4.3, 8.0	1.5, 6.0	5.0, 7.7	1.3, 6.5	4.8, 7.7	1.4, 5.9					
p value	–		0.041		0.001		<0.001		<0.001			0.008	<0.002	<0.001	<0.001
Health assessment questionnaire (0–3)	1.8	1.5	1.8	1.5	1.8	1.1	1.8	1.4	1.5	1.3	–3	–13	–29	–27	–24
IQR	1.3, 2.1	1.0, 2.0	1.4, 2.3	0.9, 2.1	1.3, 2.1	0.6, 1.9	1.3, 2.1	0.5, 1.8	1.3, 2.1	0.5, 1.8					
p value	–		0.766		0.012		0.002		0.002			0.167	<0.001	<0.001	<0.001
C-reactive protein concentration (mg/dL)	3.0	2.3	3.1	0.8	2.0	0.5	2.5	0.6	2.4	0.5	–9	–60	–61	–68	–76
IQR	1.2, 5.7	0.7, 5.1	1.3, 5.3	0.4, 2.3	0.8, 4.4	0.3, 1.4	1.0, 4.0	0.3, 1.3	1.3, 5.3	0.3, 1.1					
p value	–		<0.001		<0.001		<0.001		<0.001			<0.001	<0.001	<0.001	<0.001
Rheumatoid factor concentration (IU/mL)	188	172	143	98	231	117	178	75	177	111	0	–37	–32	–46	–31
IQR	34, 489	45, 462	44, 318	23, 192	65, 451	31, 312	49, 429	27, 209	60, 413	34, 294					
p value	"	"	"	"	"	"	"	"	"	"	"	<0.001	<0.001	<0.001	<0.001

*Median values;†the per cent reduction achieved at 30 weeks relative to baseline is calculated for every patient. Percentage change from basline shown in the table is the median of these values each treatment group. †p vs placebo. X_2 test: SVAS=visual analogue scale;IIO=no discomfort or disability, 3=maximum disability. IQR=interquartile range.
Source: Maini *et al.* (1999).

Infliximab and methotrexate in the treatment of rheumatoid arthritis.

P E Lipsky, D M van der Heijde, E W St Clair, *et al. N Engl J Med* 2000; **343**(22):1594–602.

BACKGROUND. This paper reports the 54-week follow-up data on the patients in the ATTRACT trial in which efficacy at 30 weeks was previously reported (Maini et a/., see above). Although an improvement in symptoms was clearly shown in that trial, the capacity of anti-TNF therapies to effect a more sustained benefit and prevent continuing joint damage were not known. Treatment was as before: placebo or infliximab, a chimeric monoclonal antibody against TNF-α, in intravenous doses of 3 or 10 mg/kg body weight every 4 or 8 weeks in combination with oral methotrexate for 54 weeks. Clinical responses were assessed with use of the criteria of the ACR, quality of life with a health status questionnaire, and the effect on joint damage radiographically.

The combination of infliximab and methotrexate was well tolerated and resulted in a sustained reduction in the symptoms and signs of RA that was significantly greater than the reduction associated with methotrexate therapy alone (clinical response, 51.8% vs. 17.0%; *P*<0.001). The quality of life was also significantly better with infliximab plus methotrexate than with methotrexate alone. Radiographic evidence of joint damage increased in the group given methotrexate, but not in the groups given infliximab and methotrexate (mean change in radiographic score, 7.0 versus 0.6; *P*<0.001). Radiographic evidence of progression of joint damage was absent in infliximab-treated patients whether or not they had a clinical response.

INTERPRETATION. In patients with persistently active RA despite methotrexate therapy, repeated doses of infliximab in combination with methotrexate provided clinical benefit and halted the progression of joint damage.

Comment

This is a very important further report on the ATTRACT study, principally because of the marked ability of the anti-TNF antibody to halt the progression of erosive disease. These data have now been extended by data on patients followed up for 102 weeks reported to the ACR meeting in Philadelphia; continued protection from joint erosion was evident. From a practical point of view the data produce difficulties for health-care providers trying to allocate relatively expensive anti-TNF therapies appropriately. Usually, patients who fail to make an adequate symptomatic response (ACR20) after 3 months treatment would be excluded from receiving further therapy. However, if the results of this trial are confirmed, this policy could deprive them of significant benefit as radiographic scores did not progress even in those patients who failed to make a symptomatic response.

Conclusion

Anti-TNF therapies produce both symptomatic improvements and decreased joint destruction, a result that could not necessarily have been inferred from animal models where, in some circumstances, inflammatory changes and bony erosion can be dissociated in terms of their response to anti-TNF therapies.

A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis.
J M Bathon, R W Martin, R M Fleischmann, *et al. N Engl J Med* 2000; **343**(22):1586–93.

BACKGROUND. Etanercept, which blocks the action of TNF by virtue of its action as a soluble p75 TNFR, reduces disease activity in patients with long-standing RA. However, its efficacy in reducing disease activity and preventing joint damage in patients with active early RA is unknown. In this trial 632 patients with early RA were treated with either twice-weekly subcutaneous etanercept (10 or 25 mg) or weekly oral methotrexate (mean, 19 mg per week) for 12 months. Clinical response was defined as the per cent improvement in disease activity according to the criteria of the ACR. Bone erosion and joint-space narrowing were measured radiographically and scored with use of the Sharp scale. On this scale, an increase of 1 point represents one new erosion or minimal narrowing. As compared with patients who received methotrexate, patients who received the 25 mg dose of etanercept had a more rapid rate of improvement, with significantly more patients having 20%, 50% and 70% improvement in disease activity during the first 6 months ($P<0.05$). The mean increase in the erosion score during the first 6 months was 0.30 in the group assigned to receive 25 mg of etanercept and 0.68 in the methotrexate group ($P=0.001$), and the respective increases during the first 12 months were 0.47 and 1.03 ($P=0.002$). Among patients who received the 25 mg dose of etanercept, 72% had no increase in the erosion score, as compared with 60% of patients in the methotrexate group ($P=0.007$). This group of patients also had fewer adverse events ($P=0.02$) and fewer infections ($P=0.006$) than the group that was treated with methotrexate.

INTERPRETATION. As compared with oral methotrexate, which has been regarded as the drug of choice for early RA, etanercept acted more rapidly to decrease symptoms and slowed joint damage in patients.

Comment

This trial, along with the second report from the ATTRACT trial (Lipsky *et al.,* see above), confirms the efficacy of anti-TNF therapies in both symptom control and the decreasing the rate of joint destruction by erosions. In this trial, patients had relatively early disease (<3 years by protocol, and a mean of 11–12 months in the treated groups). However ~40% had already been treated with one disease modifying drug, and 87% already had erosions. The principal comparison in this

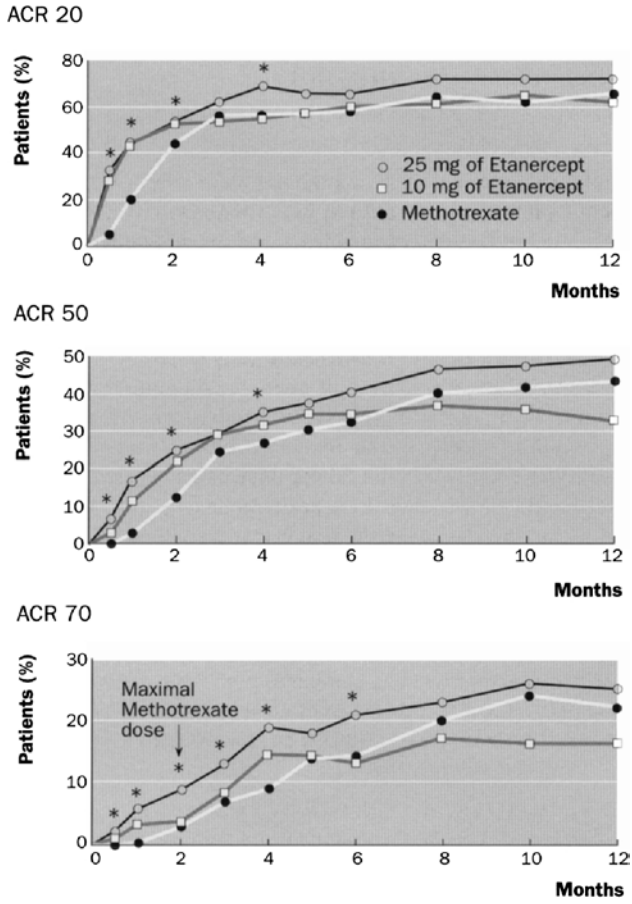

Fig. 2.2 Percentages of patients with RA who had an improvement, according to the criteria of the ACR, of 20% (ACR 20), 50% (ACR 50) and 70% (ACR 70) during treatment with 25 mg of etanercept, 10 mg of etanercept, or methotrexate. Asterisks indicate significant differences ($P<0.05$) between the methotrexate group and the group assigned to receive 25 mg of etanercept. Source: Bathon *et al.* (2000).

trial was between etanercept (two doses) and methotrexate, whereas the ATTRACT trial compared methotrexate with infliximab *plus* methotrexate. Concomitant methotrexate with infliximab is warranted because of the immunogenicity of infliximab (which incorporates part of a mouse IgG molecule); methotrexate appears to decrease the formation of antibodies to the chimeric anti-TNF antibody. Etanercept, being wholly human (p75—human Fc) does not require this co-treatment. This might explain why some degree of progression of erosion was still measurable in the etanercept group, whereas erosion scores in the ATTRACT study barely progressed over 54 and 102 weeks in the infliximab plus methotrexate group.

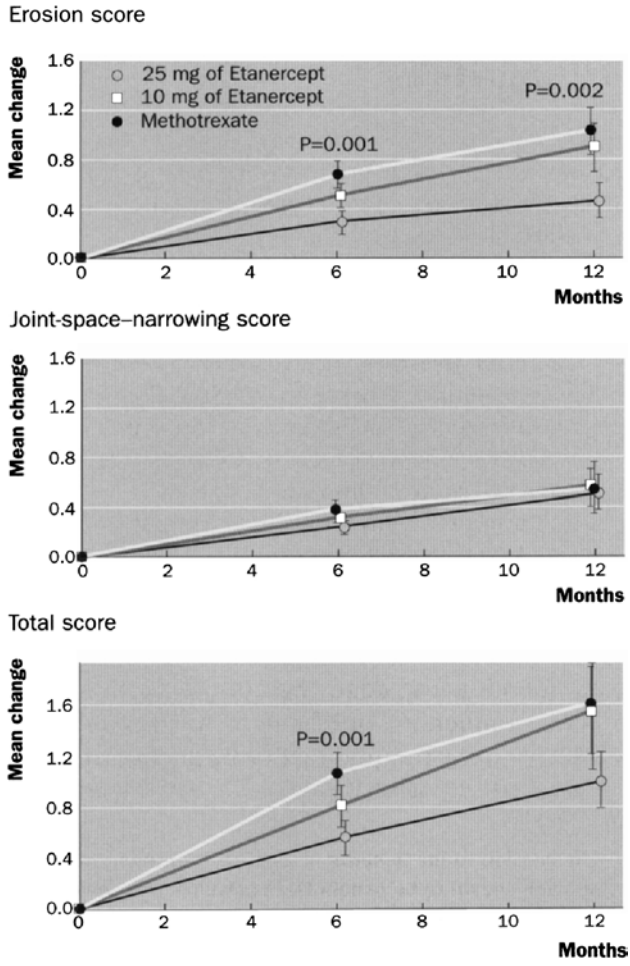

Fig. 2.3 Mean (±SE) changes from baseline in erosion scores, joint-space-narrowing scores, and total scores on the sharp scale at 6 and 12 months in patients with RA who received 25 mg of etanercept, 10 mg of etanercept, or methotrexate. *P* values indicate significant differences between the methotrexate group and the group assigned to receive 25 mg of etanercept. Source: Bathon *et al.* (2000).

Again, the effect on erosions may be a crucial point in deciding the place of anti-TNF therapies in the treatment of patients with relatively early disease. As far as symptom control is concerned, as judged by ACR criteria, methotrexate and etanercept were remarkably similar especially at 1 year, with ~20% of patients treated with either drug meeting the ACR 70 criteria. Thus treatment with etanercept could not be justified (on expense grounds) without the ‘added

benefit' of an effect on joint erosion, which predicts disability. This added benefit requires additional study.

Conclusion

Like infliximab, etanercept arrests joint erosion as well as improving inflammation and in the long term this may come to be seen as the major benefit conferred by anti-TNF therapies in the 'average' RA patient, as compared with those who have failed, or are intolerant of, treatment with conventional drugs.

Anti-tumour necrosis factor treatment in spondyloarthropathies

Overview

TNF is a pro-inflammatory cytokine produced predominantly by monocytes and macrophages. It has a broad range of pro-inflammatory actions, including lymphocyte activation, modulation of other cytokines, promotion of angiogenesis and the induction of MMPs. Over the past few years the importance of TNF in the aetiology of rheumatoid disease has been shown, both in basic science studies, and more recently, as illustrated in the ATTRACT study discussed in this chapter, in the proven efficacy of anti-TNF therapies. As a result of the clinical success of these agents, interest in their potential role in other rheumatic diseases has been generated. Ankylosing spondylitis (AS), a systemic inflammatory disease involving mainly spinal and sacroiliac joints, has been the focus of several recent studies of anti-TNF treatment. The progressive nature of this condition, coupled with a limited repertoire of pharmacological agents of proven benefit, has prompted these studies. Two recent studies have used the chimeric human/murine monoclonal antibody, infliximab, which binds to and neutralizes TNF.

Effects of a loading dose regimen of three infusions of chimeric monoclonal antibody to tumour necrosis factor α (infliximab) in spondyloarthropathy: an open pilot study.
F Van den Bosch, E Kruithof, D Baeten, *et al. Ann Rheum Dis* 2000; **59**(6):428–33

BACKGROUND. In addition to the benefits of anti-TNF treatment in rheumatoid disease, it has also been shown to be beneficial in patients with inflammatory bowel disease. Several previous studies have shown an association between subclinical intestinal inflammation and activity of the spondyloarthropathies (SPAs), particularly the peripheral joint manifestations. This, in conjunction with the finding of high levels of TNF expression in biopsies of affected sacroiliac joints led this Belgian group to

undertake a small pilot study examining both the efficacy and safety of anti-TNF treatment in a cohort of patients with SPA.

INTERPRETATION. In this small pilot study treatment with a chimeric monoclonal antibody to TNF (infliximab) resulted in rapid and significant improvement in both axial and peripheral manifestations of SPA.

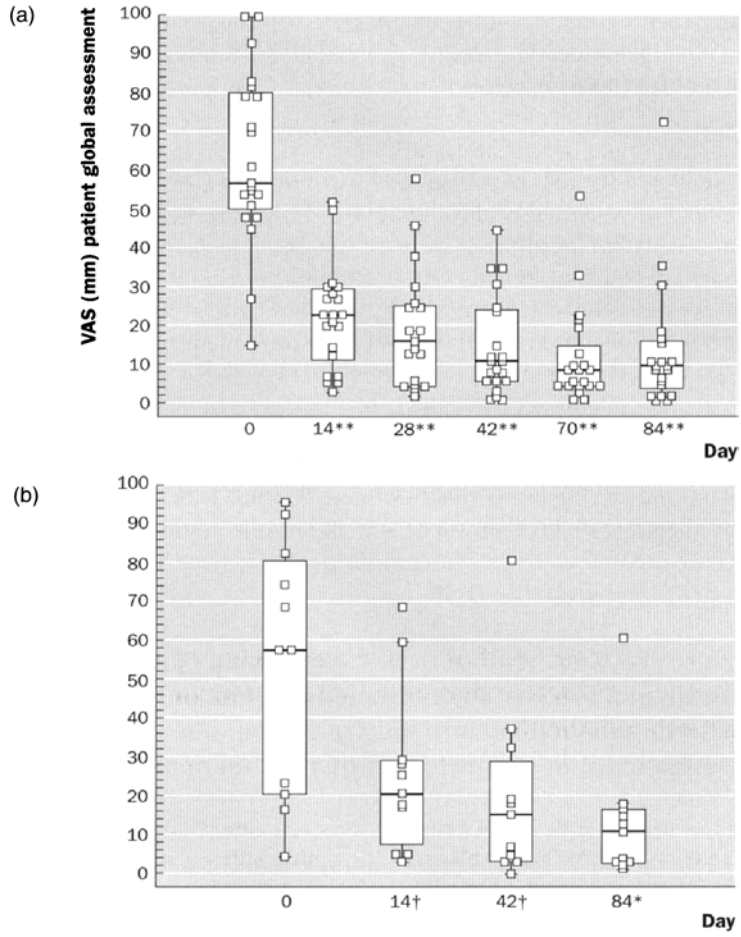

Fig. 2.4 Evolution of patient assessments over time. The box and whisker plots show the median value (horizontal line) and range (first to third quartiles in boxes, 98% of values between closed bars, individual values indicated by single dot) of the chosen parameter (*y* axis) over time (days). Significance *(P)* was calculated by Wilcoxon signed ranks test. †$P \leq 0.05$; *$P \leq 0.01$; **$P \leq 0.001$. (a) Patient global assessment (100 mm visual analogue scale [VAS]). (b) Spinal pain assessment (100 mm VAS).
Source: Van den Bosch *et al.* (2000).

Comment

The study was a small, open label, single centre, 12-week study of 21 patients who fulfilled the SPA diagnostic criteria of the European Spondyloarthropathy Study Group. The antibody was administered using the same protocol as in rheumatoid disease (three intravenous infusions, 5 mg/kg, at weeks 0, 2 and 6). As the antibodyis chimeric, it is possible that an immune response to the antibody may be generated, potentially resulting in decreased efficacy with time and an increased risk of hypersensitivity reactions to the infusion. For this reason, concomitant methotrexate treatment has been used routinely in rheumatoid studies but was not given in this study. For analysis, patients were divided into those with axial disease and those with peripheral arthritis and assessed using various validated clinical and laboratory measures. In all patients a significant reduction in all global assessments of disease activity was seen. Unlike other slow acting agents, that have been evaluated previously, this preliminary study suggested that axial as well as peripheral disease could be improved (Fig. 2.4). Benefits were also seen in the skin disease of patients with psoriatic arthropathy. In addition to the improvements in clinical variables reductions in the levels of CRP and the ESR were also seen. This improvement was extremely rapid (by day 3) and was maintained throughout the study. Whether the benefits of treatment were mediated in the joints or whether joint improvement was secondary to changes in the gut mucosa remains unresolved. As this was a small study no significant data are presented on the potential risks of treatment. Previous studies in rheumatoid disease have been associated with autoimmune phenomena (anti-DNA antibodies) and increased upper respiratory tract infections and subsequent larger studies will undoubtedly address these considerations.

Conclusion

This study, albeit small and unblinded, highlights the potential benefits of anti-TNF treatment in the SPAs. It certainly suggests the need for a larger randomized double-blind study of this treatment.

Successful treatment of active ankylosing spondylitis with the anti-tumour necrosis factor α monoclonal antibody infliximab.
J Brandt, H Haibel, D Cornely, *et al. Arthritis Rheum* 2000; **43** (6): 1346–52.

BACKGROUND. Twenty to 60% of SPA patients have gastrointestinal mucosal lesions similar to those seen in Crohn's disease. The clinical efficacy of anti-TNF treatment in this condition in conjunction with the finding of both messenger RNA for TNF and the protein itself in biopsies of the sacroiliac joints of SPA patients led this German group to undertake this small scale open pilot study of patients with AS. Unlike the parallel Belgian study this

study focused on patients with AS (as defined by the 1984 New York diagnostic criteria for AS). In addition to clinical measures and measures of CRP/ESR, assessments of spondylitis by magnetic resonance imaging (MRI) and levels of IL-6 were also undertaken.

INTERPRETATION. This short-term, small-scale study, reinforces the potential efficacy of anti-TNF treatment in AS. Improvements were seen not only in standard clinical and laboratory variables, but also in the levels of IL-6 and in the MRI measurements of disease activity.

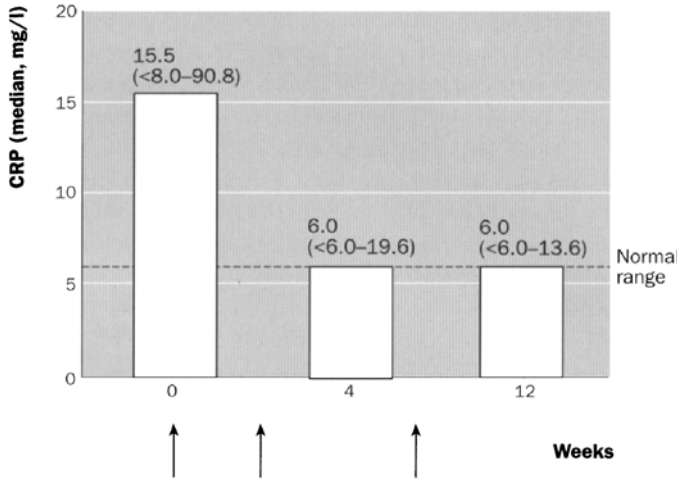

Fig. 2.5 Levels of CRP before, during and after treatment with 5 mg/kg infliximab. Arrows indicate the dates of infusion. Values are the median; ranges are shown in parentheses. Source: Brandt *et al.* (2000).

Comment

This study was an open label, single centre 12-week study of 11 patients (10 male, one female—withdrawn after first infusion) with chronic AS (mean disease duration 5 years; range 0.5–13 years). Disease-modifying antirheumatic drugs were withdrawn 4 weeks prior to treatment. The antibody was administered by three intravenous infusions (5 mg/kg at weeks 0, 2 and 6). Unlike in the RA trials, methotrexate was not given concomitantly. Patients were reviewed at fortnightly intervals and showed a significant improvement in disease activity, function and pain scores within the first 2 weeks, an effect that persisted during the course of the study. Positive benefit was seen in both axial and peripheral disease as was seen in the Belgian study of anti-TNF treatment in SPA. This improvement was reflected in decreased NSAID use by the patients. CRP measurements were similarly improved (Fig. 2.5). IL-6 has been reported to be a useful measure of disease activity in AS, and IL-6 measurements were reduced during the course of the study (median 12.4 ng/l [range 0–28.9] pre-

treatment and <5 ng/l in all patients at weeks 4 and 12). The effects on IL-6 and CRP are unlikely to be due to any placebo effect. MRI scans were undertaken in three patients before and 2–6 weeks after the third infusion. All had active spondylitis, as detected by dynamic spinal MRI pre-treatment and two of the three showed less or no spinal inflammation after therapy. The presence of chronic changes in the sacroiliac joints of all patients precluded assessment of this region. It appeared that there was a slight increase in infections, as has been seen in the rheumatoid studies; however, this study was too small to allow more definite conclusions to be drawn. Nonetheless, one patient withdrew due to urticarial xanthoma and an episode of bacterial tonsillitis, while tonsillitis was reported in one other patient. Another patient experienced a short relapse of uveitis during the study. Other adverse events reported were sinusitis (one), otitis media (one) and herpes labialis (one).

Conclusion

This study adds further weight to the suggestion that anti-TNF treatment is beneficial in the SPAs. Any long-term benefits of the treatment await evaluation but the improvements in spinal MRI measurements of inflammation are encouraging. Like the Belgian study, this study certainly suggests that there is short-term benefit from anti-TNF therapy and highlights the need for a larger multicentre randomized double-blind study over a longer follow-up period.

Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial.
P J Mease, B S Goffe, J Metz, *et al. Lancet* 2000; **356**:385–90.
BACKGROUND. Etanercept, a TNF inhibitor consisting of the p75 TNF receptor fused to the Fc portion of immunoglobulin, has shown efficacy in the treatment of RA. Psoriatic arthritis and psoriasis are disease states in which TNF, a pro-inflammatory cytokine, is present in increased concentrations in joints and in the skin. Therefore, psoriatic arthritis and psoriasis may be appropriate therapeutic targets for etanercept. This randomized, double-blind, placebo-controlled, 12-week study assessed the efficacy and safety of etanercept (25 mg twice weekly subcutaneous injections) or placebo in 60 patients with active psoriatic arthritis and psoriasis. Patients had disease for a mean of 10 years and had a median of 14 and 20 swollen and tender joints, respectively. Psoriatic arthritis end-points included the proportion of patients who met the Psoriatic Arthritis Response Criteria and who met the ACR preliminary criteria for improvement (ACR20). The Psoriatic Arthritis Response Criteria has some similarity to an ACR20; it requires improvement in two of four measurements and no deterioration in the others. The criteria are patient and physician global assessment (five-point scale—improvement defined as 1 point), and painful and swollen joint scores (30% decrease required to define improvement). Psoriasis end-points included improvement in the

Psoriasis Area And Severity Index (PASI) and improvement in prospectively identified individual target lesions. In this 12-week study, 26 (87%) of etanercept-treated patients met the Psoriatic Arthritis Response Criteria, compared with seven (23%) of placebo-controlled patients. The ARC20 was achieved by 22 (73%) of etanercept-treated patients compared with four (13%) of placebo-treated patients. Of the 19 patients in each treatment group who could be assessed for psoriasis (> or=3% body surface area), five (26%) of etanercept-treated patients achieved a 75% improvement in the PASI, compared with none of the placebo-treated patients (*P*=0.015). The median PASI improvement was 46% in etanercept-treated patients versus 9% in placebo-treated patients. Etanercept was well tolerated.

INTERPRETATION. Etanercept is safe and effective in psoriatic arthritis, and offers patients a new therapeutic option for the control of their disease.

Comment

Similar encouraging reports have appeared in abstract form on the treatment of psoriatic arthritis with infliximab. Since psoriatic arthritic may on occasion be difficult to treat and resistant to agents which are effective in RA, an additionaltherapeutic option is very welcome. The comparison with placebo, while necessary in assessing treatment, is not the most relevant one for every day practice, and comparison with methotrexate will be valuable. However it is worth noting that 50% of the patients in each group were already on methotrexate which was not discontinued; i.e. etanercept can be effect in patients with inadequate disease control by methotrexate. The improvement in skin score represents an added bonus for some of the patients (Fig. 2.6).

Overall comment on anti-tumour necrosis factor treatment in spondyloarthropathies

These studies demonstrate that anti-TNF treatment has a short-term benefit in the SPAs. Not only do they show improvements in laboratory measures but also in clinical measures of disease activity. It is interesting that both studies have demonstrated significant improvement in both axial and peripheral disease as axial disease has proven recalcitrant to treatment with other slow acting agents, a number of which may be capable of inhibiting TNF production. Undoubtedly, further large-scale studies will follow and it will be interesting to see whether this treatment is not only efficacious over the long term but whether it retards disease progression.

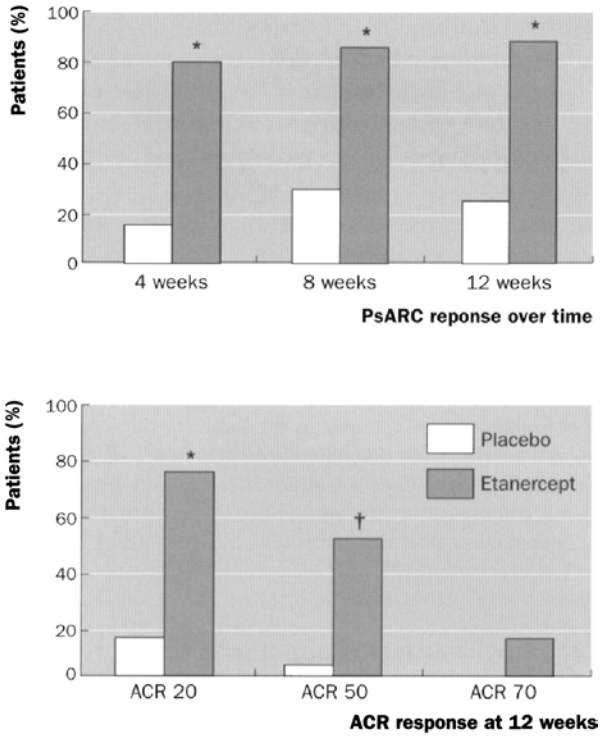

Fig. 2.6 Percentage of patients with Psoriatic Arthritis Response Criteria responses over time and with ARC 20, ARC 50 and ARC 70 responses at 12 weeks. Source: Mease *et al.* (2000).

Tumour necrosis factor—mechanisms of action

Overview

As TNF has been shown, in both animal models and recent clinical studies (such as the ATTRACT study discussed above), to be pivotally involved in the pathogenesis of inflammatory arthritis much recent interest has focused on the potential mechanisms of action of TNF. These studies, using both experimental models of inflammatory arthritis and clinical material, have explored the effects of TNF on synovial fibroblasts, on leucocyte chemotaxis, on other cytokine profiles, and on cartilage degradation. Studies have also highlighted a potentially detrimental effect of blocking TNF, inhibiting the apoptosis of synoviocytes, through reducing certain intracellular signalling cascades. These studies have further illuminated the role of TNF in the pathogenesis of RA.

A comparative study into the mechanisms of action of anti-tumour necrosis factor α, anti-CD4 and combined anti-tumour necrosis factor α/anti-CD4 treatment in early collagen-induced arthritis.

L Marinova-Mutafchieva, R O Williams, C Mauri, *et al. Arthritis Rheum* 2000; **43**(3):638–44.

BACKGROUND. Despite evidence for the involvement of CD4+ T cells in RA, therapies specifically targeted at these cells have provided only limited efficacy. The reasons for this are unclear. It may be related to the therapies being inadequate to inactivate pathogenic T cells. Alternatively, indiscriminate inhibition of CD4+ T cells may abrogate the effects of potentially regulatory CD4+ T cells. The limited benefits of anti-CD4 therapy are in marked contrast to the dramatic effects of anti-TNF treatment. In one experimental animal model of inflammatory arthritis (collagen induced arthritis) it has been shown that depleting anti-CD4 treatments effectively prevent induction of disease, but once disease is active they have little efficacy. By contrast, analogous to RA, anti-TNF treatment is effective in established disease. This study investigated the possible reasons for the differences in efficacy of the two treatments, elucidating some of the effects of anti-TNF treatment on cytokines, adhesion molecules and cellular infiltrates. It also investigated the potential benefit of combination therapy with a depleting anti-CD4 antibody and anti-TNF.

INTERPRETATION. The efficacy of the treatments tested was dependent on their ability to modulate the expression of TNF and IL-1, reduce adhesion molecules and cellularity, and inhibit cytokine activity of a T-helper (Th) 1 type.

Comment

Arthritis was induced in DBA/1 mice using a standard protocol of injection of native type II collagen in adjuvant. Upon development of arthritis, mice were treated with anti-CD4, anti-TNF or a combination of the two. The severity of arthritis was graded clinically and histologically. Anti-CD4 failed to reduce significantly either the clinical or histological scores, in contrast to anti-TNF treatment that significantly reduced both variables. Combination treatment was significantly better than either treatment in isolation. Interestingly, anti-CD4 treatment only reduced the number of CD4+ T cells by 41%, compared with a 59% reduction with anti-TNF and a 95% reduction with combination therapy. This is in keeping with the clinical studies, where it was demonstrated that anti-CD4 treatment preferentially eliminates CD45RA+ cells (naive T cells—not normally found within the synovium).

The differing efficacies of treatment were reflected in adhesion molecule expression, cytokine production and the ability of a treatment to inhibit Th1-like cytokine responses to the stimulating antigen.

The expression of two adhesion molecules (very late activation antigen 4 [VLA-4] and vascular cell adhesion molecule 1 [VCAM-1]) was assessed

immunohistochemically. These molecules were significantly upregulated in arthritic joints and this expression was unaffected by anti-CD4, whereas anti-TNF and combination treatment both significantly reduced the levels seen.

Similar results were seen with TNF and IL-1 expression, again assessed immunohistochemically. Cytokine expression was not significantly affected by anti-CD4 treatment, while anti-TNF reduced it and this effect was cumulative in the combination therapy.

The ability of lymph node cells from the draining inguinal nodes to produce interferon (IFN) -γ, when stimulated with type II collagen, was also assessed. As inflammatory arthritis is dominated by the effects of Th1 cytokines such as IFN-γ, high levels of this cytokine were produced, as expected in the arthritic mice. Again anti-CD4 treatment had no significant effect, while, somewhat surprisingly, anti-TNF produced a significant reduction, which was further decreased by combination treatment. The lack of efficacy of anti-CD4 treatment again correlates with the previous clinical data, in which Th1 T cells appeared to be resistant to treatment. The effectiveness of anti-TNF treatment on T-cell responses indicates the importance of this cytokine in T-cell responses to collagen in this particular model. How applicable this particular finding is to RA is questionable.

Conclusion

These findings suggest that anti-TNF treatment not only alters the cytokine profile but affects cellular infiltration, adhesion molecule expression and the T-cell cytokine balance. It also shows that the anti-CD4 treatment's lack of efficacy is in part attributable to a failure to eliminate CD4+ T cells from the joint and in part due to persistent IFN-γ production by activated T cells. Finally, combination treatment exerts synergistic effects in this particular model. Whether the immunosuppressive effects of such combinations would be acceptable clinically is unclear.

Reduction of chemokine levels and leucocyte traffic to joints by tumour necrosis factor a blockade in patients with rheumatoid arthritis.

P C Taylor, M Peters, E Paleolog, *et al. Arthritis Rheum* 2000; **43**:38–47.

BACKGROUND. Anti-TNF treatments have been shown to be efficacious in RA, improving both clinical and laboratory variables. Which of the many effects of anti-TNF are important in ameliorating disease is unknown. TNF blockade has been shown to interrupt the pro-inflammatory cytokine cascade in RA and this is one accepted mechanism of action of the anti-TNF treatments. However, studies such as those in the collagen-induced arthritis model discussed in this section, have additionally shown that TNF blockade results in a dose-dependent reduction in adhesion molecule expression,

within 1–3 days of initiating treatment, suggesting anti-TNF treatment may also inhibit inflammatory cell migration. In addition to requirements for vascular endothelial adhesion molecules, inflammatory cell migration is determined by gradients of particular chemotactic cytokines (chemokines). This study addressed the effects of anti-TNF treatments on both leucocyte traffic and chemokine gradients.

INTERPRETATION. TNF blockade inhibited granulocyte traffic to the joints and significantly reduced the synovial expression of the chemokines IL-8 and monocyte chemotactic protein 1 (MCP-1).

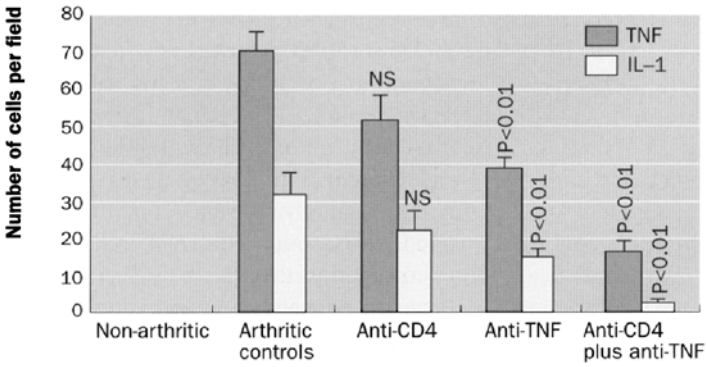

Fig. 2.7 Effect of therapy on the expression of TNF-α and IL-1β in arthritic joints (day 6 of arthritis). Mice were treated with anti-CD4, anti-TNF-α, or a combination of anti-CD4/ anti-TNF-α on days 1 and 4 of disease. Each bar represents the mean and SEM number of cells per high-power field in seven mice, each sampled at four levels throughout the joint, i.e. for each cytokine, 28 sections were assessed. NS=not significant. Source: Marinova-Mutafchieva *et al.* (2000).

Comment

Ten patients (seven male and three female) with RA (ARA 1987 revised criteria) were recruited to the study; all fulfilled the following clinical criteria: (a) at least six tender joints; (b) at least six swollen joints; (c) early morning stiffness >45 min; (d) at least one knee with active inflammation. All patients were treated with a single infusion of a monoclonal anti-TNF antibody. There was significant improvement in all clinical measures in all 10 patients following treatment (all achieved ACR20). Composite knee scores similarly showed significant improvement. An arthroscopy and biopsy of an affected knee was undertaken in each patient, prior to commencing treatment, and synovial biopsy was repeated 2 weeks later. Infiltrating synovial CD3+ T cells were significantly reduced in the synovium following treatment, in line with previous studies. This finding is consistent with previous work, showing a reduction of adhesion molecule expression, following anti-TNF treatment that would be predicted to inhibit cell migration. In line with this, B cells (CD22+) and macrophages (CD68+) were

also significantly reduced in both the lining and sublining layers. The expression patterns of several chemokines (IL-8, MCP-1, MIP-1α, MIP-1β, Groα and RANTES), in the synovium, were assessed by immunohistochemistry. Expression of IL-8 and MCP-1 was reduced in both the lining and sublining layers. IL-8 reduction was greatest in the lining layer in line with the suggested importance of chemokine gradients in cell migration. No change was seen in the synovial expression of MIP-1α, MIP-1β or RANTES. Groα (a neutrophil chemoattractant) expression levels were also reduced in the synovium, although the effects were not statistically significant. Serum levels of IL-8 and MCP-1 were also reduced, although this trend was again not significant. Granulocytes were harvested from venous blood and radiolabelled with ^{111}In-tropolonate. These were injected 24 h prior to synovial biopsies. Gamma camera imaging was undertaken 4 h and 22 h post-injection. A significant reduction in cellular infiltration with granulocytes was apparent on the gamma-camera images of both knees and hands. This reduction was paralleled by reductions in the synovial fluid granulocyte counts in the three patients from whom samples were obtainable post-treatment. These findings reinforce the evidence for inhibitory effects of TNF blockade on granulocyte migration. As granulocytes release mediators such as MMP-1 such effects may be critical in the therapeutic function of anti-TNF treatment. It is interesting that significant reductions in MMP-1 have also been seen following anti-TNF treatment, although the contribution of neutrophils to this finding is unknown.

Conclusion

In the light of previous work showing effects of TNF blockade in adhesion molecules, this study confirms an additional potentially important effect of anti-TNF treatment on leucocyte migration.

TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand.

J Lam, S Takeshita, J E Barker, *et al. J Clin Invest* 2000; **106**(12): 1481–8.

BACKGROUND. Osteoclasts are critical cells in the erosive process in RA, which leads to joint destruction and disability. The success of anti-TNF-α therapies in preventing the appearance of new erosions suggest that this cytokine is important in the generation of osteoclasts. This has been assumed to be an indirect role whereby TNF-α acts on osteoblasts to induce expression of RANKL (ligand for the receptor activator of NF-κB, another member of the TNF superfamily, sometimes termed OPGL), which in turn stimulates differentiation of RANK+ osteoclast precursors into active bone-resorbing cells. This paper documents a more direct involvement of TNF-α in this process.

A pure population of murine osteoclast precursors failed to undergo osteoclastogenesis when treated with TNF-α alone. In contrast, the cytokine dramatically stimulated differentiation in macrophages primed by less than 1% of the amount of RANKL required to induce osteoclast formation (Fig. 2.8). Mirroring their synergistic effects on osteoclast differentiation, TNF-α and RANKL markedly potentiated NF-κB and stress-activated protein kinase/c-Jun NH(2)- terminal kinase activity, two signalling pathways essential for osteoclastogenesis. This mechanism was verified in studies using stromal cells from mice lacking both p75 and p55 TNF receptors, so that indirect effects of TNF-stimulated stromal cells on osteoclast precursors could be excluded. Co-culturing these cells with bone marrow macrophages (osteoclast precursors) from TNF receptor-positive mice produced osteoclasts in response to TNF, proving a direct effect on the precursor cells. Similar effects were demonstrated *in vivo* by administration of TNF-α to chimeric animals in which genetically marked TNF-responsive macrophages were shown to develop into osteoclasts within a TNF non-responsive stromal environment.

INTERPRETATION. TNF-α alone does not induce osteoclastogenesis—RANKL-deficient mice fail to generate osteoclasts—but it does so both *in vitro* and *in vivo* by directly targeting macrophages within a stromal environment that expresses permissive levels of RANKL. Minuscule amounts of RANKL are sufficient to synergize with TNF-α to promote osteoclastogenesis; thus it would be easier to inhibit inflammatory osteolysis by blocking TNF-α rather than RANKL, as for the latter to be effective the cytokine would virtually have to be eliminated.

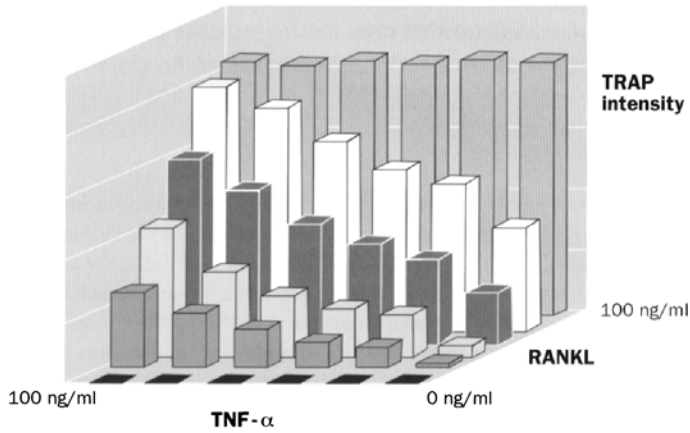

Fig. 2.8 TNF- synergizes with RANKto stimulate osteoclast differentiation. Pre populations of myeloid cells were treated with various combinations of TNF- and RANK. After 5 days the extent of osteoclastogenesis was expressed as a function of TRAPactivity, determined by a colorimetric assay. No osteoclast formation is induced in the absence of RANKwhile in its presence, TNF- augments osteoclast differentiation in a dose-dependent manner. TNF- potentiation of osteoclastogenesis is seen with all RANK dosages beneath saturating levels. Source: Lam *et al.* (2000).

Comment

These observations might explain why anti-TNF-α therapies appear to be very effective in halting erosive change in RA—even in patients whose symptom improvement fails to meet the criteria for responsiveness such as the ACR20. However, it also points to the possibility that anti-TNF therapies may interfere substantially with normal bone turnover.

The findings amplify and modify the conclusions of a paper published earlier this year suggesting that TNF-α alone could induce osteoclast differentiation |**2**|. In that paper, culture of bone marrow prior to the isolation of macrophage precursor cells allowed exposure to RANKL and hence priming for a subsequent response to TNF-α. If isolation was carried out in the presence of an antagonist of RANKL (osteoprotegerin), this priming was prevented and no osteoclastogenesis resulted.

Tumour necrosis factor a promotes the expression of stem cell factor in synovial fibroblasts and their capacity to induce mast cell chemotaxis.

H P Kiener, R Hofbauer, M Tohidast-Akrad, *et al. Arthritis Rheum* 2000; **43**:164–74.

BACKGROUND. Mast cells are pro-inflammatory cells that produce a large variety of pro-inflammatory mediators, including histamine, PGs and TNF. They have been implicated, in recent studies, in the pathogenesis particularly of RA but also of osteoarthritis (OA). Other data have demonstrated that stem cell factor (SCF), which exists in both membrane bound and soluble forms, is a major regulator of mast cells, inducing both their differentiation and their chemotaxis. As SCF is expressed on stromal cells such as fibroblasts it is possible that expression of this molecule on synovial fibroblasts promotes the infiltration and activation of these potent pro-inflammatory cells. To investigate this possibility this study investigated the expression of SCF on synovial fibroblasts and the ability of these cells to induce the migration of mast cells. It also investigated whether TNF could modify the expression of SCF, thereby modulating mast cell infiltration.

INTERPRETATION. Synovial fibroblasts of both osteoarthritic and rheumatoid joints express SCF and induce mast cell chemotaxis. TNF augments the expression of this molecule, potentially playing an important part in the mast cell infiltration seen in RA.

Comment

Synovial tissue was obtained from 29 rheumatoid joints and 25 osteoarthritic joints. Tissues were snap frozen and fixed, prior to incubating with an anti-SCF monoclonal antibody, detected colorimetrically. These sections were

subsequently stained with macrophage-specific and fibroblast-specific antibodies to determine the cell types stained with the anti-SCF antibody. In the rheumatoid samples, in contrast to those from osteoarthritic joints, SCF antibody staining was detectable on both fibroblasts and macrophages. This suggest the presence, in the rheumatoid joint, of factors that upregulate SCF expression. Following *in vitro* culture this difference disappeared and subsequent experiments were performed on these cultured cells, the phenotype of which may have been substantially altered by the culture conditions. Nonetheless, when cultured fibroblasts, from either source, were stimulated with TNF, mRNA levels of SCF (determined by semiquantitative PCR) were increased. As SCF has been shown to exist in both a membrane bound and a soluble form, an enzyme-linked immunosorbent assay (ELISA) based technique was used to determine the presence of SCF in the supernatants of cultured synovial fibroblasts to confirm that the mRNA level changes translated into protein production. Although low levels of soluble SCF were detectable without stimulation a significant dose-dependent increase was seen with TNF treatment. These findings suggest that TNF could have significant effects on the synoviocyte's chemoattractant ability. To confirm this, a chemotaxis assay was undertaken and it was shown that the synovial fibroblast supernatants induced migration of a mast cell line. Furthermore, the magnitude of the migratory response was dependent on the dose of TNF with which the fibroblasts were stimulated.

Certainly this study demonstrates the capacity for synovial fibroblasts to express and release SCF upon stimulation with TNF. Whether these *in vitro* findings translate into *in vivo* changes in both OA and RA is unclear. The findings in the non-cultured fibroblasts suggest that these effects are seen predominantly in rheumatoid disease and this is in keeping with the studies suggesting a pivotal role for TNF in this disease. Further studies are required on the role of mast cells in pannus formation, erosive change and chronic inflammation, before the significance of these findings can be appropriately interpreted.

Conclusion

Synovial fibroblasts express SCF in a TNF-dependent manner and this expression can influence mast cell migration. *In vivo* this expression is found in rheumatoid joints but not osteoarthritic joints. Further studies are required to elucidate the role of these changes in disease pathogenesis.

Synovial fluid levels of tumour necrosis factor a and oncostatin M correlate with levels of markers of the degradation of crosslinked collagen and cartilage aggrecan in rheumatoid arthritis but not in osteoarthritis.

D-H Manicourt, P Poilvache, A van Egeren, *et al. Arthritis Rheum* 2000; **43** (2):281–8.

BACKGROUND. Collagen fibrils and aggrecan are important constituents of articular cartilage. The mechanisms that control the degradation of these critical elements in RA is unknown. A number of molecular markers of cartilage degradation have been described. Pyridinoline (Pyr) is a marker of cartilaginous collagen, while lysylpyridinoline (D-Pyr) is a marker of bone collagen. Keratan sulphate (KS) is a marker of aggrecan catabolism. This study sought to determine whether levels of these breakdown products correlated with cytokine concentrations in the synovial fluid. Levels of TNF, IL-6 (a cytokine capable of inducing bone resorption) and oncostatin M (OSM) (a cytokine that in animal models induces aggrecan breakdown) were measured. Synovial fluid from patients with RA and OA were compared.

INTERPRETATION. Increased levels of TNF are associated with upregulated degradation of cartilage aggrecan and collagen in RA.

Comment

Synovial fluid was aspirated from the knees of 31 erosive rheumatoid patients (ARA 1987 criteria) and 31 OA patients. To reduce variability in the degree of synovitis all patients underwent ^{99}mTc-IgG scintigraphy and were shown to have similar levels of uptake. ELISA was used to determine the levels, of the degradation products KS, Pyr and D-Pyr, employing appropriate monoclonal antibodies and standards. Collagen breakdown products (Pyr and D-Pyr) were detected in all the synovial fluids. The median Pyr level (a marker of cartilage degradation) was significantly higher in rheumatoid patients (P<0.0001) while the median level of D-Pyr (a measure of bone collagen degradation) was significantly lower (P=0.0013). The reasons for the reduced levels of D-Pyr are unclear. No significant difference in KS levels was detected. It is worth noting, with respect to these latter two results, that all the rheumatoid patients were taking low-dose prednisolone, which could have reduced collagen and aggrecan degradation. Therefore, the figures presented for the rheumatoid patients may underestimate the true levels.

ELISA was also used to measure TNF, oncostatin M and IL-6 levels. However, no measurement of the associated soluble receptors was undertaken so these measures may not represent the whole story. Nonetheless, as would be anticipated, the median TNF level was significantly greater in RA than in OA. The IL-6 levels were similarly elevated and strongly correlated with the levels of TNF (r=0.7, P<0.0001), in the rheumatoid patients. OSM levels were elevated but did not show significant correlation with TNF. The IL-6 correlation fits with data that suggest that TNF induces expression of this cytokine in a number of synovial cells. OSM induction may in contrast occur, at least in part, independently of TNF.

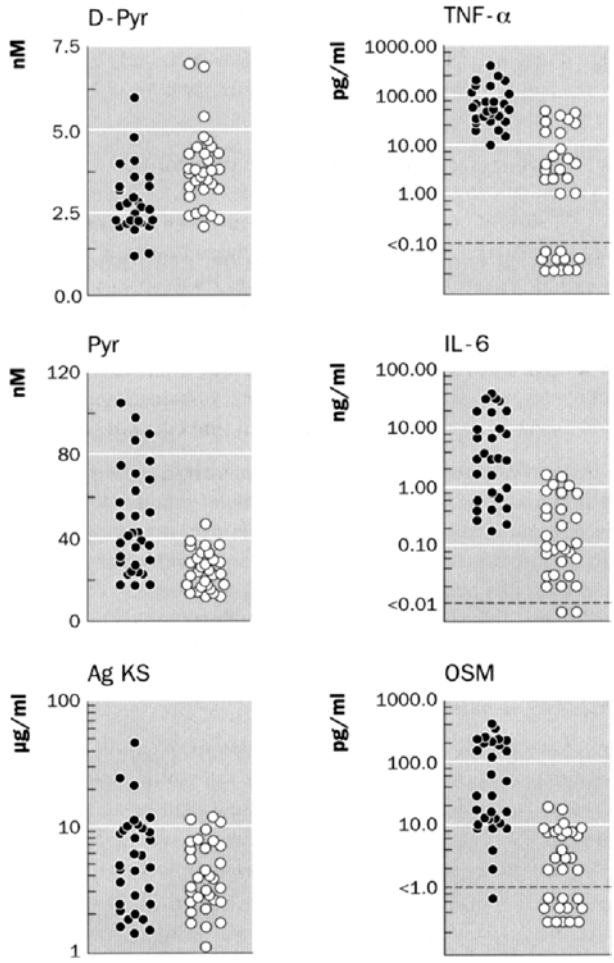

Fig. 2.9 Synovial fluid levels of lysylpyridinoline (D-Pyr), pyridinoline (Pyr), antigenic keratan sulphate (Ag KS), TNF-α, IL-6, and oncostatin M in patients with RA (closed circles) and OA (open circles). Source: Manicourt *et al.* (2000).

In the rheumatoid patients the levels of the three cytokines measured positively correlated with KS and Pyr levels. This suggests that these cytokines may be involved in the upregulation of the degradative processes in RA. OSM has recently been implicated in cartilage breakdown through the induction of MMPs and these data support such a role. However, the levels of OSM detected in the synovium in this study were considerably less than those required to stimulate cartilage breakdown *in vitro*. No similar significant correlations between cytokine levels and breakdown products were seen in the OA patients.

Conclusion

Although this study used only highly selected patients, it nonetheless supports a role for cytokine-dependent cartilage degradation in RA that is not seen in OA. This gives further insight into the potential pathogenic mechanisms of RA. It also suggests further potential benefits in inhibiting TNF, theoretically retarding cytokine-mediated collagen and aggrecan breakdown and preventing cartilage degradation.

Differential regulation of Fas-mediated apoptosis of rheumatoid synoviocytes by tumour necrosis factor α and basic fibroblast growth factor is associated with the expression of apoptosis-related molecules.

T Kobayashi, K Okamoto, T Kobata, *et al. Arthritis Rheum* 2000; **43**(5): 1106–14.

BACKGROUND. Synoviocytes and infiltrating lymphocytes within the synovium express the cell surface protein Fas (CD95). Ligation of this molecule by Fas-ligand (CD95L) induces apoptotic cell death and is an important mechanism of tissue homeostasis. In the rheumatoid joint this system seems defective as, despite adequate levels of both Fas and Fas-ligand, there is evidence that apoptotic cell death is impaired resulting in a failure to eliminate the proliferating synovial cells. This study sought to assess the effects of TNF and basic fibroblast growth factor (bFGF)—another cytokine capable of inducing synovial proliferation—on the sensitivity of synovial fibroblasts to Fas-mediated apoptosis.

INTERPRETATION. TNF induces apoptosis, while bFGF inhibits this effect. This inhibition of apoptotic cell death by bFGF was in part due to the upregulated expression of intracellular signalling molecules, particularly FLIP, an inhibitor of the pro-apoptotic caspase pathway.

Comment

Synoviocytes were isolated and cultured from rheumatoid synovium during arthroscopy. The effects of TNF and bFGF treatment on Fas-mediated apoptosis were investigated by culturing the cells in one or other cytokine for 5 days and, subsequently, attempting to induce apoptosis with an anti-Fas antibody. Both TNF and bFGF apparently induced proliferation of the synoviocytes, although data support ing this statement are not presented. TNF in addition to inducing proliferation also significantly increased the sensitivity of synoviocytes to apoptosis. This effect was not due to the induction of surface Fas expression as flow cytometry showed no differences in cells treated with the different cytokines. However, whether physiologically important differences in Fas expression would be apparent with flow cytometry is unclear.

Differences in the activities of a family of enzymes known to be involved in apoptosis (the caspases) were determined. Caspase 8 (a pro-apoptotic enzyme) was

upregulated in TNF-treated cells but not bFGF-treated cells. FLIP is an intracellular signalling molecule that inhibits caspase activation. Immunoblotting was undertaken to determine whether concentrations of FLIP were reduced. As immunoblotting is not quantitative, interpretation of the results must be cautious. Although the authors interpreted the results as indicating reduced levels of FLIP in TNF-treated synoviocytes, this conclusion is not clear-cut.

The finding that TNF increases apoptosis, suggests a potentially important regulatory role for this cytokine in preventing synovial hyperplasia. *In vivo* the balance between bFGF and TNF may be important in determining the degree of synovial proliferation. Previous work has suggested that pannus formation and subsequent erosive changes in the joint are due to impaired apoptosis of synovial fibroblasts. If that is the case this study suggests that anti-TNF treatments, despite their proven anti-inflammatory properties, may encourage the progression of the underlying damage of rheumatoid disease.

Conclusion

TNF not only induces proliferation of synovial fibroblasts but also increases their susceptibility to apoptosis a through upregulating the activity of caspase 8. This finding has implications for our understanding of the pathogenesis of RA.

Leflunomide: mode of action in the treatment of rheumatoid arthritis.
F C Breedveld, J M Dayer. *Ann Rheum Dis* 2000; **59**(11):841–9.
BACKGROUND. The principal property of leflunomide, or more accurately its active metabolite, is its ability to prevent *de novo* pyrimidine synthesis by reversibly inhibiting dihydroorotate dehydrogenase, the rate limiting step (see Fig. 2.10). For dividing cells such as T lymphocytes, *de novo* synthesis is essential because insufficient pyrimidines can be salvaged from nucleotide breakdown to allow DNA synthesis. Other effects, particularly on tyrosine kinases, which are important in cell signalling in T and B lymphocytes, require much higher concentrations and are not likely to be so important in the mechanism of the drug. Lack of sufficient pyrimidines is sensed by the p53 system, which acts as a check-point on the cells suitability to enter S phase and proceed to division. Provision of exogenous pyrimidine (uridine) reverses the effects of leflunomide.

A secondary effect involves inhibition of the activation of the transcription factor NF-κB, which is critical for mediating the effects of TNF-α. This effect can also be inhibited by providing exogenous uridine but the precise mechanism is unclear. Other mechanisms discussed include modulating the effect of T cells on macrophages so as to decrease the production of IL-1 and metalloproteinases while having a lesser effect on IL-1 receptor antagonist and the tissue inhibitor of metalloproteinases. Finally, lack of uridine diphosphate may downregulate the glycosylation of cell surface adhesion

molecules, effectively reducing cell-cell contact activation during inflammation of the molecules and altering traffic of leucocytes from blood into sites of inflammation.

The drug was first shown to be effective in animal models of autoimmune disease and also in preventing transplant rejection. It has now been proven to be effective in phase II and III clinical trials of active RA, and both improves symptoms and slows radiological progression.

INTERPRETATION. Although thought of as a drug that primarily affects T lymphocytes, the data showing an important effect on NF-κB activation and hence multiple pro-inflammatory pathways link leflunomide to the effects of the TNF-α blocking drugs.

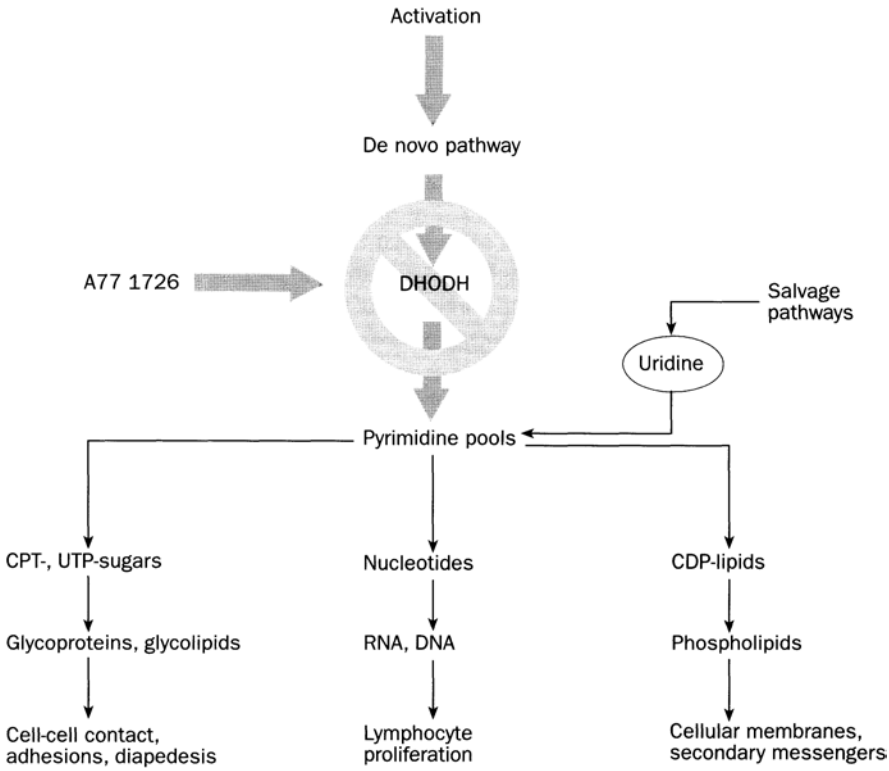

Fig. 2.10 Effect of inhibition of *de novo* pyrimidine synthesis on various mechanisms of activated lymphocytes. Adapted from Herrmann *et al.* |3|

Comment

Leflunomide is now becoming established in the treatment of RA, so this review of its modes of action and efficacy is timely and informative. There is useful speculation on the possible role of leflunomide in combination with methotrexate

(which affects purine synthesis—hence synergy with a pyrimidine synthesis inhibitor) and cyclosporin A where suboptimal doses of both drugs might effectively limit lymphocyte proliferation with a lower incidence of the side-effects seen at higher doses of cyclosporin A.

Leflunomide suppresses TNF-induced cellular responses: Effects on NF-kappa B, activator protein-1, c-Jun N-terminal protein kinase, and apoptosis.
S K Manna, A Mukhopadhyay, B B Aggarwal. *J Immunol* 2000; **165**(10): 5962–9.

BACKGROUND. Leflunomide is a pyrimidine biosynthesis inhibitor that has recently been approved for the treatment of RA. However, the mechanism of leflunomide's antiarthritis activity is not fully understood. The crucial part that TNF plays in RA led the authors to postulate that leflunomide might block TNF signalling.

They previously demonstrated that leflunomide inhibits TNF-induced activation of NF-κB; this transcription factor is maintained in an inactive state in the cytoplasm by being bound to an inhibitor, IκBα. The inhibitor can be removed by phosphorylating it so that it is tagged for degradation and allows NF-κB to move to the nucleus and alter expression of the multiple genes involved in the inflammatory response. This study showed that leflunomide could stop the removal of IκBα by suppressing the enzymes that phosphorylate it. When NF-κB reaches the nucleus, one of the genes it turns on is the NF-κB gene itself to produce more of the protein. The influence of TNF binding to its major receptor (TNFR1) on NF-κB gene transcription was prevented by leflunomide. Effects of leflunomide were not confined to NF-κB; the activation of the transcription factors AP-1 by TNF-α was also inhibited (by inhibiting an enzyme, c-Jun N-terminal protein kinase, which activates one of its components). Likewise, the ability of TNF-α to induce apoptosis by activating caspase enzymes was inhibited. All of these suppressive effects of leflunomide on TNF signalling were completely reversible by uridine, indicating a crucial part for pyrimidine biosynthesis in TNF-mediated cellular responses.

INTERPRETATION. Overall, these results suggest that suppression of TNF signalling is one of the possible mechanisms for inhibitory activity of leflunomide in RA.

Comment

The success of drugs that directly block TNF-α in treating RA naturally gives rise to speculation about whether other effective drugs might affect TNF-α indirectly. This paper shows rather convincingly that leflunomide reverses many of the signalling pathways initiated by TNF-α. In so doing, it does not, however, suggest a novel action for the drug; all its effects on TNF-α-driven actions were inhibited by providing uridine, so the availability of pyridimines such as uridine

must be critical to pro-inflammatory signalling pathways initiated by cytokines. Precisely how this comes about remains rather unclear. However, it is worth noting that the experiments reported here were mostly carried out in a T-cell line (Jurkat) or in transfected epithelial cells, and the concept that lymphocytes are uniquely sensitive to leflunomide remains. It would be useful to know whether the effects of TNF-α on macrophages and synoviocytes could also be blocked by leflunomide.

Overall comments on anti-tumour necrosis factor therapies

Over the past 12 months, the increasing use of anti-TNF therapy has reinforced the therapeutic efficacy of this novel treatment. The ATTRACT study has confirmed the benefits, reported previously with other TNF antagonists. The potential benefits of anti-TNF treatment are also being extended and recent papers highlight the potential benefit of such treatments in the SPAs, particularly AS.

Further insight is being gained into the cellular mechanisms by which the treatment works. In addition to affecting cytokine profiles (particularly IL-1 and TNF), the papers reviewed illustrate a broad immunomodulatory effect. These effects range from inhibiting cellular migration by both chemokine inhibition and adhesion molecule downregulation to alterations in T-cell function and MMP balance. Although all these effects are likely to impart benefit in inflammatory arthritis, a note of caution is sounded by the study of apoptosis, as inhibiting apoptosis may, in the long term, prove detrimental. Further studies of this aspect are required to assess whether the current short-term benefits of anti-TNF treatment translate into long-term efficacy. It is also interesting to note that the most recently introduced disease-modifying drug, leflunomide, may also act primarily in terms of its effects on TNF signalling.

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3
Additional targets of anticytokine therapy in rheumatoid arthritis

Overview

Despite the evidence in favour of anti-tumour necrosis factor (TNF) treatments, the cause of rheumatoid arthritis (RA) remains unclear. A number of other cytokines are likely to be involved in the pathogenesis of RA and these include interleukin (IL) -1, IL-12, IL-15 and IL-17. Therapies directed at, or affecting, all of these cytokines have been explored in either clinical trials or in experimental models of arthritis over the past 12 months. Work has also investigated the effects of more conventional treatments and interventions such as physiotherapy on these cytokines.

Interleukin-1 and interleukin-1 receptor antagonist

Overview

IL-1 is a prototypic pro-inflammatory cytokine, produced predominantly by monocytes, capable of stimulating the production of cyclooxygenase (COX)-2 and nitric oxide synthase (NOS), with a resulting increase in the pro-inflammatory mediators, prostaglandin (PG) E_2 and NO. Several studies in both animal models of inflammatory arthritis, and clinical studies of RA, have implicated this cytokine in disease pathogenesis. It has been particularly implicated as a mediator of bone resorption. There are three members of the IL-1 gene family: IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra). IL-1α and IL-1β are both agonists, activating target cells when they bind to the IL-1 receptor. IL-1Ra, by contrast, is an antagonist that regulates the action of the other two gene products by competitively binding to the IL-1 receptor with high affinity. Levels of IL-1Ra appear to be reduced in RA and in experimental animal models recombinant IL-1Ra has previously been shown to improve arthritis and also to suppress bone resorption. Recent work with IL-1Ra knockout mice has confirmed the importance of IL-1Ra, while results from ongoing clinical studies exploring the efficacy of treatment with IL-1Ra have been published this year.

Furthermore, an alternative therapeutic delivery system for IL-1Ra, using viral gene therapy, has been investigated in another animal model. Other methods of inhibiting IL-1 have also been explored—the theoretical efficacy of antibodies to IL-1 has been assessed in a novel *in vitro* model of synovitis, and the anti-IL-1 effects of physical therapy have also been investigated.

Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice.
R Horai, S Saijo, H Tanioka, *et al. J Exp Med* 2000; **191**(2):313–20.

BACKGROUND. IL-1Ra is a naturally occurring inhibitor of IL-1 that competes for the IL-1 receptor. Although previous work in experimental animal models has demonstrated that IL-1Ra can ameliorate inflammatory arthritis, the role of IL-1Ra in normal physiology has not been completely elucidated. Using mice deficient in the gene encoding for IL1-Ra, this study investigated the effects of IL-1Ra deficiency on these animals.

INTERPRETATION. IL-1Ra deficiency in BALB/cA mice was associated with the development of a spontaneous chronic inflammatory arthropathy, with synovial and periarticular inflammation and articular erosion that histologically closely resembled RA in humans. IL-1β, IL-6 and TNF-α were overexpressed in the affected joints, indicating an important role for IL-1Ra in the regulation of cytokine networks.

Comment

Gene targeting was employed to produce IL-1Ra-deficient mice, and pathology was assessed in mice with either a BALB/cA or C57BL/6J genetic background. All 56 of the BALB/cA IL-1Ra-deficient mice spontaneously developed a chronic inflammatory arthropathy by 13 weeks of age. The synovitis was most marked in the hindlimbs, particularly the ankle, but also affected the forelimbs. Histologically, there was marked synovial and periarticular inflammation, with articular erosion, a picture similar to that seen in RA. The mice also showed significant levels of IgG rheumatoid factor. Total RNA was isolated from the affected joints and Northern blot hybridization was used to quantify the expression of IL-1β, TNF-α, IL-6 and COX-2. All these molecules showed augmented expression, indicating that IL-1Ra deficiency causes increased expression of a number of pro-inflammatory mediators. IL-1 β was, however, detectable in normal joints, suggesting that IL-1β is normally suppressed by IL-1Ra under physiological conditions. Mice on the C57BL/6J genetic background had a low incidence of arthritis, suggesting an important role for other, as yet unidentified, genetic factors.

Conclusion

IL-1Ra is clearly important in the normal cytokine homeostasis of the immune system. The demonstration, in RA patients, that there is a reduced ratio of IL-1Ra to IL-1β, might suggest that a similar deficiency is contributing to the disease process in these individuals. However, the limited efficacy of specific IL-1Ra treat ments so far reported might imply that the major effects are mediated prior to the development of clinical features. If IL-1Ra deficiency contributes to the onset of RA, it would be interesting to screen families with a strong history of RA for polymorphisms in this gene, as targeted treatment might be beneficial in these individuals.

A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis.

Y Jiang, H K Genant, M Cobby, *et al. Arthritis Rheum* 2000; **43**(5): 1001–9.

BACKGROUND. Previous experimental animal models have indicated that IL-1Ra treatment can reduce bone resorption. Furthermore, in preliminary clinical studies, it was demonstrated that, in addition to its safety and efficacy, treatment with recombinant IL-1Ra reduced the Larsen erosive joint count. This study sought to evaluate, in a large double-blind placebo-controlled study, whether the radiological improvements seen in pre-clinical studies were reproducible.

INTERPRETATION. Treatment, for 24–48 weeks, with IL-1Ra, reduced the radiological progression of disease, evaluated by two different radiographic scoring techniques.

Comment

In this large, multicentre study, 472 patients with RA (American Rheumatism Association 1987 revised criteria) of between 6 months and 8 years' duration, that was currently active (10 or more swollen joints, plus two of 10 or more tender joints, physician's global assessment as severe or very severe, and C-reactive protein >1.5mg/dl) were recruited to one of four groups: placebo, 30 mg/day, 75 mg/day and 150 mg/day of subcutaneous IL-1Ra. It is potentially relevant that the selection criteria may have favoured newly diagnosed patients with more severe disease. Treatment was continued for 48 weeks, although after 24 weeks placebo-treated patients were randomly assigned to other treatments. Radiographs of each hand and wrist were obtained at baseline and after 24 and 48 weeks of treatment. Radiographs were scored in random sequence using two different previously validated scoring techniques (the Genant and the Larsen scoring methods). The two scoring techniques were significantly correlated for all the variables assessed (P<0.0001). However, the correlation was lower when the scores were used for the assessment of disease progression, possibly reflecting

Table 3.1 The Genant scoring method*

Erosion†	Joint space narrowing‡
0 = normal	0 = normal
0+ = subtle change	0+ = questionable or subtle
1 = mild	1 = mild
1+ = mild worse	1+ = mild worse
2 = moderate	2 = moderate
2+ = moderate worse	2+ = moderate worse
3 = severe	3 = severe
3+ = severe worse	3+ = severe worse
	4 = ankylosis or dislocation

* +=0.5.
†Fourteen joints are examined: interphalangeal (IP) of digit 1, proximal IP (PIP) of digits 2–5, metacarpophalangeal (MCP) of digits 1–5, carpometacarpal (CMC) of digit 1, scaphoid, distal radius, and distal ulna. Maximum score is 98.
‡Thirteen joints are examined: IP of digit 1, PIP of digits 2–5, MCP of digits 1–5, combination of CMC of digits 3–5, combination of capitate, scaphoid lunate, and radiocarpal joint. Maximum score is 104.
Source: Jiang *et al.* (2000).

Table 3.2 The Larsen scoring method*

0 = normal
1 = slight abnormality, including 1 or more of the following lesions: periarticular soft tissue swelling, periarticular osteoporosis, and slight joint space narrowing
2 = definite early abnormality, including definite erosion, with or without joint space narrowing
3 = medium destructive abnormality
4 = severe destructive abnormality
5 = mutilating abnormality (the original articular surfaces have disappeared)

*Fifteen areas are examined: interphalangeal of digit 1, distal and proximal interphalangeal of digits 2–5, metacarpophalangeal of digits 1–5, and the wrist. Dislocation and bony ankylosis are considered; if they are present, the scoring is based on the concomitant bone destruction. Maximum score (total for both hands) is 150.
Source: Jiang *et al.* (2000).

the increased sensitivity of the Genant scoring method, which is more time consuming. Nonetheless, a significant reduction was seen in radiographic disease progression (total scores) when all treatment doses were grouped and compared with placebo. Subgroup analysis of the individual doses was less clear-cut, possibly due to the smaller numbers being analysed. Changing from placebo to active treatment at 24 weeks also resulted in a significant slowing of disease progression (total scores).

This is the first study to demonstrate a reduction in radiographic change using a 'biological agent'. Although other outcome measures can give an improved assessment of a patients' overall improvement, the radiographic changes offer an objective assessment of a defined endpoint. The speed of reduction in radiographic

progression, with treatment was remarkably fast. Changes were demonstrable by 24 weeks, although the rate of change slowed thereafter, a finding in keeping with the slowing in radiographic progression after the first year of disease.

Conclusion

In this group of rheumatoid patients treatment with IL-1Ra retards the radiological progression of the disease. Further studies will be required to determine the position of this treatment in the armamentarium of biologics available to the rheumatologist. It may beneficial used in combination treatments with therapies such as the anti-TNF compounds, and further studies will be required to elucidate the role of this agent.

Therapy and prevention of arthritis by recombinant adeno-associated virus vector with delivery of interleukin-1 receptor antagonist.
R-Y Pan, S-L Chen, X Xiao, *et al. Arthritis Rheum* 2000; **43**(2): 289–97.

BACKGROUND. A theoretical alternative to the repetitive injections required by treatments such as the IL-1Ra is local gene therapy. The gene products can then be translated locally resulting in targeted production of the appropriate treatment. Viral gene delivery systems have been developed utilizing both adenoviral vectors and retroviral vectors. Adeno-associated viral (AAV) systems allow long-term transduction of the vector product, and previous studies have shown that transgene expression can correlate with the severity of inflammation. In this study the efficacy of an AAV vector encoding for the IL-1Ra (AAV-IL-1Ra) is assessed in an experimental animal model of inflammatory arthritis (lipopolysaccharide [LPS] induced arthritis).

INTERPRETATION. The production of the AAV-IL-1Ra transgene was upregulated by LPS-induced joint inflammation and proved to be efficacious in both therapeutic and preventative protocols. A single injection of AAV-IL-1Ra prevented not only primary but also recurrent arthritis. This stable, efficacious disease-regulated gene expression system provides a valuable and novel approach to arthritis treatment.

Comment

Human IL-1Ra complementary DNA was isolated by reverse transcription-polymerase chain reaction (RT-PCR) from a cell line (U937) and the PCR product sequenced to confirm appropriate amplification. The IL-1Ra sequence was then placed under the transcriptional regulation of the human cytomegalovirus immediate early promoter in the AAV and purified. Experimental arthritis of the knee was induced in rats by intra-articular injection of LPS and after 12 h either AAV-IL1Ra or a control AAV encoding an

irrelevant protein (LacZ) was injected. IL-1Ra levels were determined in synovial washings by enzyme-linked immunosorbent assay (ELISA) and subsequently in synovial tissue by RT-PCR. Significantly higher levels were demonstrable in arthritic knees treated with AAV-IL-1Ra than in nonarthritic knees (treated with AAV-IL-1Ra), non-treated arthritic knees or arthritic knees treated with the control AAV. These results indicated that expression of the IL-1Ra was effective *in vivo* and was inducible by inflammation. The reasons for the inducibility of the gene product with inflammation were not clear, but they are most likely related to characteristics of the cytomegalovirus promoter. Nonetheless these findings indicated potential therapeutic usefulness and subsequent assessments of efficacy were undertaken.

Histological assessments of synovitis were undertaken post-treatment, along with leucocyte counts and radiolabelled citrate absorption (a read-out of extracellular fluid accumulation). The prominent inflammation, assessed by all three of these measures, associated with LPS treatment, was significantly reduced with AAV-IL1Ra therapy, but was unaffected by the control AAV. This indicated that the AAVIL-1Ra could not only produce the gene product, but that the IL-1Ra was capable of ameliorating LPS-induced arthritis.

For future clinical application it would be important to be sure that the genes were stable. In order to assess the longevity of the effects a further challenge with LPS (without further AAV treatment) was undertaken, in an additional cohort of rats, 80 days after the initial injection. Comparable results were seen in this study. Furthermore, pre-treatment of rats with AAV-IL-1Ra significantly reduced the severity of arthritis, induced 100 days later by injection with LPS.

One concern regarding gene therapy such as this is the diffusion of the vector to other organs. In this study the investigators undertook PCR assessments on a number of other organs (brain, lung, liver, spleen and gonads) and found no evidence of spread. However, no data on the timing of these assessments are given.

A further concern is the possible pro-inflammatory effects of the vector itself, but in this model system no such effects have been noted.

Conclusion

The gene delivery of IL-1Ra, in this model, could be achieved in normal tissue and the transgene remained inducible after dormancy of at least 100 days. The findings show that a transgene can be stably maintained and suggest that this delivery system might be of benefit in the treatment of arthritis. LPS-induced arthritis is a self-limiting arthritis, analogous to reactive arthritis, and the results of this study will need to be confirmed in other more chronic experimental models of arthritis such as collagen-induced arthritis. However, if the findings are reproducible, these data suggest a possible future role for gene therapy in inflammatory arthritis.

Anti-interleukin-1 and anti-CD44 interventions producing significant inhibition of cartilage destruction in an *in vitro* model of cartilage invasion by rheumatoid arthritis synovial fibroblasts.

M Neidhart, R E Gay, S Gay. *Arthritis Rheum* 2000; **43**(8):1719–28.

BACKGROUND. Although animal models are frequently used to investigate the pathogenesis and treatment of inflammatory arthritis, an *in vitro* model of the disease would be an attractive research tool. This paper uses a novel *in vitro* model of rheumatoid synovial cartilage destruction, in which human chondrocytes are stimulated to synthesize a radiolabelled collagenous matrix in sponges. These sponges can then be co-cultured with rheumatoid synovial fibroblasts that induce matrix degradation, which can be assessed by release of the radioisotope. In this paper the effects of antibodies to IL-1 and CD44 in addition to the effects of IL-1Ra on this process were investigated. IL-1 is a prototypic pro-inflammatory cytokine implicated in RA. While IL-1Ra, as discussed in other presented papers, has been shown to be inhibitory in other models of arthritis, this study assesses the potential benefits of antibody treatment directed at IL-1. Antibodies to CD44 were also studied. CD44 is an adhesion molecule upregulated by pro-inflammatory cytokines and, because of its ability to bind to hyaluronic acid, it has been implicated in cartilage destruction.

INTERPRETATION. The rheumatoid synovial fibroblasts degraded the human cartilaginous matrix, a process stimulated by IL-1β and TNF. Furthermore both anti-IL-1 and IL-1Ra inhibited this. Pre-treatment of cultures with anti-CD44 also inhibited the degradation. These data demonstrate the possible uses of this model and suggest that both anti-IL-1 and anti-CD44 antibody treatments may be therapeutically useful in RA, particularly in the prevention of cartilage destruction.

Comment

Human chondrocytes, isolated from the cartilage of patients undergoing joint replacement surgery, were cultured with collagen sponges pre-treated with bovine extracellular matrix isolated from bovine placenta. The cartilage matrix was then labelled with ^{35}S prior to use in culture. Fibroblasts were isolated from rheumatoid, osteoarthritic and normal synovium and cultured with the radiolabelled sponges for 21–28 days and ^{35}S release into culture supernatant was determined. A significantly greater release was seen when the sponges were co-cultured with rheumatoid synovial fibroblasts than when cultured with osteoarthritic ones, although no data were given on normal synoviocytes. This finding is in keeping with a comparable mouse model (the SCID mouse model) in which human articular cartilage and synovium are implanted into the mice and cartilage degradation is monitored histologically. Blocking IL-1 with either the antibody or IL-1Ra inhibited the destruction mediated by rheumatoid synovial fibroblasts. This mirrors the finding in the SCID mouse model that IL-1 might

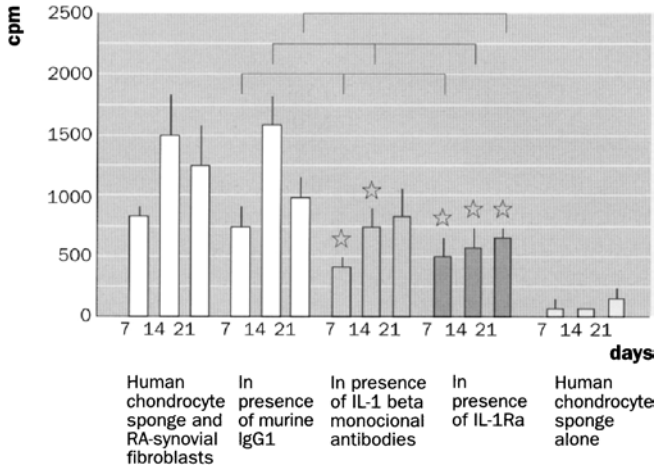

Fig. 3.1 Weekly release of ^{35}S into the medium from bovine embryonic extracellular matrix/human chondrocyte sponges after the addition of RA-synovial fibroblasts in the absence or presence of 1.0 μg/ml murine IgG1, murine IL-1β monoclonal antibodies, or 100 ng/ml IL-1Ra for 7 days in culture. Bars show the mean and SD of three sponge cultures (days 7, 14 and 21). ☆Significant decrease compared with the reaction with synovial fibroblasts treated with murine IgG1 (P<0.05 by paired t-test). Source: Neidhart *et al.* (2000).

favour cartilage invasion, and correlates with the potentially beneficial effects seen on erosive changes in clinical studies. Treatment with both IL-1 β and TNF significantly increased ^{35}S release although this was only demonstrable at one time-point. Since the SCID mouse model showed less benefits on cartilage invasion when TNF was blocked as compared with when IL-1 was blocked, it was unfortunate that no experiments in the sponge model were presented using anti-TNF therapies. However pre-treatment of the cultures with anti-CD44 also inhibited the cartilage destruction. Previous experimental models have shown that anti-CD44 treatment inhibits synovial fibroblast adhesion and the inhibition seen in the presented data indicates a need for cell to cell contact for mediating destruction.

Conclusion

This novel model may prove beneficial for developing future treatments for RA. The data presented imply that anti-IL-1 therapy (be it antibodies or IL-1Ra) and anti-CD44 therapy may prevent cartilage destruction in RA.

Cyclic tensile strain acts as an antagonist of IL-1β actions in chondrocytes.
A Xu, M J Buckley, C H Evans, S Agarwal. *J Immunol* 2000; **165**:453–60.

BACKGROUND. IL-1β induces catabolic responses in chondrocytes by stimulating the expression of NOS (inducing NO that inhibits proteoglycan synthesis), COX-2 and collagenase. Physiotherapy is beneficial in aiding the recovery of inflamed joints. This has previously been attributed to increased local circulation and the dissemination of inflammatory mediators. This study investigated the effects of mechanical strain (cyclic tensile strain) on chondrocyte function *in vitro*.

INTERPRETATION. Mechanical strain is a potent antagonist of IL-1β, exerting its effects by regulating the transcription of IL-1 response elements. Strain suppresses transcription of collagenase, NOS and COX-2, probably through interactions with the signal transduction cascade; therefore, controlled physiotherapy interventions in inflammatory arthritis may be anti-inflammatory.

Comment

Rabbit chondrocytes were isolated and cultured by standard techniques. Confluent chondrocyte monolayers were subjected to cycles of strain and relaxation of varying intensities in culture, in the presence or absence of IL-1β. Levels of NOS, COX-2 and collagenase (matrix metalloproteinase-1) mRNA induction by IL-1β were significantly reduced by cyclic tensile strain. Collagenase levels and PGE_2 levels (a read-out of COX-2 function) were assessed by Western blotting and radio-immunoassay, respectively, and were similarly inhibited. Cyclic tensile strain treatment also restored proteoglycan synthesis as determined by ^{35}S incorporation. This demonstrates that the mechanical strain of chondrocytes can effectively reverse a number of the potentially detrimental effects of IL-1β. This is in keeping with the beneficial effects documented with continuous passive motion in patients with inflamed joints. Whether these clinical interventions exert similar levels of strain on the chondrocytes *in vivo* is unclear. The level of strain is important as, although relatively low magnitude levels were used in these experiments, previous studies with greater intensities of strain have shown pro-inflammatory effects.

In order to assess whether these effects were due to downregulation of the IL-1 receptor, chondrocytes were pretreated with cyclic tensile strain to assess whether this reduced the response to IL-1β. Pre-treatment had no significant effect implying that the strain was not exerting its effects through IL-1 receptor levels. The effects of strain were therefore only demonstrable when it occurred in the presence of IL-1ß.

Conclusion

These data provide evidence that the benefits of continuous passive motion in inflammatory arthritis may occur through immunomodulation. These findings are of relevance to the management of inflammatory arthritis, suggesting that carefully designed exercise, far from being detrimental, may be an important therapeutic modality.

Interleukin-17

IL-17 is a T-cell-derived cytokine that stimulates a variety of cell types to produce pro-inflammatory products such as PGs, NOS, chemokines and other cytokines, including IL-6 and IL-8. It has been shown to induce osteoclastogenesis and, therefore, may be important in the subchondral osteoporosis and erosive changes of RA. Immunostaining studies have previously demonstrated elevated levels of this cytokine in rheumatoid joints when compared with osteoarthritic controls.

These findings have stimulated interest in IL-17 as a T-cell-derived mediator of the inflammatory process in RA. Recent studies have investigated the effects of IL-17 on proteoglycan degradation and explored whether other cytokines can inhibit this process. Work investigating IL-17 in patients with RA has also been published, indicating that levels of this cytokine can be modified with cyclosporin.

Effect of interleukin 17 on proteoglycan degradation in murine knee joints.
J Dudler, N Renggli-Zulliger, N Busso, M Lotz, A So. *Ann Rheum Dis* 2000; **59**:529–32.

BACKGROUND. Previous *in vitro* studies have shown that IL-17 may potentiate matrix degradation through the upregulation of NO, stromelysin and IL-1β in chondrocytes. This study evaluated the effects of IL-17 *in vivo* following injection of the cytokine into murine knee joints.

INTERPRETATION. The findings confirm *in vivo* the catabolic effects of IL-17 on chondrocytes, potentially implicating this cytokine in the pathogenesis of cartilage degradation in inflammatory arthritis.

Comment

This is the first study to investigate the effects of IL-17 *in vivo*. One knee of a cohort of C57BL/Ola mice was injected intra-articularly with either recombinant murine IL-17 or recombinant murine IL-1β. The contralateral knee was injected with an identical volume of phosphate-buffered saline. Joint inflammation was assessed by the accumulation of radiolabelled technetium, measured by gamma-counter, and the severity was expressed as a ratio between experimental and

control knee. Synovitis was subsequently also scored. Using either measure little in the way of leucocyte infiltration or synovitis was detectable 24 or 48 h after injection. However, statistically significant proteoglycan depletion, assessed by safranin O staining, was detectable after 48 h using either IL-1β or IL-17. As little cellular infiltration was detectable it is likely that this effect was due to the effects of IL-17 on chondrocytes. Using a protocol that incorporated three repeated injections, a slight increase in inflammation was detectable. However, given the repetitive trauma, such a finding would be anticipated, and the knees of the control animals also had slightly increased inflammatory changes. More severe safranin O depletion, correlating with increased proteoglycan degradation, was seen in the animals injected repetitively. Given the increased levels of cellular infiltration the interpretation of these results is more difficult as other cellular products, released due to the inflammation, could have been mediating the effects. As proteoglycan levels, as determined by safranin O staining, are a dynamic balance of synthesis and degradation, levels of proteoglycan synthesis were assayed by radiolabelled sulphate(^{35}S) incorporation into IL-1β or IL-17 treated cultured patella cartilage. Co-culture with IL-1β reduced proteoglycan synthesis, in line with previous studies that have shown prolonged suppression of proteoglycan synthesis with this cytokine. However, in contrast to IL-1β no decrease was seen with IL-17. It, therefore, appears that IL-17 is increasing degradation of IL-17 without stimulating biosynthesis. It is, however, noteworthy that this finding contradicts another study of IL-17 published this year (see below), in which IL-17 treatment did inhibit ^{35}S incorporation into cultured patellar cartilage. The reason for this difference is most likely due to the lower concentrations of IL-17 used *in vitro* in this study as the later study demonstrated that 100 times greater concentrations of IL-17 are required to mediate the same effects. Nonetheless, both studies agree that the end-result of IL-17 treatment is proteo- glycan depletion, whether due to increased degradation or decreased synthesis.

Conclusion

This study did not seek to address the previously documented, potentially detrimental, effects of IL-17 on other T cells, mesenchymal cells, macrophages and osteoclasts, but nonetheless, these data support the need for further investigations targeting this particular cytokine with blocking agents in other experimental models of inflammatory arthritis. If studies showed such blocking to be efficacious, this cytokine might represent a novel therapeutic target in RA.

Reduction of interleukin-17-induced inhibition of chondrocyte proteoglycan synthesis in intact murine articular cartilage by interleukin-4.
E Lubberts, L A B Joosten, F A J van de Loo, L A M van den Bersselaar, W B van den Berg. *Arthritis Rheum* 2000; **43**(6):1300–6.

BACKGROUND. IL-4 is a T-cell-derived cytokine that, although not detectable in rheumatoid synovium, protects animals from cartilage erosion in experimental models. Furthermore, *in vitro* studies have shown that IL-4 can suppress cartilage proteoglycan degradation. This study investigated the inhibitory effects of two cytokines, IL-1 (previously well described) and IL-17 (a novel cytokine implicated in cartilage proteoglycan degradation) on chondrocyte proteoglycan metabolism, and explored a possible role for NO in mediating these effects. The potential for IL-4 to inhibit these effects was also investigated

INTERPRETATION. IL-17, like IL-1 can inhibit chondrocyte PG synthesis. Although IL-4 has no effects on basal chondrocyte metabolism, it can protect the cartilage from the IL-1-and IL-17-mediated suppression. This effect is probably mediated through modulation of inducible NOS (iNOS).

Comment

Whole patellae, isolated from C57BL/6 mice, were incubated with different concentrations of IL-17 for 48 h, in insulin-like growth factor-1 (IGF-1) (an essential growth factor for chondrocytes, which also serves as a control). Proteoglycan synthesis in these explant cultures was assessed by ^{35}S incorporation during the final 3 h of culture. IL-17 significantly inhibited proteoglycan synthesis (compared with IGF-1 alone), although 100 times greater concentrations of IL-17 were required compared with IL-1 to mediate the same effect. This is in keeping with another study published this year (see above) that showed no effect of IL-17 at doses equivalent to the IL-1 doses used in this study. Nonetheless, as IL-17 has been shown in other studies not only to induce IL-1 production from macrophages but also to increase the effectiveness of IL-1 on synoviocytes, it is possible that IL-17 secreted by T cells in the joint is serving a pivotal role. The finding that both these cytokines can inhibit proteoglycan synthesis, demonstrates the potential for them to directly induce cartilage degradation. To investigate a possible mechanism for this effect, nitrite levels in the supernatant of the cartilage explant cultures were measured colorimetrically using the Greiss reaction. The inhibition of cartilage metabolism seen with both IL-1 and IL-17 was associated with an increase in nitrite levels. As previous studies have demonstrated that both these cytokines can upregulate iNOS in chondrocytes this finding suggests that the inhibitory effects on proteoglycan synthesis are also being mediated by NO. Indeed, when explants were co-cultured with an inhibitor of iNOS the IL-17-induced inhibition of chondrocyte proteoglycan synthesis was abrogated. Patellae from iNOS knockout C57BL/6 mice were also cultured with IL-17. Although significantly less inhibition of proteoglycan synthesis was seen with IL-17 there was nonetheless some reduction, suggesting the involvement of other pathways.

IL-4 has previously been shown to suppress NO production in macrophages in response to inflammatory stimuli such as LPS. No direct effect of IL-4 on

proteoglycan metabolism was seen, but pre-incubation of cartilage explants with IL-4 reduced the inhibitory effects of both IL-17 and IL-1. Furthermore nitrite production was reduced in parallel, as might be predicted from the previously reported effects of IL-4 on monocytes.

Conclusion

This study confirms a potentially important part for IL-17 in cartilage damage. The protective effects of IL-4 suggest that this cytokine could be an important modulator of this process. As IL-4 is not detectable in the rheumatoid synovium, despite cartilage destruction, it is possible that treatment with IL-4 could be efficacious. Moreover, blocking IL-17 may also be beneficial.

IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. E Lubberts, L A B Joosten, M Chabaud, *et al. J Clin Invest* 2000; **105**(12): 1697–710.

BACKGROUND. Bone destruction is the most difficult target in the treatment of RA. This paper shows that local overexpression of IL-4, introduced by a recombinant human type 5 adenovirus vector (Ad5E1mlL-4) prevents joint damage and bone erosion in the knees of mice with collagen-induced arthritis. No difference was noted in the course of collagen-induced arthritis, as judged by incidence of arthritis and severity of swelling in the injected knee joints between those treated with Ad5E1mlL-4 or the control vector. However, radiographic analysis revealed impressive reduction of joint erosion and more compact bone structure in the group given gene therapy with Ad5E1mlL-4. Although severe inflammation persisted in treated mice, Ad5E1mlL-4 prevented bone erosion and diminished tartrate-resistant acid phosphatase activity. This indicates that local IL-4 inhibits the formation of osteoclast-like cells. Messenger RNA levels of IL-17, IL-12 and cathepsin K in the synovial tissue were suppressed, as were IL-6 and IL-12 protein production. Osteoprotegerin ligand expression was markedly suppressed by local IL-4, but no loss of osteoprotegerin expression was noted with Ad5E1mlL-4 treatment. To assess the significance of these results derived from an experimental model, studies were undertaken of bone from patients with arthritis, both RA and osteoarthritis (OA). *In vitro* studies revealed consistent suppression by IL-4 of type I collagen breakdown in bone samples, measured by the release of type I collagen C-telopeptide breakdown products into supernatants of cultured bone fragments. IL-4 also enhanced synthesis of type I procollagen, suggesting that it promoted tissue repair.

INTERPRETATION. These results point to a potential role for IL-4 in protecting arthritic joints from undergoing bony erosion and destruction. This was achieved without influencing inflammation in the joint. The feasibility of delivering IL-4 by a gene therapy approach was also shown, although there are

still significant hurdles to overcome before this kind of delivery system can be used in human studies.

Comment

As well as showing an inhibition of bony erosion by IL-4 the study also gives interesting information on the mechanism. Erosion is carried out by osteoclasts, and their differentiation from precursor cells requires osteoprotegerin ligand (the same molecule is called RANK ligand and TRANCE in other work). These are further examples of the involvement of members of the TNF/TNFR superfamily of cytokines and receptors in the pathogenesis of arthritis. As with other cytokines the influence of osteoprotegerin ligand is modulated by the release of a soluble receptor, osteoprotegerin, which binds it and prevents it signalling to the osteoprotegerin ligand receptor, RANK, on osteoclast precursors. Production of this regulatory molecule was maintained while osteoprotegerin ligand itself decreased, thus amplifying the effect. IL-17 also influences osteoclast production probably via the osteoprotegerin ligand system, and this was also modulated by IL-4. With increasing knowledge of the molecular mechanisms of bony destruction, new therapeutic targets can be identified. These findings may have significant implications for the prevention of bone erosion in arthritis.

High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A sensitive mechanism.

M Ziolkowska, A Koc, G Luszczykiewicz, *et al. J Immunol* 2000; **164**: 2832–8.

BACKGROUND. Recent interest in IL-17 has elucidated a potentially important part for this T-cell-derived cytokine in triggering synoviocytes to produce pro-inflammatory mediators (IL-6, IL-8 and PGE_2), as well as mediating direct effects on chondrocytes. IL-15 is another T-cell and monocyte-derived cytokine that has been found to be elevated in rheumatoid synovial fluid. This study addresses the hypothesis that IL-15 triggers the production of IL-17 that in turn stimulates the production of other inflammatory mediators.

INTERPRETATION. Levels of IL-17 and IL-15 are correlated in patients with RA and OA and IL-15 is shown to induce IL-17 production; both methylprednisolone and cyclosporin are capable of inhibiting this induction. This may, in part, explain the therapeutic efficacy of cyclosporin in RA.

Comment

Peripheral blood and synovial fluid samples were obtained from 15 patients with RA (American Rheumatism Association 1987 criteria; mean disease duration 75 months) and eight patients with OA. Peripheral blood samples were also

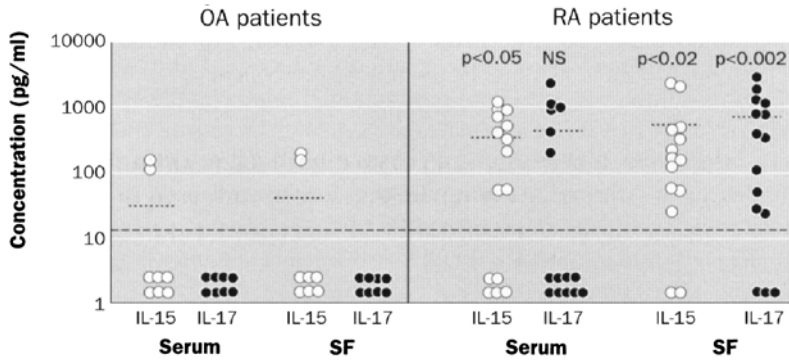

Fig. 3.2 Levels of IL-17 and IL-15 in serum and synovial fluids (SF) from RA and OA patients. The levels of IL-17 (●) and IL-15 (○) were measured by ELISA. -----, detection limits of the assays; ·····, mean value of each group. Statistical comparison between each group is shown as a *P*-value; NS=not significant (two-tailed Mann-Whitney *U*-test). Source: Ziolkowska *et al.* (2000).

obtained from 20 healthy individuals. Using an ELISA technique (incorporating controls to eliminate effects of rheumatoid factor), significantly elevated levels of both IL-15 and IL-17 were detected in the synovial fluid of rheumatoid patients. In the peripheral blood only IL-15 was significantly elevated with a non-significant trend detected for IL-17 (Fig. 3.2). Levels of IL-15 and IL-17 were significantly correlated in the rheumatoid patients ($p<0.02$).

In vitro assays demonstrated that 72 h of IL-15 treatment (at varying concentrations) induced IL-17 production from peripheral blood mononuclear cells and anti-IL-15 inhibited this effect. Other cytokines, previously implicated in RA (IL-6, IL-8 and TNF-α) failed to trigger IL-17 production. IL-1β also triggered IL-17 pro duction but was 30% less potent. This is the first report that IL-17 production is induced by IL-15. Given the evidence that both these cytokines contribute to the pathogenesis of RA this finding is important.

Peripheral blood mononuclear cells were pre-treated for 1 h with either cyclosporin or methylprednisolone at varying concentrations and both treatments were capable of abrogating the IL-15-induced secretion of IL-17. Inhibiting the downstream effects of IL-17 may be therapeutically important in RA. Cyclosporin has been shown to be beneficial as a disease-modifying antirheumatic drug (DMARD) at doses of 2–5 mg/kg per day. The effects observed in this study were at concentrations easily obtainable *in vivo* with these therapeutic doses. It is, therefore, likely that inhibition of IL-17 production is one of the therapeutic effects of cyclosporin.

Conclusion

IL-15 induces the production of IL-17, which itself can induce pro-inflammatory and cartilage damaging effects. The ability of cyclosporin to inhibit this effect represents another reason for using the DMARD in the treatment of RA.

Interleukin-12

IL-12 is a potent pro-inflammatory cytokine, secreted predominantly by monocytes and dendritic cells, which polarizes T-cell responses towards the T-helper (Th) 1 (cell-mediated immunity) phenotype, a profile associated with RA. It is a potent stimulator of interferon (IFN) -γ and TNF-α production and has been shown in experimental models of arthritis to accelerate disease progression. Synovial tissue from patients with RA has also been shown to express high levels of mRNA for IL-12. One animal study and one clinical case report published during 2000 have highlighted the potential involvement of this cytokine in RA and suggested that blocking this cytokine might be therapeutically beneficial.

Blockade of endogenous interleukin 12 results in suppression of murine streptococcal cell wall arthritis by enhancement of interleukin 10 and interleukin 1Ra.
L A B Joosten, M M A Helsen, W B van den Berg. *Ann Rheum Dis* 2000; **59**:196–205.

BACKGROUND. IL-12 is a produced by phagocytic cells, dendritic cells, B lymphocytes and natural killer (NK) cells. It is pro-inflammatory and has previously been shown to be capable of exacerbating collagen-induced arthritis. In this study the potential effects of blocking IL-12 were investigated in another experimental model (streptococcal cell wall-induced arthritis).

INTERPRETATION. Anti-IL-12 treatment improved chondrocyte function, suppressed arthritis and upregulated IL-10 and IL-1Ra levels, improving the balance of pro-inflammatory and anti-inflammatory cytokines.

Comment

Unilateral knee arthritis was induced in C57/B16 mice by injecting streptococcal cell wall preparations. Inflammation was measured by ^{99}mTc uptake and compared with the untreated knee, while chondrocyte proteoglycan synthesis (a read-out of IL-1 activity) was determined by patellar cartilage ^{35}S uptake. In accordance with previous work streptococcal cell wall injection caused an acute inflammatory response with inhibition of chondrocyte proteoglycan synthesis. IL-12 levels, measured by ELISA, increased rapidly following intra-articular injection of streptococcal cell wall preparations, along with TNF-α and IL-1 levels. A cohort of mice was pre-treated with an intraperitoneal injection of anti-IL-12 2 h prior to the knee injection. Although this did not alter inflammation in

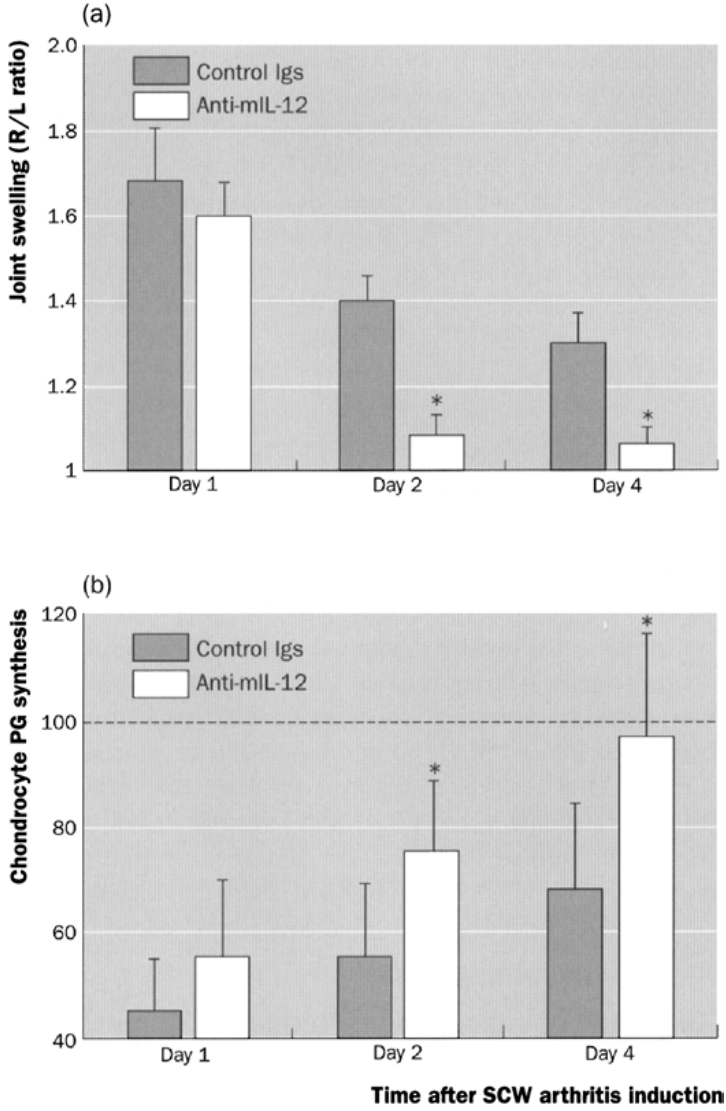

Fig. 3.3 Effect of anti-IL-12 treatment on joint swelling and chondrocyte PG synthesis. (a) Mice were given an intraperitoneal injection 2 h before induction of arthritis with either 0.5 mg rat anti-murine IL-12 or rat immunoglobulins. At days 1, 2 and 4 joint swelling was determined by ^{99m}TC uptake and expressed as R/L ratio. The data represent the mean (SD) of at least seven mice per group. (b) Chondrocyte PG synthesis was measured by ^{35}S-sulphate incorporation at days 1, 2 and 4 after induction of arthritis. The data represent mean (SD) percentage chondrocyte PG synthesis of the left control patella. *P<0.05, Mann-Whitney U-test, compared with rat immunoglobulin group. Source: Joosten *et al.* (2000).

the first 24 h, thereafter it significantly reduced the severity of the synovitis and improved proteoglycan synthesis. Subsequently, it was demonstrated that anti-IL-12 therapy was still efficacious up to 24 h following intra-articular injection of streptococcal cell wall preparations. Anti-IL-12 treatment had no effect on synovial TNF-α levels but did significantly reduce both IFN-γ and IL-1 levels. As would be anticipated it also significantly reduced IL-12 levels. Furthermore, synovial IL-1Ra and IL-10 levels, again determined by ELISA, were significantly increased by treatment. The findings in this experimental model suggest an important part for IL-12 in the early stages of inflammatory arthritis.

Conclusion

In this experimental arthritis model anti-IL-12 therapy appears efficacious. This provides further insight into the pathogenesis of inflammatory arthritis, but is less likely to be clinically relevant to RA. IL-12 is considered to be pivotal in controlling bacterial infection and has been shown to be critically important in the control of *Leishmania* infection in animal models. Humans deficient in IL-12 are at risk of infection by atypical *Mycobacteria* and *Salmonella,* both intracellular bacteria. The risks of infection and those of malignancy (highlighted below) might preclude long-term blockade of this cytokine as a therapeutic option. However, the effects of anti-IL-12 therapy on IL-10 and IL-1Ra levels support the need for continued investigation of these as therapeutic options (see above for recently published work on IL-1Ra).

Rheumatoid arthritis exacerbation caused by exogenous interleukin-12.
E Peeva, A D Fishman, G Goddard, S Wadler, P Barland. *Arthritis Rheum* 2000; **43**(2):461–3.

BACKGROUND. IL-12 is pro-inflammatory, stimulating and activating T cells and NK cells. In addition, in murine models of malignancy, interleukin-12 has been shown to mediate anti-tumour and anti-metastatic effects. As a result of this IL-12 therapy is being trialled in malignant disease. This case report highlights the potential involvement of IL-12 in RA.

CASE. A 53-year-old woman with mild seropositive RA, stable for two years and managed with non-steroidal anti-inflammatory drugs (NSAIDs), was admitted for IL-12 therapy for stage IV cervical carcinoma. Following treatment with intravenous IL-12 she developed a marked flare of her RA with diffuse synovitis, markedly elevated inflammatory markers and an increase in her rheumatoid factor titre. This settled following discontinuation of her trial treatment.

INTERPRETATION. Previous experimental models of inflammatory arthritis have implicated IL-12 in the pathology of arthritis. This case highlights the potential part this cytokine might play in clinical rheumatoid disease. It further suggests that anti-IL-12 therapy may be therapeutically beneficial.

However, given the theoretical anti-tumour role of IL-12, blocking its action may be associated with an unacceptable risk of malignancy.

The role of IL-12 in inflammatory activity of patients with rheumatoid arthritis (RA).
W-U Kim, S-Y Min, M-L Cho, *et al. Clin Exp Immunol* 2000; **119**:175–81.

BACKGROUND. To investigate the involvement of the monocytic cell derived pro-inflammatory cytokine, IL-12, in rheumatoid inflammation, a cohort of individuals with RA were studied during conventional antirheumatic treatments, and improvements were correlated with cytokine levels including those of IL-12.

INTERPRETATION. IL-12 was more frequently detectable in the serum, and levels were greater, in the patients with RA as compared with healthy controls or patients with OA. Furthermore, IL-12 levels reflected the activity of the RA and the results suggest that IL-12 is involved in the production of other pro-inflammatory cytokines.

Comment

One hundred and fifty-two patients (mean disease duration 78.4 months) with RA (American Rheumatism Association 1987 criteria) were recruited to the study. The patients were assessed clinically (using the American College of Rheumatology core set measures), and with laboratory measures, at study entry and 4 months later. The patients were compared with OA patients (69 patients) and healthy controls (50). Unfortunately, no information on the patients' treatment at the initial assessment is given. Furthermore, it is not clear whether recruitment to the study was dependent on disease activity, failed treatment or the need to change treatment.

Sera were isolated from the patients at commencement and after 4 months of treatment, although information on how treatment was allocated is not given. In addition, synovial fluid from patients with joint effusions was collected. IL-12, IL-2, IFN-γ, TNF-α, IL-6, IL-4 and IL-10 levels were measured by cytokine-specific ELISAs, using standard techniques.

Sixty-four (42.2%) of the sera of rheumatoid patients had detectable IL-12, compared with only one (1.4%) of the OA patients and five (10%) of the healthy controls ($p<0.001$). The median circulating levels of IL-12 were also significantly greater in the rheumatoid patients compared with the other groups. Similar resultswere obtained when comparing rheumatoid synovial fluid with fluid aspirated from patients with OA. These findings are in keeping with the detrimental effects associated with IL-12 in animal models of RA, and suggest an involvement of this cytokine in the clinical disease.

The rheumatoid patients were divided into those that had detectable levels of IL-12 (51 patients) and those that did not (57 patients). Elevated IL-12 levels

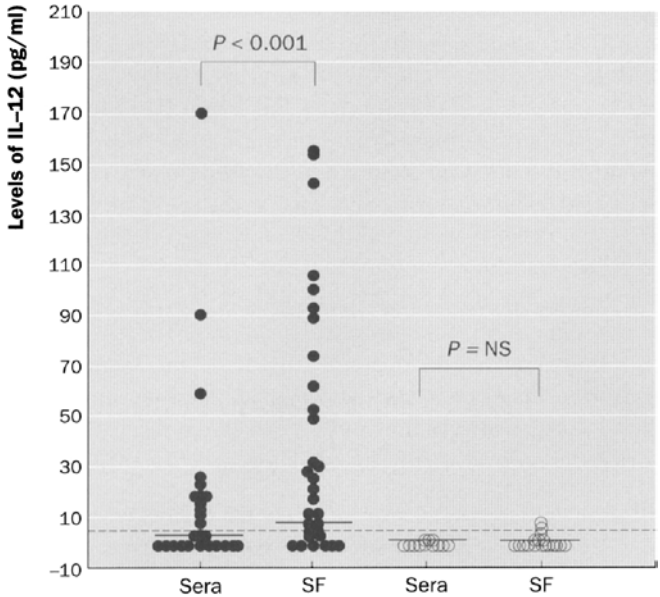

Fig. 3.4 The levels of IL-12 in paired sera and synovial fluid (SF) obtained simultaneously from patients with RA (●; n=53) and osteoarthritis (○; OA) (*n*=22). Broken line indicates the limit of detectable concentration of IL-12 (5 pg/ml). Bars represent median levels. Comparison of median IL-12 levels between sera and SF was performed using paired Wilcoxon signed ranks test. NS, not significant. Source: Kim *et al.* (2000).

significantly correlated with tender joint counts, swollen joint counts, visual analogue scores, physicians' global assessments, patients' global assessments and C-reactive protein. Curiously, no correlation was found between IL-12 and erythrocyte sedimentation rate. As no information is given on treatments at the baseline assessments, these results are difficult to assess as they may merely reflect well-controlled disease in a proportion of the patients studied. However, these results are in keeping with those in juvenile chronic arthritis where elevated levels of IL-12 again correlated with disease activity.

Fifty-seven of the patients were selected for further study. Although no information is given on the selection criteria, information on disease activity, demographics and previous therapy for these individuals is provided. The clinical variables were studied over a 4 month period of treatment with low-dose prednisolone, NSAIDs and a variety DMARDs, including methotrexate, antimalarials, sulphasalazine, bucillamine and gold. 46.5% of these patients were improved following treatment and the improvement correlated with reductions in the serum IL-12 levels. This suggests that currently available treatments can effectively influence the levels of IL-12, although this study did not address which of the above treatments may be mediating this effect. No data are

currently available on the IL-12 modulation associated with DMARDs, but other recently published work (also reviewed in this chapter) suggests that corticosteroids can inhibit IL-12 production.

To investigate possible effects of IL-12 on other cytokines, correlations between IL-12 and changes in other cytokines were determined. There was a positive correlation between IL-6 and TNF-α, suggesting that IL-12 production is closely linked to the production of other pro-inflammatory cytokines. A negative correlation with IL-10 was found, consistent with previous reports that indicate that IL-12 is impor- tant in favouring the production of Th1 cytokines.

Conclusion

Despite some methodological limitations, this study suggests that IL-12 levels reflect clinical activity in RA and implies that IL-12 production is linked to the secretion of other pro-inflammatory cytokines, in keeping with previously published work.

Inhibition of Th1 immune responses by glucocorticoids: dexamethasone selectively inhibits IL-12-induced Stat4 phosphorylation in T lymphocytes.

D Franchimont, J Galon, M Gadina, *et al. J Immunol* 2000; **164** (4): 1768–74.

BACKGROUND. T-helper cells are divided into two broad subsets (Th1 and Th2), dependent on the cytokines they produce. Th1 cells secrete predominantly IFN-γ while Th2 cells produce mainly IL-4, IL-5 and IL-10. Models of inflammatory arthritis and clinical studies of RA suggest that Th1 cells promote the development of disease while Th2 cells are protective and may attenuate arthritis. Previous studies have indicated that glucocorticoids inhibit Th1 immune responses and enhance the secretion of Th2 cytokines. This study examined the effects of glucocorticoid treatment on the signalling pathway of IL-12, the cytokine principally responsible for the development of Th1 T cells. Given the documented benefits of glucocorticoids in RA this study is of rheumatological relevance.

INTERPRETATION. Glucocorticoids can block the effects of IL-12 by inhibiting the receptor-associated intracellular signalling pathway, without altering the signalling of Th2 cytokines, thereby shifting the cytokine profile of T cells towards a Th2 phenotype. As IL-12 has been shown to have detrimental effects in RA, this inhibitory effect of glucocorticoids supports a potentially disease-modifying role for steroid treatment.

Comment

Glucocorticoids have previously been shown to inhibit IL-12 production from monocytes. This study investigated whether it also affects the ability of cells to

respond to IL-12. A line of NK cells (NK3.3) and T lymphocytes were used in this study of the cellular effects of IL-12.

Recombinant human IL-12 (10–20 ng/ml) was used to stimulate both NK cells and T cells. The production of IFN-γ, a pro-inflammatory cytokine released following IL-12 stimulation, was measured by ELISA. Pre-treatment of cells with dexamethasone for 6 h significantly inhibited the production of IFN-γ.

To determine whether these effects of IL-12 were mediated by downregulation of the IL-12 receptor, mRNA levels for this receptor were measured using an RNase protection assay, in T cells and NK cells, with and without up to 18 h dexamethasone treatment: no changes were detected. This is in contrast to a previous study in which dexamethasone did reduce the levels of the IL-12 receptor but in that study cells were incubated for up to 3 days with glucocorticoid. The findings in this study implied that dexamethasone was exerting its effects downstream of the receptor.

To assess intracellular signalling, which initially occurs following IL-12 receptor ligation by the phosphorylation of Jak2 and Tyk2, the phosphorylation status of these proteins was assessed by immunoblotting. No difference in phosphorylation status was detected following dexamethasone treatment.

The next component of the IL-12 receptor signalling pathway is the recruitment and phosphorylation of the Stat (Signal Transduction and Transcription) protein, Stat 4, which can initiate gene transcription. The phosphorylation status of this protein was also assessed by immunoblotting ,and dexamethasone was shown to inhibit the phosphorylation of Stat 4. This effect was seen in both T cells and NK cells and was noted using doses of dexamethasone as low as 10^{-10}M. The mechanism by which dexamethasone exerts this effect on Stat 4 was not elucidated. IL-4, a Th2 cytokine, with potentially beneficial effects in RA signals through another Stat protein, Stat 6. Phosphorylation of this protein following IL-4 treatment was unaffected by pre-treatment of the cells with dexamethasone. This is in keeping with the previously reported ability of glucocorticoids to shift the cytokine profile from Th1 to Th2.

Conclusion

This study indicates that glucocorticoids can inhibit not only the production of IL-12, but also the ability of cells to respond to this cytokine. The ability of glucocorticoids to inhibit Th1 responses has been well described previously but this study suggests a mechanism for this action. Lowering T-cell responsiveness to IL-12 in RA might have considerable therapeutic benefit and this supports the need for further clinical studies of corticosteroids to assess the lowest doses at which therapeutic efficacy can be elicited.

Contribution of 0X40/0X40 ligand interaction to the pathogenesis of rheumatoid arthritis.
T Yoshioka, A Nakajima, H Akiba, *et al. Eur J Immunol* 2000; **30**(10): 2815–23.

BACKGROUND. Anti-TNF-α antibodies are assuming a major role in the treatment of RA. However, there is a large family of TNF-α-like factors and their corresponding receptors, most of which have functions that are important to very aspects of the immune system. Several are potential targets of future therapies. This paper investigates one of the least well characterized TNF-like molecules, 0X40 ligand (OX40L) and its receptor 0X40. Interaction of OX40L with its receptor 0X40 is thought to be important in T-cell activation through T-cell/antigen-presenting cell interaction. However, involvement of these molecules in the pathogenesis of RA remains unclear. To explore the contribution of OX40/OX40L interaction to the pathogenesis of RA *in vivo,* the effect of a neutralizing anti-OX40L monoclonal antibody (MoAb) on the development of collagen-induced arthritis in DBA/1 mice was evaluated. Administration of anti-OX40L MoAb into type II collagen (CII)-immunized DBA/1 mice dramatically ameliorated the disease severity. *In vivo* treatment with anti-OX40L MoAb did not inhibit the expansion of Cll-reactive T cells, but suppressed IFN-γ and production of antibodies to type II collagen of the IgG2a subclass (this subclass requires IFN-γ producing T cells, often termed Th1 cells). In addition, in studies of synovial fluid and synovial tissue from RA patients T lymphocytes expressed OX40, while OX40L was expressed on sublining cells in synovial tissue.

INTERPRETATION. The OX40/OX40L interaction appears to play a crucial part in the development of collagen-induced arthritis by enhancing Th1-type autoimmune response. The finding that the same molecules are present in the RA joint raises the possibility that their interaction may play a crucial part in the development of RA.

Comment

An interesting paper, albeit with a misleading title. All the functional data relate the collagen-induced arthritis in the mouse and, although these are convincing, it is possible to ameliorate arthritis in this model by any number of immunological interventions. The data from human RA are only observational, but the presence of OX40+ T cells in the synovial fluid and not in matched peripheral blood combined with the expression of OX40L in synovial tissue (but not synovial fluid) does suggest that this interaction might well play an important part in RA. An attractive possibility is that interference with the interaction, especially if it could be achieved only locally, might be minimally immunosuppressive, as in the animal model T-cell and antibody responses to antigen were not abolished, merely modulated to be less pro-inflammatory.

Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function.
J Parrish Novak, S R Dillon, A Nelson, *et al. Nature* 2000; **408** (6808): 57–63.

BACKGROUND. Novel cytokines continue to be described; the starting point nowadays is not a biological activity but an expressed sequence tag, which is found to have some similarity with known cytokines or cytokine receptors. In this case a receptor with homology to known class I cytokine receptors (those for IL-2–7, 9, 11–13 and 15) was discovered and characterized, and later used to isolate the ligand, the novel cytokine IL-21. The receptor is selectively expressed in lymphoid tissues and is capable of signal transduction. The cytokine was produced by activated human CD3+ T cells supported proliferation of a assay cell line, which was transfected with the IL-21 receptor. Structurally, IL-21 is most closely related to IL-2 and IL-1, and it is mainly produced by CD4+ T cells. *In vitro* assays suggest that IL-21 plays a part in the proliferation and maturation of NK cell populations from bone marrow, in the proliferation of mature B-cell populations co-stimulated with anti-CD40, and in the proliferation of T cells co-stimulated with anti-CD3.

INTERPRETATION. A novel cytokine and its receptor have been cloned and characterized. Initial results suggest that its activity is confined to lymphoid tissues.

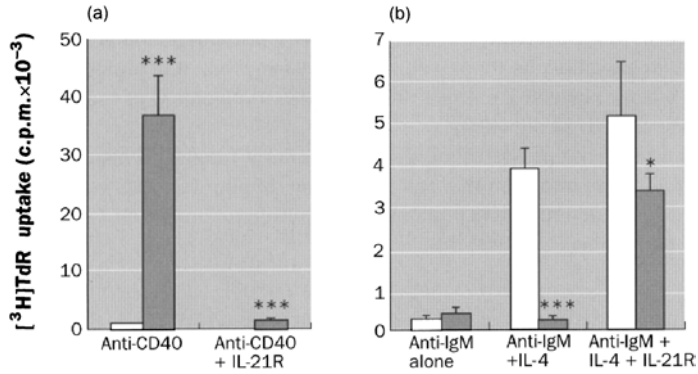

Fig. 3.5 Effect of IL-21 on the proliferative responses of human B cells. B cells were purified from peripheral blood of normal human donors and cultured either in the absence (open bars) or in the presence (filled bars) of 10 ng/ml^{-1} IL-21. (a) Anti-CD40 and 25 μg/ml^{-1} slL-21R were used as additional stimuli as indicated. (b) Combinations of immobilized anti-lgM, IL-4 and 25 μg/ml^{-1} sIL-21R were used as additional stimuli as indicated. Values plotted represent the mean value (±SD) obtained from triplicate cultures. Similar results were obtained in four independent experiments. *P<0.05; **P<0.005; ***P<0.001. Source: Parrish *et al.* (2000).

Comment

There are no studies to indicate whether this cytokine plays any part in inflammatory arthritis. It would not be surprising if it were expressed in the T-lymphocyte-rich tissue of the rheumatoid synovium, and its activities would appear to modulate other components of the immune system, including other T cells. Interestingly, it can have both positive and negative effects on B cells, according to the other stimuli that the B cells receive and may, therefore, modulate autoantibody production. Results in cytokine and receptor gene targeted mice will provide further information on its potential to be involved in inflammatory processes.

Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12.

B Oppmann, R Lesley, B Blom, *et al. Immunity* 2000; **13**:715–26.

BACKGROUND. As with IL-21 (see above) the starting point was the identification of a novel sequence discovered in a computational screen of a molecule distantly related to one of the subunits of IL-12 (p35)—IL-12 consists of two subunits, p35 and p40, with p40 acting as a 'soluble receptor' for p35 and allowing binding to the cell surface bound IL-12 receptor. The new molecule, termed p19, shows no biological activity by itself, but also combines with the p40 subunit of IL-12 to form a novel, biologically active, composite cytokine, which we term IL-23. Activated dendritic cells secrete detectable levels of this complex. The receptor for IL-12 also has two chains: IL-12Rβ1 and IL-12Rβ2; IL-23 binds to IL-12Rβ1 but fails to engage IL-12Rβ2. The second chain of the IL-23 receptor has not yet been identified, and is likely to be the one involved in signalling as there was no effect seen when IL-23 bound IL-12Rβ1. However, a similar signalling pathway is used, with involvement of the factor, Stat 4, which is critical to IL-12 signalling.

IL-23 induces strong proliferation of mouse memory T cells, a unique activity of IL-23 as IL-12 has no effect on this cell population. Similar to IL-12, human IL-23 stimulates IFN-γ production and proliferation in mitogen-stimulated T cells, as well as in CD45RO+ (memory) T cells.

INTERPRETATION. A novel cytokine closely related to IL-12 has been described—one chain of both the dimer cytokine and its receptor is shared with IL-12.

Comment

As IL-12 plays an important part in infection and autoimmune inflammation, IL-23 may prove to be implicated in inflammatory joint disease (as has the other IL-12-related cytokine, IL-18). It is made by the same kinds of cell that make IL-12, particularly antigen-presenting cells such as dendritic cells. There is a

suggestion that IL-23 production may precede IL-12 production, because the p19 molecule is not N-glycosylated, unlike the p35 molecule of IL-12, which requires this modification for activity. Unpublished results show that uncontrolled expression of p19 (and therefore IL-23) in transgenic mice, leads to systemic inflammation and death. Because IL-23 and IL-12 share a subunit, it will be necessary to reinterpret experiments using blocking antibodies that bind the shared subunit, as the effects of these antibodies will be due to blocking both IL-12 and IL-23. Such antibodies block collagen-induced arthritis, so IL-23 may well play a part in this experimental joint disease. Likewise the phenotype of patients with defects in p40 or IL-12Rβ1 (susceptibility to intracellular infection) represents a combination of IL-12 and IL-23 deficiency.

Overall conclusions on anticytokine therapy

An increasing number of cytokines have been implicated in the pathogenesis of RA and interest over the past 12 months has focused on three of these, in addition to TNF-α. A potential model, albeit simplified, as to how all these cytokines are involved in RA is illustrated in Fig. 3.6. Blockade of each of the cytokines discussed above (IL-1, IL-17 and IL-12) appears to be therapeutically beneficial. However, at least for IL-12, the promalignant effects potentially associated with such a blockade may preclude clinical use.

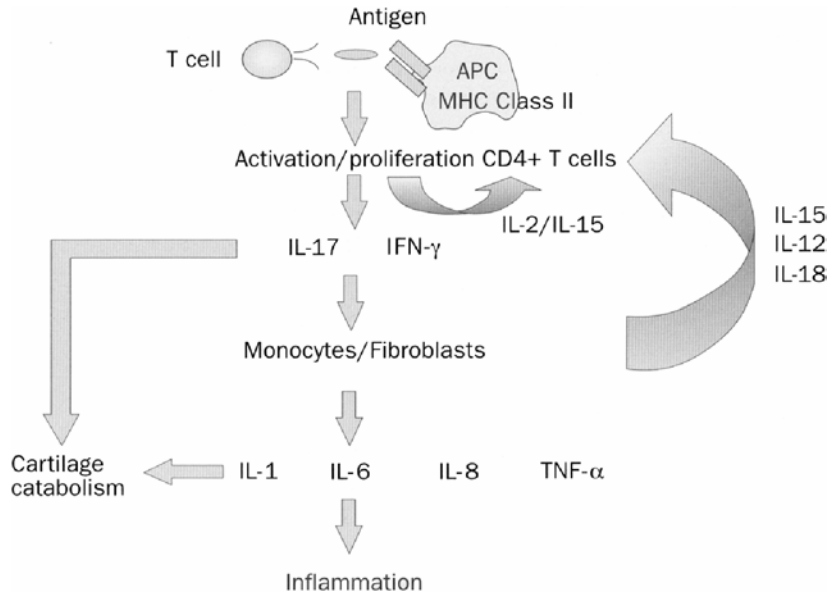

Fig. 3.6 A simplified diagram of the potential interactions of different cytokines in the pathogenesis of RA.

Work over the past 12 months has given insight into the roles of these cytokines in disease pathogenesis. The occurrence of a spontaneous inflammatory arthritis in IL-1Ra knockout mice suggests an aetiologically important part for this cytokine and it will be interesting to assess whether a subgroup of rheumatoid patients is similarly genetically predisposed. The finding that treatment with IL-12 (for malignancy) resulted in a flare of disease (albeit only in a single report), suggests that upregulation of this cytokine could be an important trigger to disease flares and this assumption is supported by the elevated levels of IL-12 that correlated with disease activity in another study. The effects of both IL-1 and IL-17 on cartilage metabolism indicates that these cytokines may be particularly important in this aspect of rheumatoid disease and the finding that treatment with IL-1Ra retards radiographic changes is a promising development.

While the efficacy of anti-TNF therapies has highlighted the importance of monokines in the pathogenesis of rheumatoid disease, several of the papers reviewed also emphasize a potential role for T-cell-derived cytokines such as IL-17 and IL-4, and of other TNF family members involved in T-cell activation.

In addition to highlighting potentials for new therapeutic interventions the recently published work indicates that current treatment modalities can also be used to impact these cytokines. Prednisolone treatment could inhibit both IL-12 and IL-17 production. Appropriate physiotherapy can antagonize the effects of IL-1 β in inflammatory arthritis, while cyclosporin can inhibit IL-17 production. An increased understanding of the mechanisms of action of the current therapies may enable more tailored use of these treatments in future.

Part II

Pathogenesis of rheumatoid arthritis

4
Genetics of rheumatoid arthritis

In 1978 Stastny |**1**| first reported an association of HLA-DR4 with rheumatoid arthritis (RA). Subsequent studies of the HLA class II region have shown several HLA-DRB1 genes (DRB1*0401, *0404, *0405, *0408, *0101, *0102, *1001, *1402) to be associated with disease. Sequencing of these genes has shown that all the alleles associated with disease share an amino acid sequence (QKRAA, QRRAA or RRRAA) in a region of the gene critical for the protein's function in the presentation of antigens to T cells. This conserved sequence is commonly termed 'the shared epitope' (SE). The presence of the SE appears to be associated with more severe disease outcome as hospital-based studies have shown a stronger association than community-based studies. However, RA is a complex polygenic disease and at least 25% of patients with RA lack the SE. Other recent studies have endeavoured to localize other genes that may be involved in disease. Candidate genes studied in the past 12 months include those encoding tumour necrosis factor (TNF), corticotrophin-releasing hormone (CRH) and mannose binding lectin (MBL), all of which may contribute either to susceptibility or severity of RA.

Several recent papers have explored the relationship between the SE and aspects of rheumatoid disease. These have addressed the following issues:

1. Does knowledge of the SE early in disease allow one a better understanding of prognosis and therefore contribute to clinical decision making?
2. What aspects of the disease are affected by the presence of the SE?
3. How does presence of the SE influence the radiographic changes of RA?
4. Could the SE be a marker for other genetic polymorphisms which influence the disease?

Further papers have investigated similar questions for the other candidate genes:

1. Is there evidence for involvement of a particular gene?
2. What aspects of the disease are affected by a particular gene?

The influence of HLA-DRB1 alleles and rheumatoid factor on disease outcome in an inception cohort of patients with early inflammatory arthritis.

B Harrison, W Thomson, D Symmons, *et al. Arthritis Rheum* 1999; **42**(10): 2174–83.

BACKGROUND. The association of certain HLA-DRB1 alleles with rheumatoid disease has been attributed to a conserved sequence in the third hypervariable region of these alleles known as the SE. As the association has been greatest in hospital-based studies this has led to the suggestion that the epitopes presence is a marker of disease severity rather than susceptibility. The Norfolk Arthritis Register (NOAR), established in 1989, documents all patients presenting to primary care in Norfolk, UK with newly diagnosed arthritis. This study used patients on the NOAR to assess whether diseases outcome at 2 years was significantly influenced by the SE.

INTERPRETATION. No influence of the SE was seen in early arthritis on the likelihood of disease persistence and only a modest effect on functional disability was documented. The SE did significantly increase the risk of development of erosions, but did not affect the severity of radiological damage.

Comment

In order to investigate whether the presence of the SE (QKRRAA) can be used to predict progression of RA 532 patients were recruited from the NOAR. The disease outcome at 2 years was assessed in order to determine the influence of HLA-DRB1 alleles. Disease outcome was measured by: (a) presence of persistent disease; (b) functional disability—health assessment questionnaire; and (c) radiological damage—Larsen score. The median duration of disease at recruitment was 28 weeks and 77% of patients had a disease duration of <1 year. After 2 years 79% of the recruited patients had persistent disease. There was no significant influence of the SE on disease progression or on disability (as measured by the health assessment questionnaire). There was, however, an association with the risk of developing erosions (relative risk 1.9; 95% CI 1.4–2. 6) and the presence of at least one SE allele. This effect was greater in individuals homozygous for the sequence. However, this effect was only apparent in individuals seronegative for rheumatoid factor at baseline and had no influence on the amount of damage beyond the presence of erosions.

Conclusion

This study highlights the potential involvement of the SE as a disease severity marker rather than a marker of susceptibility. Specifically in rheumatoid factor negative patients it is a risk factor for erosive change. However, in terms of clinical practice, the findings do not suggest that a knowledge of SE status would

Table 4.1 Positive predictive values for the development of erosions according to baseline rheumatoid factor (RF) and shared epitope (SE) status

Risk group	No. (%) of patients with erosions (PPV*)
RF positive (n = 118)	77 (65)
RF negative (n = 241)	69 (29)
RF positive/SE positive (n = 80)	54 (68)
RF positive/SE negative (n = 34)	19 (56)
RF negative/SE positive (n = 134)	52 (39)
RF negative/SE negative (n = 106)	17 (16)

*PPV=positive predictive value.
Source: Harrison *et al.* (1999).

influence decision making and its routine testing would therefore not be justified.

The influence of HLA-DRB1 alleles encoding the DERAA amino acid motif on radiological outcome in rheumatoid arthritis.

D L Mattey, A B Hassell, M J Plant, *et al. Rheumatology* 1999; **38**(12):1221–7.

BACKGROUND. In addition to the susceptibility epitope (QKRRAA) there is also evidence that certain other HLA-DR alleles are protective. These potentially protective epitopes are characterized by another shared amino acid sequence (DERAA). This study explored the radiological severity of RA, as measured by the Larsen score, in association with HLA-DRB1 typing to assess whether combinations of alleles encoding susceptibility and/or protective motifs modulate radiological outcome.

INTERPRETATION. HLA-DRB1 alleles encoding the DERAA motif may be protective against severe erosive disease, but any beneficial effect is overridden by the presence of a single, SE-containing allele.

Comment

A cohort of 315 Caucasian patients from North Staffordshire (UK) with long-standing rheumatoid disease (mean disease duration 12.2 years; SD 5.2) was recruited to this study. Patients were studied over at least 5 years of follow-up. HLA-DRB1 typing was undertaken by polymerase chain reaction (PCR) using a panel of sequence-specific oligonucleotide probes. Patients homozygous for the

Table 4.2 Influence of the RAP epitope (DERAA) and shared epitope (SE) on Larsen score

	n	**RAP − Larsen score mean (S.D.)**	*n*	**RAP + Larsen score mean (S.D.)**
All patients	281	94.4 (44.1)	34	89.1 (48.0)
SE −	51	88/2 (54.0)*	14	65.6 (45.9)
SE +	230	95.8 (41.5)‡	20	105.6 (43.2)†

P values (uncorrected)
**P*=0.05
†*P*=0.02
‡*P*=0.003
P values corrected for multiple testing.
*P=0.*05 (vs RAP+/SE−)
*P=0.*04 (vs RAP+/SE−)
*P=0.*009 (vs RAP+/SE−)
RAP=*0103, *0402, *1102, *1103, *1301, *1302.
Source: Mattey *et al.* (1999).

SE had significantly higher Larsen scores (mean 96.9; SD 39) than those negative for that sequence (mean 83.3; SD 53) (*P*=0.004). No significant conclusions could be drawn about those individuals with only a single allele of the SE. The association of more severe radiographic changes with the SE in this large study suggests the need for further investigation of its potential role in the development of erosive change. The presence of the protective amino acid sequence (DERAA) in the absence of the SE reduced the severity of radiographic change suggesting an additional level of complexity of HLA-DRB1 allele function in disease susceptibility. However, it is noteworthy that there was no evidence that the protective sequence could modify the effects of the SE. In SE heterozygotes there was no beneficial effect associated with having an additional protective motif encoded by the other allele. The failure to demonstrate protection may be related to a lack of appropriate patients as even in this large cohort the number of DERAA allele positive patients was still relatively small. Alternatively, it may reflect a dominant pro-erosive effect of the SE. Previous studies of the DERAA motifs have suggested that its presence prevents the onset of rheumatoid disease. As, in this study, patients with RA, carrying the protective sequence (DERAA), could be defined, it suggests that any protective effects associated are not adequate to prevent disease development. This study did not endeavour to address any potential mechanism by which the DERAA motif could mediate protection and this is an area for future research.

Conclusion

In addition to the detrimental effects of the SE, other HLA-DRB1 alleles can exert beneficial effects. The detrimental effects appear to be dominant as any

beneficial effects are abrogated by a single SE allele. Whether genotyping, of rheumatoid patients, HLA-DRB1 genes would allow prognostic prediction to be undertaken at an individual level remains an unanswered question.

The predictive value of the HLA shared epitope for severity of radiological joint damage in patients with rheumatoid arthritis. A 10 year observational prospective study.
A Valenzuela-Castano, A Garcia-Lopez, D Perez-Vilches, *et al. J Rheumatol* 2000; **27**(3):571–4.

BACKGROUND. **Recent studies have suggested that the presence of SE containing alleles predicts the subsequent development of erosive change. This study sought to address the question of whether the presence of the SE offers useful prognostic information by following a cohort of early arthritis patients.**

INTERPRETATION. This study showed that the SE is generally not a good predictor of radiographic progression over the first 10 years of disease, but did not address influences of the allele on events in the intervening period.

Comment

Eighty-two Spanish patients, with a mean disease duration of 8.1 months (95% CI 6.7–9.6) at baseline, were analysed over a mean follow-up period of 9 years. HLA-DRB1 typing for the SE and 14 patients were homozygous for a SE associated HLA-DRB1, 36 carried a single allele while 32 were negative for the sequence. At the study entry clinical data, laboratory data (rheumatoid factor, erythrocyte sedimentation rate [ESR], haemoglobin) and radiographic data were collected. Radiographic progression was assessed by a modified Sharp score. After 10 years of follow-up the factors associated with (later development of) severe disease were the ESR, seropositivity for rheumatoid factor and modified Sharp score at baseline. In this study the presence of the SE had no overall effect on the severity of joint damage, although the lack of significance demonstrated may be due to the relatively small number of patients involved. Other work has suggested that patients with the SE develop erosive disease earlier, but subsequent progression is not affected. This study may have missed such an effect by using assessments separated by 10 years. Analysis of large joint involvement showed increased erosive change in those patients carrying two SE alleles. The finding that large joint involvement is increased in those homozygous for the SE is in agreement with previously published data indicating a higher frequency of joint replacements in this group.

Conclusion

Factors such as the ESR and rheumatoid factor, and initial radiographic assessments, were better prognostic predictors than the HLA-DRB1 status in this study.

Interaction between tumor necrosis factor microsatellite polymorphisms and the HLA-DRB1 shared epitope in rheumatoid arthritis.

D L Mattey, A B Hassell, P T Dawes, W E R Ollier, A Hajeer. *Arthritis Rheum* 1999; **42**(12):2698–704.

BACKGROUND. TNF-α is considered to be one of the most important cytokines in rheumatoid disease and the clinical effectiveness of current anti-TNF treatments supports this. The TNF gene is situated on chromosome 6 in close proximity to the HLA-DR locus within the major histocompatibility complex (MHC). Polymorphisms in a number of microsatellite markers in the TNF locus have been described. Previous studies have suggested involvement of two of these polymorphisms (TNF-α6 and TNF-α11) in rheumatoid severity, albeit in different patient subgroups. Using the defined microsatellite markers within the TNF gene, this paper investigated whether the previously described polymorphisms within these microsatellites influence disease development and whether the influences of the SE on disease severity may itself be modified by such polymorphisms.

INTERPRETATION. The association of the SE with disease severity in RA is influenced by an interaction with the TNF-α6 microsatellite polymorphism.

Comment

One hundred and fifty-seven patients from North Staffordshire (UK) were studied and disease severity was assessed using measures, including the Larsen scoring of radiographs, health assessment questionnaires and Visual Analogue Scale assessment of global disease. TNF-α microsatellite and HLA-DRB1 genotyping was undertaken and interactions, between TNF-α polymorphisms and the SE, were examined using multiple regression analysis. No single TNF-α polymorphism, in isolation, was associated with disease severity in RA in line with previous studies. However, polymorphisms in the TNF-α6 allele in association with the TNF-α11 allele influenced various outcome measures, most significantly the functional outcome (as measured by the Health Assessment Questionnaire) generally being associated with more severe disease. Studying the interaction between the SE and the TNF-α6 allele showed a significant effect that could not be accounted for solely by linkage disequilibrium. This effect was most marked in females, although a similar trend was seen in males; however, smaller numbers may have prevented the effects in males reaching statistical significance. As both these genes are encoded on chromosome 6 it is conceivable that these two genes constitute part of an extended haplotype contributing to

disease severity. Certainly the increasing genetic complexity indicated by this study suggests that measurement of the SE in isolation is likely to be unproductive.

Conclusion

The interaction between the SE and polymorphisms in the TNF-α gene shown in this study, implicates a gene, the product of which is known to be pivotal in rheumatoid disease. As the effects demonstrated are not fully explained by linkage disequilibrium, other mechanisms by which the SE influences disease still require exploration.

High serum levels of pro-matrix metalloproteinase-3 are associated with greater radiographic damage and the presence of the shared epitope in patients with rheumatoid arthritis.

N T Cheung, P T Dawes, K V Poulton, *et al. J Rheumatol* 2000; **27**(4):882–6.

BACKGROUND. The matrix metalloproteinases (MMPs) are zinc-dependent enzymes capable of degrading the extracellular matrix and potentially contributing towards the cartilage destruction of RA. MMP-3 (stromelysin 1) is the most abundant MMP in the rheumatoid synovium where it is found predominantly as an inactive precursor— proMMP-3. In addition to its identification in the synovium, this group and others have shown that levels of the precursor are elevated in the serum of patients with RA. This study sought to determine whether there was a relationship, in rheumatoid patients, between serum proMMP-3 levels, radiographic damage and the presence of the SE.

INTERPRETATION. Serum proMMP-3 levels were consistently correlated with radiographic damage. In addition, serum proMMP-3 levels were significantly higher in patients homozygous for the SE compared with SE negative patients, an effect most noticeable in early disease.

Comment

This was a large study with blood samples taken from 337 patients with RA (American Rheumatism Association [ARA] 1987 revised criteria) recruited from the North Staffordshire region, UK. Patients were divided into those with early disease (<3 years; *n*=45) and those with established disease (*n*=292). Patients were radiographically assessed and scored using the Larsen scoring system. Functional (Health Assessment Questionnaire), clinical (objective and subjective visual analogue scales) and laboratory (C-reactive protein [CRP], ESR and rheumatoid factor) assessments were also undertaken. ProMMP-3 was measured by a double antibody enzyme-linked immunosorbent assay (ELISA) and the SE by genotyping. For SE analysis patients were stratified into homozygotes (SE+/+),

heterozygotes (SE+/−) and negatives (SE−/−). Correlations were estimated using the Spearman rank method and proMMP-3 levels were found to correlate significantly with the Larsen score (unlike CRP and ESR) even after corrections for multiple testing ($P< 0.0001$). This result is surprising as only a single measure of proMMP-3 is made and erosive change may take many months to develop. Nonetheless, in a large cohort such as this, it is unlikely such a result would have arisen by chance. As in other studies, proMMP-3 levels were correlated with the CRP, perhaps reflecting the ability of TNF-α and interleukin-1 to induce the production of both. However, the correlation of proMMP-3 with joint destruction suggests it is more than just a marker of the acute phase response.

In the SE+/+ patients significantly higher levels of proMMP-3 were also found and this correlated with greater erosive change in these patients. This correlation was independent of the stage of the disease, but more noticeable in the early arthritis patients. This study, although illuminating a possible reason for the previously documented associations between the SE and erosive change does not clarify the mechanism that links the two. Another gene in linkage disequilibrium with the SE, such as the TNF-α gene, may be important.

From a practical perspective it is noteworthy that, although correlations were demonstrable on a population basis, large variations in the levels measured in different individuals were reflected in the wide standard deviations; this suggests measurement of proMMP-3 by this method would have only limited clinical usefulness. Longitudinal studies of proMMP-3 levels, in early arthritis patients, over time would be of interest to assess whether persistent elevation was predictive of subsequent erosive change and, therefore, applicable to therapeutic decision making.

Conclusion

This study shows that proMMP-3 levels are associated with both erosive disease and the presence of the SE. This finding may partly explain the association between the SE and more erosive disease. It is, however, not clear how the SE influences proMMP-3 levels and the possibility of other genes in linkage disequilibrium with the SE requires further study. The TNF-α gene would be a good candidate as TNF-α is known to influence MMP-3 expression.

Primary association of tumour necrosis factor-region genetic markers with susceptibility to rheumatoid arthritis.
A Martinez, M Fernandez-Arquero, D Pascual-Salcedo, *et al. Arthritis Rheum* 2000; **43**(6):1366–70.

BACKGROUND. Despite the interest in the SE as a susceptibility marker for RA, in a significant percentage of rheumatoid patients (at least 25%) it is not present. As the cytokine TNF-α appears to serve a pivotal role in rheumatoid disease, this study sought to determine whether polymorphisms

in the gene for TNF (particularly microsatellites α6/β5) were associated with susceptibility, independently of the HLA-DR SE. The genes encoding TNF-α are found within the MHC, and linkage disequilibrium of genes across this entire region has previously made interpretation of the influence of other genetic polymorphisms, in addition to the SE, difficult.

INTERPRETATION. The TNF-α6/β5 microsatellite pair is an independent marker of RA susceptibility and increases the susceptibility associated with the SE.

Comment

Several previous papers have highlighted associations between TNF-α/β microsatellites, particularly TNF-α6/β5, and disease susceptibility. This group, therefore, studied the inheritance of TNF-α6/β5 in a set of families with RA to determine whether there was an HLA-DR independent association with disease. Fifty-two Spanish families with an affected member were studied, by examining both the patient and his/her parents. In 19 of the 52 families one of the parents was also affected. The study entry criteria depended on both parents being alive; therefore, the study focuses primarily on patients with an early age of disease onset. DNA was extracted from peripheral blood leucocytes and the TNF-α and β microsatellites amplified with suitable primers. Twelve alleles of TNF-α (TNF-α1–α11 and α13) and five alleles of TNF-β (TNF-β2 and β6 were not present in this study) were detected. HLA-DRB1 typing was undertaken using PCR and hybridization allowing determination of the presence or absence of the SE. TNF promoter genotyping was also undertaken using probe hybridization. A total of 202 haplotypes were deduced and the allele frequencies of transmitted (any haplotype in a heterozygous parent as well as in the patient) and non-transmitted haplotypes compared by a χ^2 test.

The α6/β5 pair was transmitted from heterozygous parents 22 times, and not transmitted four times, a significant association (P=0.0002). To exclude an influence of the SE, a stratified analysis was undertaken in patients without the SE. In this case the α6/β5 pair was transmitted in nine of the SE negative and non-transmitted in three, again a significant association (P=0.004). This was the first time such an association has been shown in patients negative for the SE, possibly because SE negative rheumatoid patients are observed relatively frequent in southern European countries allowing appropriate levels of statistical significance to be obtained. The data show that TNF-α6/β5 not only confers susceptibility independent of the SE (odds ratio 6.71), but also increases the susceptibility conferred by the SE (from an odds ratio of 5.13 to 8.80). Interestingly, none of the other TNF-α/β alleles was predominantly transmitted, in contrast to previous studies which have observed preferential transmission of α2/β3. To exclude a linkage of α6/β5 with TNF promoter polymorphisms the TNF promoter was also studied and no association was found between promoter polymorphism and susceptibility to RA. The associations described support a

role for TNF in disease pathogenesis, but do not exclude an involvement of other nearby genes, in linkage disequilibrium, such as those coding for lymphotoxin. This study does not address the potential functional implications of the TNF-α6/β5 pair on levels of TNF or on responses to anti-TNF treatments, questions that future studies may address.

Conclusion

This study not only confirms the polygenic nature of RA, but also reinforces the role of TNF in the disease pathogenesis of RA. Furthermore, it demonstrates that polymorphisms in the genes encoding for TNF provide an additional susceptibility locus, independent of the SE. Further studies will undoubtedly elucidate the functions and implications of the TNF-α6/β5 association described in this study.

Class II MHC antigens in early rheumatoid arthritis in Bath (UK) and Madrid (Spain).
A Balsa, N J Minaur, D Pascual-Salcedo, *et al. Rheumatology* 2000; **39**: 844–9.

BACKGROUND. A number of studies have suggested that RA is a less severe disease in Mediterranean countries than it is in northern Europe. The objective of this study was to investigate whether there are differences in the frequency of class II alleles and the SE that may account for this observation.

INTERPRETATION. The SE was considerably more common in the general population in the UK. Differences in the alleles encoding the SE (predominantly DR4 in Bath, both DR4 and DR1 in Madrid) were apparent in the two populations that may, in part, explain the variability noted in rheumatoid disease between the UK and Spain.

Comment

This large study, based in Bath (UK) and Madrid (Spain), recruited 136 early rheumatoid patients (68 in Bath, 68 in Madrid) and 1155 controls (226 in Bath, 929 in Madrid) and HLA genotyped HLA-DR and DQ in all the individuals, using both low and high resolution PCR. Allele frequencies were compared using the χ^2 test.

In Madrid 38% of the control population carried one or two alleles encoding the SE. This was significantly lower than the 48% of controls in Bath (P=0.0001). In line with previous studies, in the UK population the SE was encoded predominantly by HLA-DR4 alleles while in Spain it was encoded at similar levels by DR1 and DR4 (Table 4.3). The DR4-DQ*0301 haplotype was also much more prevalent in the controls in Bath (P=0.00007).

Table 4.3 Number and type of SE alleles in Spanish and British control subjects

	Madrid	Bath
Total no. of alleles	1858	452
**01*	213 (11.4%)	50 (11%)
**0401*	40 (2.1%)	53 (11.7%)
**0404*	57 (3%)	22 (4.8%)
**0405*	45 (2.4%)	1 (0.2%)
**0408*	4 (0.2%)	3 (0.6%)
**1001*	31 (1.6%)	2 (0.4%)
**1402*	0	0
Total no. of SE alleles	369 (19.8%)	131 (28.9%)

Global χ^2 test: χ=76.12, P=1×10^{-7}.
Source: Balsa *et al.* (2000).

As might be anticipated, in the rheumatoid patients the SE was observed significantly more frequently than in controls (in Bath 72% versus 48% and in Madrid 51% versus 38%). In the Bath population SE alleles accounted for 42.6% of all the alleles in the RA population compared with 31.6% in the Madrid RA population; in both cases this is a significantly higher proportion of alleles than found in the control populations. These frequencies are lower than have been reported in previous studies of chronic RA in both the UK and Spain and this may reflect an influence of the SE on severity rather than initiation of disease. More patients in Bath were SE positive than in Madrid, although the risk of SE carriers developing RA was similar. Like the control populations the SE was encoded predominantly by HLA-DR4 alleles in the UK rheumatoid patients while the Spanish rheumatoid patients' SEs were equally encoded by HLA-DR1 and HLA-DR4. These differences in the alleles encoding the SE may be important in that more severe disease has been associated with HLA-DRB*0401 in previous studies in both countries while HLA-DRB*0101/2 has been associated with milder disease. These findings are consistent with previous work showing that RA is a less severe disease in Spain. However, before drawing too many inferences from this result it is noteworthy that African-Americans and Hispanic-Americans frequently do not express the SE and, in these populations, disease severity appears independent of its presence or absence.

As had been found in controls, the DR4-DQB*0301 haplotype was more frequent in the Bath rheumatoid patients (60% versus 37%). As these alleles are in strong linkage disequilibrium and the DR4 alleles were similarly more frequent in the Bath population it is difficult to determine whether this finding is important. Follow-up of this subgroup of patients may, however, be informative.

Conclusion

Class II MHC gene expression, and the frequency of the SE, is different in early rheumatoid patients from Bath and Madrid. These variations may, in part,

explain the observed differences in disease severity and progression in the two populations.

Multipoint linkage analysis of a candidate gene locus in rheumatoid arthritis demonstrates significant evidence of linkage and association with the corticotropin-releasing hormone genomic region.

M S Fife, S A Fisher, S John, *et al. Arthritis Rheum* 2000; **43**(8): 1673–8.

BACKGROUND. It is clear that the HLA-DR association of RA does not fully account for the genetic susceptibility. Another strong candidate gene for rheumatoid disease is the CRH gene, as CRH is a key regulator of anti-inflammatory glucocorticoid release from the adrenal gland. Animal models have shown that inadequate CRH production is associated with arthritis, although the genetic basis for this effect is unknown. This study examined the potential involvement of the region containing this gene in multicase families with RA.

INTERPRETATION. The CRH gene locus is both linked to and associated with RA.

Comment

Using microsatellite markers in the vicinity of the CRH gene, linkage analysis was undertaken in 295 families with at least two affected siblings using DNA from the Arthritis Research Campaign national repository. In 64% of the families data were also obtained from at least one unaffected relative. Genotyping of chromosome 8q13 (the CRH gene region) was undertaken in all 295 families and linkage analysis undertaken, estimating the probability that an affected sibling shares 0, 1 or 2 of the marker alleles by descent.

The estimated contribution to sibling risk of the CRH locus was 1.14. The total sibling risk for rheumatoid is between 5 and 7.2. Hence the CRH locus accounts for <10% of the risk in comparison with ~30% for HLA. A significant distortion of transmission was also shown, in addition to the sibling risk, indicating an association for the CRH microsatellite marker among affected siblings. There was also preferential transmission from parents to affected offspring. However, the maximum logarithm of odds (LOD) score reached was only 1.78. The failure of the locus to reach the arbitrary LOD score threshold of three suggests that had this study been part of a genome-wide screen, such a score would most likely have been overlooked. This highlights the problem of localizing genes exerting minor effects using genome screening.

Conclusion

Given the background data on the role of CRH in inflammation, the CRH gene locus is a strong candidate gene. This study confirms that the locus, or one close

by, does contribute to the genetic aetiology of RA, even though its effect is minor.

The associations of variant mannose binding lectin genotypes with radiographic outcome in rheumatoid arthritis.

N A Graudal, H O Madsen, U Tarp, *et al. Arthritis Rheum* 2000; **43**(3): 515–21.

BACKGROUND. MBL is a liver-derived acute phase protein that constitutes a part of the innate immune system. It is similar to the complement component C1q, can activate the complement cascade and is capable of opsonizing a multitude of microorganisms, including yeast, mycobacteria, Gram-negative bacteria and human immunodeficiency virus, mediating their subsequent phagocytosis. Insufficiency in MBL is associated with recurrent infections and several point mutations have been described in the first exon of the gene, located on chromosome 10. Normal alleles are termed A while variant alleles, which occur at a frequency of 1/5 are designated 0. Individuals with A/A (normal genotype) have six to eight times higher serum concentrations of MBP than heterozygotes (A/0) whilst homozygotes (0/0) show virtually undetectable levels. Recent studies have suggested that low levels of MBP may be associated with a poor prognosis of RA and this study sought to investigate the possible association of MBL genotypes with radiographic outcome of rheumatoid disease.

INTERPRETATION. Patients heterozygous or homozygous for the variant alleles had a worse outcome of RA, with destructive radiographic features being demonstrable approximately nine years earlier than similar changes in normals.

Comment

Six hundred and eighty-five Danish patients with RA (ARA 1958 criteria) recruited between 1966 and 1978 had been regularly assessed radiographically. Of these 140 (119 female, 21 male) who fulfilled the 1987 American College of Rheumatology criteria for RA were recruited, retrospectively, to this study. Patients were genotyped and the distribution of the three genotypes was: A/A 60. 7%, A/0 35.7% and 0/0 3.6%. This distribution was not significantly different to that found in the 250 healthy donors assessed, in agreement with previous studies, which suggests that MBL insufficiency is not a risk factor for disease development. Radiographs were assessed during follow-up and scored (Larsen scoring method). Radiographs were then divided, according to the percentage of the maximum radiographic score, into four radiographic outcome event (RE) groups: RE10 (10%), RE20 (20%), RE30 (30%) and RE40 (40%).

Patients with 0/0 genotype had a much worse radiographic outcome (defined as time to reach RE30) than heterozygote (A/0) patients who themselves had slightly worse outcomes than A/A patients (P=0.0011) (Fig. 4.1). Similar patterns were seen with time to reach RE10, RE20 and RE40.

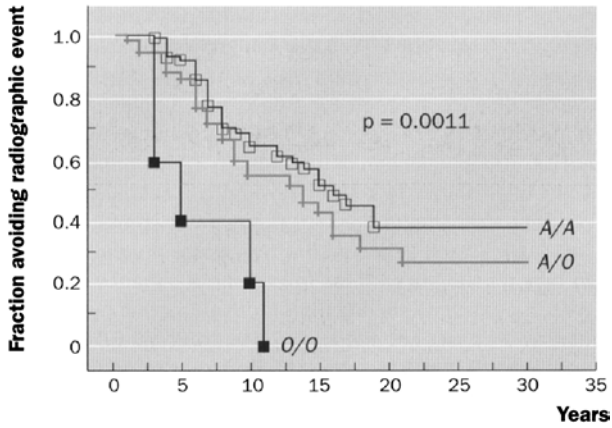

Fig. 4.1 Gene-dose effect in patient groups defined by structural MBL genotypes. Shown are Kaplan-Meier plots of severe radiographic outcome events, defined as at least 30% of maximum radiographic destruction. Source: Graudal *et al.* (2000).

In addition to the variant alleles, promoter sequences for MBP were also assessed and a low-expression promoter defined. Heterozygotes (A/0) with a low-expression A promoter (XA) had unmeasurable levels of serum MBL. XA/0 and 0/0 patients were, therefore, grouped as an MBL insufficient group and compared with the remaining individuals. A relative risk of 3.1 (P<0.0001) of reaching RE30 was associated with the MBL insufficient group, despite similar treatments. This suggests that MBL insufficiency either due to homozygosity for the variant alleles or heterozygosity of a low-expression promoter (for a normal allele) and a variant allele, predisposes to severe RA. Whether previous studies, which suggested no association of variant alleles with disease development, would be interpreted differently if heterozygotes were similarly divided is unclear.

Conclusion

RA patients with a defective (0/0) or low expression (XA/0) MBL genotype have significantly worse disease outcome as measured by radiographic progression, implying MBL insufficiency is a risk factor for poor outcome. Further studies will be required to assess whether early measurement of MBP levels enables prognostic predictions to be undertaken, or whether genotyping would be required.

Two edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study.
P Garred, H O Madsen, H Marquart, *et al. J Rheumatol* 2000; **27** (1): 26–34.

BackGROUND. Several point mutations have been described in the first exon of the MBL gene, located on chromosome 10. Normal alleles are termed A while variant alleles, which occur at a frequency of 1/5 are designated 0. Low serum levels of MBL and genotypes associated with impairment MBL gene transcription have both been associated with worse disease outcomes in RA. MBL is a component of the innate immune response, functioning in a manner similar to complement C1, in activating the complement cascade. The mechanism of its effect in RA is unknown. Serum IgG from patients with RA has been shown to contain fewer oligosaccharides terminating in galactose. This leads to exposure of N-acetylglucosamine (termed IgG-G0), which is a ligand for MBL. IgG-G0 has been associated with a worse prognosis in RA and autoantibodies to type II collagen with the IgG-G0 motif enhance joint inflammation. It is possible that MBL is somehow serving a protective function by binding this motif possibly functioning as a scavenger molecule, opsonizing IgG-G0 containing antibodies and facilitating their removal. This study investigated whether MBL gene polymorphisms were associated with the onset of RA and assessed whether MBL levels, in conjunction with the IgG-G0 motif, may be associated with different disease phenotypes.

INTERPRETATION. MBL variant alleles were associated with an early age of onset of RA. No association could be found between IgG-G0 concentrations, MBP genotypes and different disease phenotypes.

Comment

One hundred and eighty-nine Danish rheumatoid patients (1987 ARA criteria) were recruited and assessed using clinical (tender/swollen joints), functional (Health Assessment Questionnaire), laboratory (ESR, rheumatoid factor) and radiographic (presence and size of erosions) measures. All patients were genotyped for MBL alleles (A/A 57.1%, A/0 36%, 0/0 6.9%) and no difference was seen between patients and controls. This is in agreement with previous studies, suggesting MBL insufficiency is not a risk factor for disease development. Different genotypes did, however, associate with variable median ages of onset of disease (A/A median age 54.1, A/0 47.0, 0/0 38.3) and this was statistically significant. Lower serum MBL, measured by a double ELISA, was similarly associated with heterozygotes (A/0) and was virtually undetectable in variant homozygotes (0/0), in line with previous studies. In this study, in contrast to the other study of MBL and radiographic progression, no association was seen between different radiographic outcomes and genotype. The reasons for this are unclear but may be related to the differing lengths of follow-up and different outcome measures used (time to development of significant radiographic damage in the other study and presence or absence of erosions in this study).

IgG-G0 levels were assessed by measuring MBL binding to IgG-G0. This was undertaken by employing a novel technique utilizing radiolabelled MBL (purified from human plasma) and assessing its ability to bind to the serum IgG

(from patients and controls) bound to microtitre plates. To confirm the validity of this test serum concentrations of IgG-G0 were also assessed in 12 patients and six controls, using a standard chromatographic analysis and a significant correlation was demonstrated between IgG-G0 concentrations, measured by this method and the MBL binding assay results. Applying this assay to serum from patients with different MBL alleles showed no significant differences, despite several stratifications and subgroup analyses.

Conclusion

MBL may be important in RA, as alleles associated with low serum levels of MBP are associated with an earlier onset of disease. The molecular mechanisms of this association remain elusive and, although this study found no association with IgG-G0 levels, it does not exclude an interaction between these two factors being important in disease pathogenesis.

Mannose binding lectin and rheumatoid arthritis in southern Chinese.
W K Ip, Y L Lau, S Y Chan, *et al. Arthritis Rheum* 2000; **43**(8): 1679–87.

BACKGROUND. Insufficiency in MBL has been associated with both recurrent infections and a susceptibility to RA. The *MBL* gene is situated on chromosome 10 and three variant alleles (B, C and D), associated with impaired production of MBL have been described. Normal alleles are termed A, while variant alleles (either B, C or D), which together occur at a frequency of 1/5, are designated 0. This study sought to examine the role of the variant allele B (the most common variant allele) in Chinese with RA.

INTERPRETATION. Variant allele B and promoter polymorphisms of the *MBL gene,* which are associated with MBL deficiency, were associated with RA. Furthermore, a low serum level of MBL predisposed to the development of RA and was a risk factor for severe disease in southern Chinese.

Comment

Two hundred and eleven patients (176 female, 35 male) with RA (ARA 1987 revised criteria) and 196 controls were recruited from southern China. Patients were assessed clinically, radiographically and using a screen of laboratory tests (ESR, CRP, rheumatoid factor, complete blood count, renal and hepatic function). Serum MBL levels were measured by ELISA and the median level was found to be significantly lower in patients than in controls (Fig. 4.2). This is in contrast to previous studies in which allele distribution and serum MBL levels have been similar in patients and controls, but in which serum levels have correlated with severity of disease. One of the variant alleles of the *MBL* gene was assessed, alongside genotyping of the promoter region. This study found a greater prevalence of individuals with the variant allele (both homozygous and heterozygous) in the RA subgroup (68 of 211 [32%] compared with 43 of 153

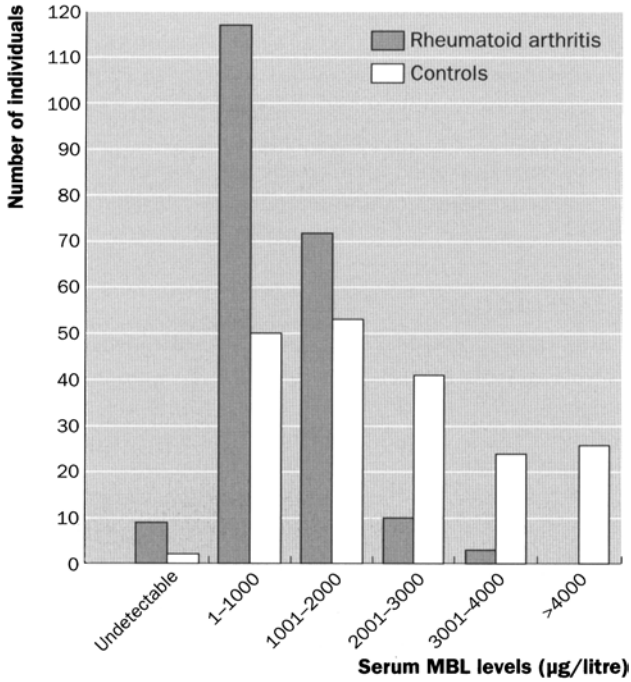

Fig. 4.2 Distribution of serum MBL levels in patients with RA (*n*=211) and controls (*n*=196). Source: Ip *et al.* (2000).

[28%] in the control group [*P*=0.027]). This is apparently in contrast to other studies. However, other studies have generally grouped the three variant alleles into one group while this study focused only on the B allele. The recent study of Garred *et al.* (discussed above) assessed frequencies of different variant alleles, including the B allele. In that study 60 of 189 (32%) of the rheumatoid patients were B allele positive compared with 54 of 250 (22%) of the controls, which is comparable with this study. Certain promoter polymorphisms were similarly associated with low MBL levels and haplotypes associated with increased MBL were significantly less commonly observed in rheumatoid patients.

Neither serum MBL or variant alleles were associated with different treatments; however, lower serum MBL levels were significantly associated with erosive disease as has been observed in previous studies. Variant B was also associated with erosive disease. These associations may be secondary to earlier onset of disease as patients with erosive disease had a significantly longer duration of disease. Extra-articular manifestations were not associated with MBL, except in a subgroup analysis, ex- cluding patients with sicca syndrome.

Conclusion

In agreement with other recent published studies, these data confirm an important role for MBL insufficiency (and genetic variants that predispose to it) in rheumatoid disease. This study suggests that in the Chinese, MBL insufficiency may not only result in more aggressive erosive disease, but may also increase disease susceptibility.

Overall comments

Exploration of the role of the SE in the pathogenesis of RA remains a fruitful area of research. Recent papers have confirmed previous work that has suggested it functions as a severity rather than a susceptibility marker. Work focusing on radiographic changes has shown that carrying the SE allele is associated with earlier development of erosions. However, the studies also show that once erosions have occurred, the SE exerts little influence over radiographic progression and, therefore, over prolonged follow-up, as in the 10-year study mentioned, little in the way of significant influence can be discerned. Although useful in understanding the progression of the disease and its genetic background the current data do not support the use of HLA typing as a clinical tool, outside of research studies. The data published to date do not suggest it would add useful prognostic information on an individual patient basis. Furthermore, the finding that other HLA-DRB1 allele motifs, such as the DERAA motif, can exert protective effects illustrates the increasing complexity of the HLA class II paradigm and makes the genotyping of patients even less attractive as a routine clinical test. Nonetheless, the findings published have raised many interesting questions that require further investigation. RA is a polygenic disease and the role of other candidate genes has been explored.

The finding of an association of the CRH gene with RA is a salutary reminder of the limitations of genome screening. It is a promising candidate gene and, although its effects appear to be minor, they are significant and would have been missed in a genome-wide screening.

Interest in the TNF gene is perhaps more predictable, not only because of the proven benefits of anti-TNF therapy but also because of the gene's proximity to HLA-DR4 allowing possible linkage disequilibrium effects with this allele. The studies of the microsatellite polymorphisms in the TNF gene suggest that TNF-α6 (with and without β5 pairing) and possibly TNF-α11 are associated with severe disease. This requires further study, both to determine the mechanism and to assess whether such polymorphisms influence a response to treatment with anti-TNF therapies. As TNF is capable of inducing the potentially destructive MMPs, it is interesting that levels of proMMP-3, a precursor of one of the MMPs implicated in rheumatoid disease, are upregulated in patients with the SE. A potentially unifying hypothesis would suggest that the SE is in linkage disequilibrium with TNF gene polymorphisms that allow upregulated expression

of this gene. This in turn would, in certain circumstances, promote increased MMP production and, therefore, pre-dispose to more aggressive rheumatoid disease. This notion would allow for the SE influencing severity without affecting disease susceptibility, fit with current clinical data on treatment efficacy, and explain the involvement of MMPs. Such a model is without doubt too simplistic, but can provide a framework for further study.

MBL deficiency has previously been shown to be associated with poor prognosis in RA. Certain polymorphisms in both the *MBL* gene and its promoter are associated with low/undetectable levels of the protein. The studies published this year have disagreed on whether these deficiency-associated polymorphisms occur more frequently in rheumatoid patients than in controls, although one of the variant alleles (B) does seem to be over-represented in rheumatoid disease. Whether these polymorphisms impart susceptibility or are merely associated with severity is an unresolved question. However, deficiency-associated polymorphisms have certainly been linked with more rapidly progressive disease (both in terms of age of onset of disease and development of radiographic erosions). The mechanisms underlying this association between MBL deficiencies and aggressive disease outcomes, remain elusive. Although no association with immunoglobulin glycosylation states was shown in the study discussed this remains an attractive hypothesis. Furthermore, studies of MBL's role in immune complex clearance would be interesting, as it could be serving a function analogous to the proposed role of complement in systemic lupus erythematosus.

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5
Matrix metalloproteinases in rheumatoid arthritis

Cartilage destruction and associated bone erosion, in rheumatoid arthritis (RA), leads to a loss of normal joint function. A family of zinc-dependent degradative enzymes, the matrix metalloproteinases (MMPs), are capable of breaking down the extracellular matrix. These enzymes are involved in the normal turnover of the cartilage matrix allowing balanced remodelling to be undertaken by the chondrocytes. However, there is increasing evidence that they also play a key part in the joint destruction seen in rheumatoid disease. Eighteen members of the MMP family have been described and they are classified into five subgroups:

1. collagenases
2. gelatinases
3. stromelysins
4. membrane type (MT) MMPs
5. Other MMPs.

The activity of the MMPs is tightly controlled by a family of specific inhibitors—the tissue inhibitors of metalloproteinases (TIMPs). The balance between the enzymes and their inhibitors may, therefore, be important in the pathology of cartilage destruction. Several recent papers have examined aspects of this balance in patients with RA to answer the following questions:

1. Which MMPs may be involved in disease pathogenesis?
2. Does imbalance in the MMPs/TIMPs predict joint destruction and what factors control this balance?
3. Could any imbalance be manipulated therapeutically?

Differential expression pattern of membrane-type matrix metalloproteinases in rheumatoid arthritis.
T Pap, Y Shigeyama, S Kuchen, *et al. Arthritis Rheum* 2000; **43** (6):1226–32.

BACKGROUND. The MT-MMPs are a recently described subgroup of the MMPs. Five different MT-MMPs have been described—MT1, MT2, MT3, MT4 and MT5. They are characterized by a transmembrane domain

and function at the cell surface. Three of these MT-MMPs (MT1, MT2 and MT3) have been shown to be capable not only of degrading extracellular matrix, but also of activating MMP-2 and MMP-13, two metalloproteinases that previous studies have suggested to be associated with RA. In this study synovial tissue was taken from patients with RA and expression of MT-MMPs 1–4 compared with the expression seen in post-mortem synovial tissue from subjects without arthritis.

INTERPRETATION. Expression of MT-MMP-1–4 was shown in all synovial tissues supporting a role for these enzymes in normal metabolic processes. However, differential distribution patterns and intense expression of MT1-MMP and MT2-MMP in the rheumatoid synovium suggests a possible role for these two enzymes in disease pathogenesis.

Comment

Using a reverse transcriptase-polymerase chain reaction (RT-PCR) based technique to amplify mRNA, it was shown that synovial tissue, from both normals and rheumatoid patients, was capable of expressing MT1, MT2, MT3 and MT4, suggesting involvement of these enzymes in normal synovial function. Experiments were undertaken using both cultured synovial fibroblasts and total synovial tissue indicating this universal expression was not just an artefact of *in vitro* culture. Quantitative or semiquantitative PCR was not undertaken so conclusions regarding differential expression could not be drawn. However, using identical concentrations of cDNA there appeared to be more MT1-MMP expression in both normals and rheumatoid patients, while MT2-MMP was barely detectable in normals. Although expressed in both normal and rheumatoid synovium, the distribution of that expression, analysed by *in situ* hybridization, was different. In rheumatoid synovium, MT1-MMP was expressed in a pattern similar to that of other disease associated MMPs, in contrast to a more uniform distribution in normal synovium. A similar differential expression was seen for MT3-MMP but not MT2 or MT4-MMP.

Conclusion

This study suggests that both MT1-MMP and MT3-MMP may play a part, not only in normal synovial remodelling, but also in the cartilage degradation of RA. More studies will be required investigating the co-localization with other disease associated MMPs, as these data are only suggesting ‘guilt by association’. Studies at the cellular level may elucidate whether MT1-MMP can activate other MMPs allowing cellular interplay in the cartilage degradation.

Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis and osteoarthritis.
Y Yoshihara, H Nakamura, K Obata, *et al. Ann Rheum Dis* 2000; **5**: 455–61.

BACKGROUND. Although osteoarthritis (OA) and RA are markedly different diseases, cartilage destruction is a common feature. As an imbalance between MMPs and TIMPs may be important in both diseases this group looked for variability in the expression of different metalloproteinases and their inhibitors to explore possible differences between the two different diseases. Different MMPs are produced from different sources. MMP-1 and MMP-3 are produced by synovial lining cells, MMP-8 is produced by neutrophils, while MMP-9 is produced by both neutrophils and macrophages. Previous reports have shown that all these MMPs can be detected in rheumatoid synovial fluid. This group, therefore, investigated the differential expression of these enzymes in addition to other MMPs that have not previously been reported.

INTERPRETATION. This study confirms previous work indicating that MMP-1, MMP-2 and MMP-3 are elevated in rheumatoid disease. Increased synovial fluid levels of MMP-8 and MMP-9 in the RA patients in this study suggests an important function for neutrophils in cartilage degradation.

Comment

Synovial fluid samples were taken from the knee joints of 97 rheumatoid patients (American College of Rheumatology 1987 revised criteria) and 103 OA patients. Levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1 and TIMP-2 were measured using an enzyme-linked immunosorbent assay (ELISA) based technique. MMP-1, MMP-2 and MMP-3 could be detected in rheumatoid and OA synovial fluid but levels were significantly higher in patients with RA. MMP-8 and MMP-9 were consistently demonstrable in the rheumatoid patients but only detectable in 2% of the OA patients. Comparing patients with early (Larsen grade 0–I) versus late (Larsen grade II–III) RA, it was shown that MMP-1 and MMP-3 were significantly greater in early disease, while MMP-8 and MMP-9 showed a trend to increase with later disease. MMP-2 showed no change with disease duration. By measuring TIMP-1 and TIMP-2 levels an estimated ratio of MMP/TIMP was calculated and shown to be significantly greater in RA than OA (Fig. 5.1). MMP-7 and MMP-13 proved more difficult to detect and no definite conclusions could be drawn about their significance in disease.

Conclusion

This large study both confirms and extends previous work on the MMPs and TIMPs. The finding that MMP-1 and MMP-3 are both elevated in rheumatoid

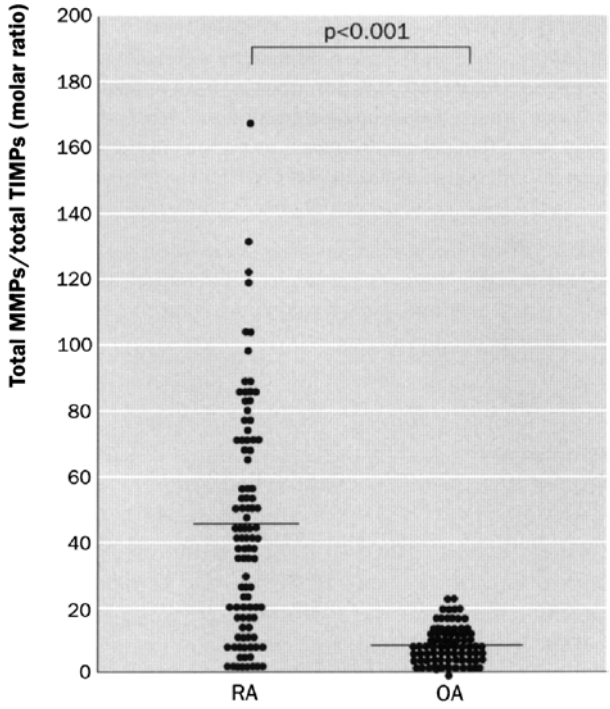

Fig. 5.1 Molar ratios of MMPs to TIMPs. Molar ratios of total MMPs to total TIMPs were calculated. Bars indicate mean values in RA and OA synovial fluids (SFs). Source: Yoshihara *et al.* (2000).

disease is consistent both with previous data in rheumatoid disease and with other data showing co-ordinated mRNA expression of both these enzymes. As levels were readily detectable in the synovial fluid, these findings support the hypothesis that the rheumatoid synovium is the source of these enzymes, although obviously they do not exclude a contribution from the chondrocyte. The elevated levels of both MMP-8 and MMP-9 in rheumatoid synovial fluid also suggest an important part for neutrophils in cartilage degradation, potentially more dominant in later disease. Overall, this study adds further weight to the suggestion that it is the balance of enzymes that is important in the processes of cartilage degradation, but it does not elucidate the mechanisms that are controlling these enzymes.

Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement in patients with early rheumatoid arthritis.
H Yamanaka, Y Matsuda, M Tanaka, *et al. Arthritis Rheum* 2000; **43**(4): 852–8.

BACKGROUND. MMP-3 (stromelysin 1) is considered to be one of the critical MMPs in the cartilage breakdown seen in RA. It has been reproducibly shown to be increased in the rheumatoid synovial fluid and analysis of proteoglycan breakdown products in the synovial fluid reveals cleavage at a site susceptible to MMP-3. Furthermore, MMP-3 has been shown to be expressed in the synovium early in disease and has stimulated interest as a potential marker of subsequent cartilage breakdown. If expression of this MMP does indeed correlate with subsequent joint destruction it may have clinical utility as a marker of worse prognosis and, therefore, enable selection of a cohort of individuals in whom early aggressive treatment is warranted.

INTERPRETATION. In this study, MMP-3 levels were shown to correlate with subsequent radiological damage. This suggests an involvement in the process of matrix destruction in RA, which if suppressed may be an effective therapeutic intervention. However, the association was not sufficiently strong to allow prognostic predictions to be made, suggesting routine clinical measurement would not be beneficial.

Comment

Serum samples were collected from 267 healthy volunteers and 82 patients with RA. Of the rheumatoid patients, 26 had early disease (polyarthritis <1 year, mean disease duration 0.4 years). The remaining 56 rheumatoid patients had a disease duration >5 years. Patients were followed-up for 3 years and treated with either gold (oral or intramuscularly), penicillamine or bucillamine. Clinical, laboratory and radiological evaluation of the patients was undertaken and a one-step sandwich ELISA was used to determine MMP-3 levels. In the healthy controls, MMP-3 levels were higher in men (64.5±29.4 ng/ml) than women (29±12.7 ng/ml). MMP-3 levels were significantly higher in patients with both early RA (246.6±267.7 ng/ml) and late RA (224.6±237.3 ng/ml). However, the broad standard deviations indicate the variabilities in serum concentration that result in low sensitivity and specificity for the test (Fig. 5.2). Although at baseline C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) correlated strongly with MMP-3 levels, joint destruction was only significantly associated with MMP-3 levels in the group with long-standing rheumatoid disease. After 3 years of follow-up, radiological damage (assessed by Larsen scoring) was found to correlate strongly with finding a single high MMP-3 reading at any point during the 3 years follow-up. Furthermore, an analysis of patients with early arthritis showed that MMP-3 levels at baseline correlated with the Larsen score at both 6 and 12 months after study entry. Neither CRP levels or ESR demonstrated a similar correlation.

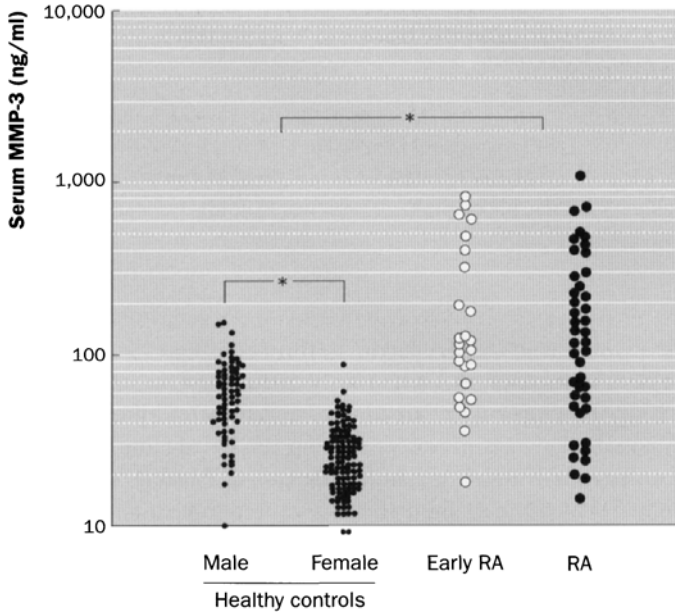

Fig. 5.2 Distribution of serum MMP-3 levels in patients with RA and healthy controls. The mean±SD level of serum MMP-3 in each group was male controls 64.5±29.4 ng/ml (n=94), female controls 29.0±12.7 ng/ml (n=173), early RA 246.4±267.7 ng/ml (n=26) and long-term RA 224.6±237.3 ng/ml (n=56). Significant differences were noted between male and female control subjects (P<0.0001) and between male or female controls and patients with early or long-term RA (P<0.00001). A significant difference was not identified between patients with early RA and those with long-term RA. Source: Yamanaka *et al.* (2000).

Conclusion

Although this study suggests that MMP-3 levels may be associated with subsequent radiological progression of disease, it does not currently offer a clinically useful test. MMP-3 levels show both poor sensitivity and specificity (Fig. 5.2). Furthermore, they have shown that the levels fluctuate with time and would therefore be inappropriate for clinical decision making in an individual. Nonetheless, the study does highlight an association between MMP-3 levels and joint progression on a population basis that merits further study, and suggests this enzyme may be a useful therapeutic target.

Modulation of inflammation and metalloproteinase expression in synovial tissue by leflunomide and methotrexate in patients with active rheumatoid arthritis. M C Kraan, R J Reece, E C Barg, *et al. Arthritis Rheum* 2000; **43** (8): 1820–30.

BACKGROUND. Leflunomide is a recently developed slow-acting antirheumatoid drug, the proposed primary mode of action of which is inhibition of the enzyme dihydroorotate dehydrogenase. It has also been shown to interfere with the phosphorylation of tyrosine kinases and to affect signal transduction. Both leflunomide and methotrexate have been shown, in recent clinical trials, to control the clinical manifestations of RA to a similar degree. Methotrexate has also been shown in previous studies to reduce both the cartilage and bone destruction associated with rheumatoid disease. As MMPs are capable of breakdown of the extracellular matrix this study investigated the effects of these two drugs on the synovial expression of the MMPs, particularly MMP-1.

INTERPRETATION. These data suggest potential beneficial effects of both methotrexate and leflunomide on matrix degradation through alterations in the levels of both MMP-1 and TIMP. However, whether such changes translate directly into clinically beneficial effects requires further study.

Comment

Thirty-five patients with active RA who were enrolled in a double-blind randomized trial of leflunomide (20 mg/day following three days loading with 100 mg) versus methotrexate (15 mg/week) underwent knee arthroscopy and synovial biopsy at baseline and after four months of treatment. Sixteen were taking leflunomide, 19 were taking methotrexate, and both groups experienced similar clinical efficacy. Synovial samples were stained immunohistochemically for MMP-1 and TIMP-1 (an inhibitor of MMP-1). Staining was also undertaken for a number of other relevant proteins (intercellular adhesion molecule [ICAM] -1, vascular cell adhesion molecule [VCAM] -1, interleukin [IL] -1β and tumour necrosis factor [TNF] -a). Paired samples from each patient were compared before and after treatment. ICAM-1, VCAM-1, and TNF-α were all reduced in patients who responded to treatment (methotrexate or leflunomide), although this reduction only reached statistical significance for ICAM-1 levels. IL-1β was reduced in the methotrexate patients, but not the leflunomide-treated patients. MMP-1 levels were significantly reduced in both groups, while TIMP-1 levels were reduced only in those patients taking leflunomide. Nonetheless, a significant reduction in the MMP-1/TIMP-1 ratio was observed irrespective of treatment and irrespective of clinical response (Fig. 5.3).

Conclusion

This study provides an interesting insight into the potential mechanism of action of slow-acting antirheumatoid drugs. It is of interest that if the ratio of MMP-1 to its inhibitor TIMP-1 is important in cartilage and bone degradation, a reduction in this ratio could be seen irrespective of clinical response as other work has shown that suppression of inflammation in isolation does not prevent

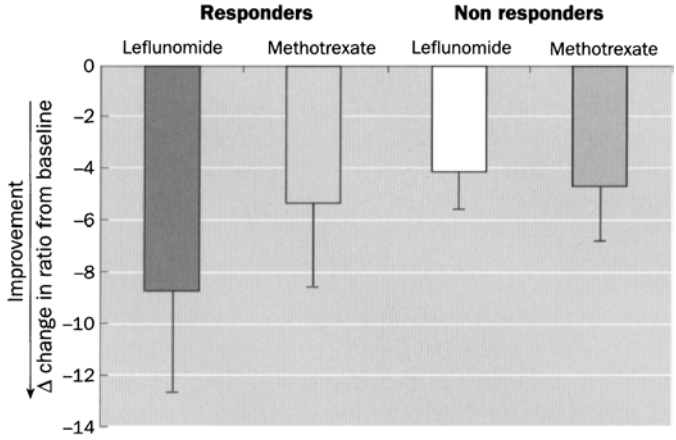

Fig. 5.3 Mean and SEM change in the Δ MMP-1 to TIMP-1 ratio after 4 months of treatment in relation to the clinical response. Source: Kraan *et al.* (2000).

development of erosive changes. As the expression of MMP-1 and MMP-3 is co-regulated, one could speculate that similar effects would be demonstrable on MMP-3 levels, although this study does not address that question. Whether other slow-acting agents produce similar effects and whether such changes correlate with protection from progressive joint damage is unclear and further study of this question is required. Nonetheless, this study does show that the balance of TIMPs and MMPs can be modulated by treatments that, in the case of methotrexate, have been shown to reduce joint damage.

Enhanced production of tissue inhibitor of metalloproteinases by peripheral blood mononuclear cells of rheumatoid arthritis patients responding to methotrexate treatment.

M Seitz, J-M Dayer. *Rheumatology* 2000; **39**:637–45.

BACKGROUND. Macrophages are critical to the pathogenesis of RA. They are the primary source of pro-inflammatory cytokines such as TNF-α and IL-1, which themselves are capable of inducing MMP production by synovial fibroblasts. Previous studies have shown that methotrexate treatment can depress IL-1 production, decrease the expression of MMP-1 and reduce protein production in the inflammatory joint. These findings suggest a beneficial effect of methotrexate on macrophages, in addition to previously documented effects on neutrophils and processes of neovascularization. This study extends these previous studies by examining the effects of methotrexate on both the circulating levels, and the *ex vivo* production by peripheral blood mononuclear cells (PBMC), of MMP-1 and TIMP-1 as well as IL-6, a pleiotropic cytokine produced by monocytes, with both pro- and anti-inflammatory properties.

INTERPRETATION. Methotrexate can increase the levels of TIMP-1 through upregulated IL-6 production *in vitro;* an effect that could potentially modify the MMP-1/TIMP-1 ratio if translated *in vivo,* theoretically impairing matrix degradation in the joint.

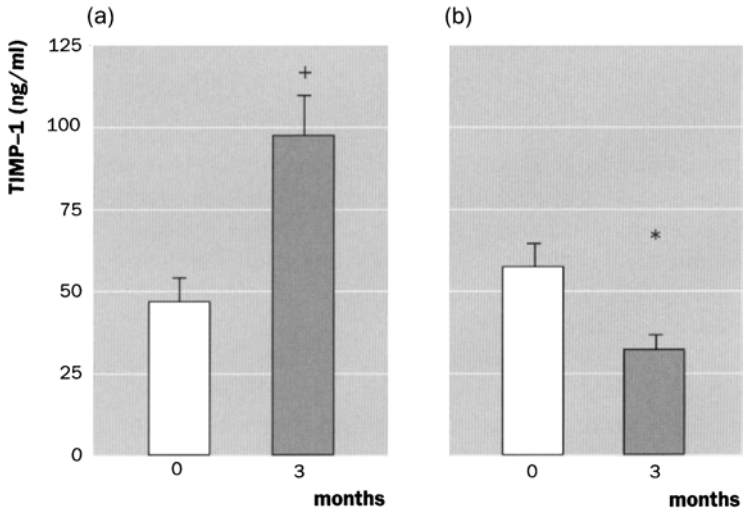

Fig. 5.4 Spontaneous TIMP-1 release by PBMC of patients with RA. TIMP-1 release before methotrexate treatment (open columns) and after 3 months of treatment (filled columns) in responding ((a); n=16) and non-responding patients ((b); n=11). The results represent mean ±SEM. $^{*}P<0.01$; $^{+}P<0.001$. Source: Seitz *et al.* (2000).

Comment

A cohort of 27 patients with active RA were recruited and treated with up to 12 weeks of weekly methotrexate (15 mg intramuscularly). PBMC were isolated both prior to treatment and 24 h after the last injection. To measure TIMP-1 and MMP-1 production, PBMC were cultured with or without lipopolysaccharide (LPS) for 48 h prior to collection of the supernatants and measurement of TIMP-1 and MMP-1 levels by ELISA. Patients were retrospectively divided into responders (>20% improvement in Paulus index) and non-responders (<20%) for analysis and it was shown that the PBMC isolated from the responders produced significantly more TIMP-1 after treatment than before, irrespective of stimulation by LPS. Furthermore, PBMC from non-responders not only failed to increase TIMP-1 levels but produced less TIMP-1 after treatment (Fig. 5.4). By contrast serum levels of TIMP-1 were not affected by treatment, and MMP-1 production was not affected in either group in any of the assays. As these assays were using whole PBMC and not isolated monocytes, the cellular basis of this effect cannot be determined. Interestingly, *in vitro* IL-6 levels were significantly increased in responders and decreased in non-responders, in parallel with the

levels of TIMP-1. Furthermore, in co-culture experiments, neutralizing IL-6 *in vitro* with appropriate monoclonal antibodies reduced the levels of TIMP-1.

These findings suggest that, in methotrexate responders, the drug can increase TIMP-1 levels without affecting MMP-1 levels, thereby potentially favourably altering the MMP-1/TIMP-1 ratio. This beneficial effect appears, at least in part, to be mediated by IL-6, which, paradoxically, previous work has shown to be reduced in the serum of patients responding to methotrexate treatment. This study did not address the mechanisms of any IL-6-mediated effect; specifically, it did not examine the levels of other cytokines potentially involved, such as IL-1 and TNF-α, which can both be reduced by IL-6.

Conclusion

This is the first study to show a drug-mediated effect on TIMP-1, although the findings suggest this is due to an upstream alteration in IL-6 production. The findings also serve as a reminder that gross changes in cytokine levels in the serum may not reflect the underlying cellular processes. Further studies are required to see if the changes seen in this study are reflected in long-term radiographic and clinical outcome measures.

Effects of sulphasalazine and its metabolites on steady state messenger RNA concentrations for inflammatory cytokines, matrix metalloproteinases, and tissue inhibitors of metalloproteinase in rheumatoid synovial fibroblasts.

P P Minghetti, W D Blackburn Jr. *J Rheumatol* 2000; **27**(3):653–60.

BACKGROUND. The sulphasalazine molecule consists of sulphapyridine and 5-amino salicylic acid connected by an azo bond that is metabolized *in vivo,* allowing release of its constituent molecules. Although a recent study highlighted effects of sulphasalazine on the transcription factor, NF-κB, its major mechanism of action in RA remains unclear. This study examined whether sulphasalazine, or its metabolites, exerts its effects through altering rheumatoid synovial fibroblast production of a variety of proteins, including the MMPs and TIMPs.

INTERPRETATION. Sulphasalazine and its metabolites can, in this *in vitro* model, at suitably high concentrations, favourably alter the mRNA levels of both TNF-α and TIMP-2.

Comment

Rheumatoid synovial fibroblasts, from an immortalized cell line, were cultured with or without activation by phorbol myristate acetate (PMA) in the presence or absence of varied dilutions of sulphasalazine and its metabolites. The study focused on the responses seen with the highest concentrations that were three times the median serum concentration in patients taking 2 g/day of sulphasalazine.

Following culture, mRNA was extracted and levels of mRNA for the relevant proteins quantified by Northern blotting and hybridization using radiolabelled cDNA probes. Levels of mRNA were compared with those of a housekeeping gene (GAPDH or 18S rRNA) the levels of which were shown to remain constant. As this was a transformed cell line, no comparison was made with synovial fibroblasts from either normals or OA patients. Nonetheless, using this particular *in vitro* model system, 40% suppression of TNF-α production was demonstrable with high dose treatment. Only modest effects were seen on levels of mRNA for TNF receptor, IL-1β, MMP-1, MMP-2, MMP-3 or TIMP-1 with, at best, a 24% change with highest dose treatments. No statistical data were given on the significance of these changes. The only other strong change that was seen was with respect to TIMP-2 levels. The TIMP-2 transcript levels that were analysed in this study (the 3.5 kb transcript) were increased by between 40 and 50% using the highest doses of either of the sulphasalazine metabolites but not when using the parent compound.

As previous work has shown the ability of sulphasalazine to inhibit NF-κB, the effects seen on TNF-α production are to be expected but, if this were the case, a reduction in IL-1β levels, not clearly seen in this study, would also be expected.

The findings in this study relate to cells from an immortalized line of rheumatoid synovial fibroblasts. Comparisons using short-term cultured synovial fibroblasts from a cohort of rheumatoid patients would be of interest as rheumatoid disease in some individuals is more responsive to sulphasalazine than in others. Furthermore, it would be of interest to note whether these modest changes in mRNA levels translate into significant alterations in protein production, by using techniques such as ELISA.

Conclusion

Accepting the limitations of this study, the data suggest that sulphasalazine may be capable of favourably altering levels of TNF-α and TIMP-2. These findings give further insight into the potential mechanisms of action of this therapeutically useful compound. The reductions seen in TNF-α production with sulphasalazine may, in part, explain its therapeutic benefit. If the ratio of MMPs to TIMPs is important in cartilage degradation, the effect on TIMP-2 mRNA, assuming it is translated into an effect on protein synthesis, implies an additional role for this drug in reducing the erosive changes of RA.

Increased expression of extracellular matrix metalloproteinase inducer in rheumatoid synovium.
Y T Konttinen, T-F Li, J Mandelin, *et al. Arthritis Rheum* 2000; **43**(2): 275–80.

BACKGROUND. Although the MMPs have been implicated in the cartilage degradation associated with RA, the exact mechanism that causes

their upregulated expression is not fully understood. Extracellular MMP inducer (EMMPRIN) is an enzyme that previous studies have shown helps mediate the tissue invasion and metastasis of malignant cells by stimulating MMP production by fibroblasts. This study examined the expression of EMMPRIN in the synovial membrane of rheumatoid joints and compared this with samples from patients with OA. EMMPRIN immunoreactivity was both more intense and more widespread in rheumatoid synovium than in synovium from OA joints. This difference was statistically significant. EMMPRIN expression in the RA joint was confirmed by both RT-PCR and immunoblotting.

INTERPRETATION. The expression of EMMPRIN is upregulated in the rheumatoid synovium where it could induce the local production of MMP-1, -2 and -3, thereby contributing to the joint destruction in RA.

Comment

Ten rheumatoid synovial biopsies from hip joints of rheumatoid patients were compared with 10 samples from patients with OA. Unfortunately, given the cartilage degradation that occurs in OA, no comparison was made with synovium from normal individuals. It is noteworthy, in this regard, that EMMPRIN has previously been identified in a range of normal tissues.

A mouse monoclonal antibody specific for EMMPRIN, subsequently visualized using a peroxidase-based method, was used to stain tissue sections. The intensity of staining was independently scored by three of the researchers and shown to be significantly greater in the rheumatoid specimens (Fig. 5.5). The distribution of the staining was dependent on the presence of hypertrophy and macrophage infiltration, in which case intense staining was demonstrated between the superficial layers and the sublining cells. However, given the small sample size it was not clear whether the distribution pattern was significant.

Five of the rheumatoid synovial biopsies were used to study EMMPRIN mRNA expression using RT-PCR with EMMPRIN-specific primers. Expression of EMMPRIN mRNA in the rheumatoid synovia was confirmed and the specificity of the RT-PCR verified by sequencing the PCR product. However, no comparison was made with the OA synovium and given that 35 cycles of amplification were used it is probable that mRNA could similarly have been detected in these specimens. Confirmation that protein is translated from the message was obtained by immunoblotting synovial extracts from both OA and rheumatoid samples. As immunoblotting, as described, is not quantitative, the interpretations of the relative abundance of the proteins must be viewed cautiously, but they do demonstrate that EMMPRIN is detectable by immunoblotting in both OA and RA samples.

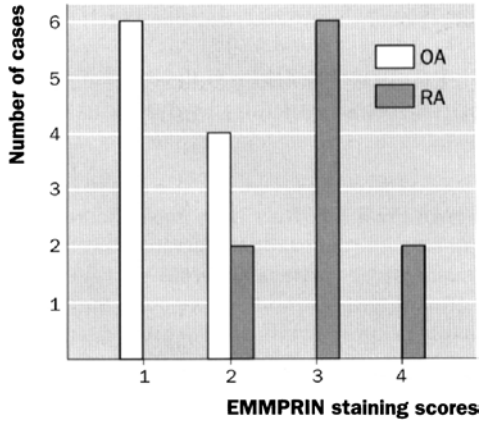

Fig. 5.5 Intensity of extracellular matrix metalloproteinase inducer (EMMPRIN) staining in OA and RA synovial membranes. Source: Konttinen *et al.* (2000).

Conclusion

EMMPRIN, although previously considered as a tumour-associated factor, has also been identified on activated leucocytes and granulocytes and is constitutively expressed on monocytes. The present study shows it is present in the synovium, although this may primarily reflect infiltration with EMMPRIN expressing cells. Nonetheless, EMMPRIN was shown to be significantly upregulated in the rheumatoid samples and as previously it has been shown that EMMPRIN induces both MMP-1 and MMP-3, through a tyrosine kinase-dependent mechanism, this finding gives further insight into the possible dysregulation of matrix metabolism in RA.

Overall comment

The studies published during the year 2000, investigating the role of MMPs in RA, have given further insight into the involvement of these proteins in the degradative processes and raised further questions that require investigation. In answer to the questions raised at the start:

1. Which MMPs may be involved in disease pathogenesis? Work published during 2000 has confirmed the importance of MMP-1 and MMP-3 in RA. Studies have consistently shown elevated levels of these enzymes in RA and the correlation of serum MMP-3 levels with subsequent radiographic damage suggests a possible target for future therapies. MMP-8 and MMP-9 also appear to be involved in cartilage degradation, although further studies will be required to confirm the importance of these metalloproteinases.

2. Does imbalance in the MMPs/TIMPs predict joint destruction and what factors control this balance? The absolute levels of the MMPs do not appear to be as important as the balance of the enzymes and their inhibitors. A number of up-stream molecules have been shown to have the potential to influence this balance in RA. The MT-MMPs, particularly MT1-MMP and MT3-MMP are capable not only of matrix degradation, but of activating other MMPs and their pattern of expression in the rheumatoid synovium suggests an important part in the disease that merits further investigation. The finding that extracellular MMP inducer is upregulated in the rheumatoid synovium suggests another level of dysregulation that may be a target for future therapy, as this enzyme is capable of upregulating both MMP-1 and MMP-3 without effect on their inhibitor TIMP-1.

3. Could any imbalance be manipulated therapeutically? The recent studies have provided interesting insights into additional therapeutic benefits of current treatments. The ability of both methotrexate and leflunomide to inhibit MMP-1, without, in the case of methotrexate, affecting TIMP-1 levels suggests true disease modifying potential. As leflunomide inhibits tyrosine kinase-dependent signalling (though whether this occurs at therapeutically relevant concentrations is still disputed), it is possible that some of these effects are mediated through inhibition of EMMPRIN signalling, but further studies will be required to answer this. It is also of interest that this potentially beneficial effect was not dependent on clinical responses. Sulphasalazine has now similarly been shown to favourably alter TIMP-2 levels. Whether specifically targeted therapies are able to exert longer-term beneficial effects is another question for future research.

Part III

Spondyloarthropathies and paediatric rheumatology

6
Spondyloarthropathies

Introduction

The spondyloarthropathies (SPA) should be the most tractable form of inflammatory arthritis in view of their exceptionally strong association with HLA-B27, and the involvement of infection by defined bacteria in one form of the disease, reactive arthritis. All this should surely lead to clear elucidation of pathogenesis and adoption of rational therapy. Unfortunately, we are not quite at that stage; however, important progress continues to be made and new insights have appeared in the last 12 months.

The sacroiliac joint is the *sine qua non* of many forms of SPA, but the joint has received relatively little investigation. Two important studies have appeared: one challenges the assumption that the primary site of pathology in SPA is the enthesis, while the other obtained sufficient material to perform immunohistochemistry and identify the cells associated with inflammation at this site; interestingly even at such an 'unpromising' site T lymphocytes were evident. The role of T cells is also highlighted by the huge increase in the incidence of SPA that has followed the human immunodeficiency virus (HIV) infection epidemic in sub-Saharan Africa, now documented for psoriatic arthropathy. However, contrary to initial expectation, disease is associated with early impairment of CD4+ T cells rather than of the CD8+ T cells, which would be expected to interact with B27 in most models of SPA. In fact B27 is not a factor in the occurrence of SPA in the African HIV+ population.

The importance of factors in addition to B27 is underlined by the recent study of the immunogenetics of SPA associated with inflammatory bowel disease (IBD), where associations with HLA antigens other than B27 were revealed following accurate clinical classification of the arthritis. It was encouraging to find the same antigens associated with other related forms of SPA.

Nevertheless, B27 still merits a good deal of attention and is the major influence in ankylosing spondylitis (AS), although analysis of B27 subtypes that do not confer susceptibility to SPA might be rewarding in terms of suggesting mechanisms, the picture is still far from clear. Likewise, characterization of B27-

restricted T cells that might be involved in the pathogenesis of SPA has been difficult, but there were two new contributions. In one B27-restricted T cells in *Yersinia*-triggered reactive arthritis were described, while in the other a B27-restricted response to a self-peptide appeared to be more frequent in AS patients. It has generally been easier to investigate the role of B27 in animal models. A new feature of this work has been the discovery that arthritis in animal models may reflect physical properties of B27, which can be mimicked by other abnormalities of class I HLA antigens and the class I major histocompatibility complex (MHC) processing pathways. These ideas are likely to be pursued vigorously in the coming years.

As infection is critical in at least some forms of SPA, the influence of B27 on host-bacteria interactions may be relevant to disease. For *Salmonella,* B27 had no effect on the susceptibility to infection or the ability to clear it, but a major effect on incidence of musculoskeletal symptoms post-infection was evident, mainly on their duration and severity. Similar data have been reported for *Campylobacter* infection. Following infection, organisms or their antigens traffic to the joint and can be detected by various means. For enteric organisms such as *Salmonella* and *Yersinia,* antigens are readily detected, but nucleic acids (DNA, mRNA) only with great difficulty and in a minority of patients. The situation for *Chlamydia* is opposite—a high frequency of demonstrating nucleic acids and relative difficulty in showing the presence of *Chlamydia* antigens. A similar involvement of bacteria may underlie the pathogenesis of chronic recurrent multifocal osteitis, which clearly resembles other forms of SPA.

If bacteria reaching the joint are important in the pathogenesis of reactive arthritis, surely antibiotics should ameliorate the disease? Unfortunately, the answer appears to be negative, as judged by most of the published series, including the data on ciprofloxacin from a Finnish group—two similar papers appeared in 1999. There are still issues to be resolved as different antibiotics and regimens of treatment might still have useful effects. Other treatments in SPA include the anti-tumour necrosis factor (TNF) agents (reviewed elsewhere in this volume), and mesalazine; the practical usefulness of sacroiliac joint injection has also been assessed but with differing conclusions, although the opportunity to obtain biopsy tissue is certainly welcome.

Somewhat anomalously, this chapter includes a report on therapy for Behçet's disease. Although not now classified as an SPA, there are similarities—association with a class I HLA antigen, and a similar range of involved tissues. As eye disease is one of the most serious consequences of Behçet's disease, the report of efficacy with interferon (IFN) -α is encouraging, and the possible mechanism of its action worth exploring further.

Histopathologic evidence that sacroiliitis in ankylosing spondylitis is not merely enthesitis—Systematic study of specimens from patients and control subjects.
R J Francois, D L Gardner, E J Degrave, E G L Bywaters.

Arthritis Rheum 2000; **43**(9):2011–24.

BACKGROUND. There has been relatively little published on the histopathology of the sacroiliac joint. The joint is inaccessible and biopsied relatively infrequently, and post-mortem studies of patient with AS inevitably tend to deal with end-stage disease. This paper reports a systematic study of the histopathology of sacroiliitis in AS at five different stages of the disease, as compared with controls. Two independent observers blindly assessed 75 microscopic features in the sacroiliac joints in 12 cases of AS (five biopsies, seven autopsies) and in 22 control cases (all autopsies). In AS, synovitis, pannus formation, myxoid marrow (an excess of stellate fibroblasts or myofibroblasts resembling young scar tissue), superficial cartilage destruction, enthesitis, intra-articular fibrous strands, new bone formation and bony ankylosis were significantly more frequent than in control cases, in which there was more endochondral bone within deep-zone articular cartilage. Cartilaginous fusion occurred in both groups, but much earlier in AS. There was no residual synovium when the joint lumen was totally occluded. Mild but destructive synovitis and myxoid subchondral bone marrow were the earliest changes identified in AS. These lesions destroyed the adjacent articular tissues, a loss that was followed to varying degrees by fibrous scarring, woven bone and new cartilage. The original cartilages also fused, and chondral fusion was the predominant mode of ankylosis. Both the original and the reparative cartilaginous tissues were replaced by bone. Active enthesitis occurred in two advanced and three late cases; fibrous scar tissue, presumed to represent previous enthesitis, was observed in all stages except the earliest. Para-articular bone was at first dense, and later porotic.

INTERPRETATION. In the sacroiliitis of AS synovitis and subchondral bone marrow changes are observed that offer a more rational explanation for widespread joint destruction than does enthesitis; an unusual form of chondroid metaplasia contributes to the ankylosis that this joint undergoes.

Comment

This paper illustrates in useful detail the histopathology of the sacroiliac joint. Since involvement of this site is invariable in AS, an understanding of the pathology at this site is crucially important. The paper contributes to the recent stimulating debate on the centrality of enthesitis to the pathology of the SPA as suggested by McGonagle *et al.* |**1**|, who regard enthesitis as the primary pathology to which a secondary synovitis can sometimes be added. The paper does not support this idea, emphasizing the presence of rheumatoid-like destructive synovitis. Nevertheless, enthesitis and evidence of previous enthesitis were recorded. A useful view of the progression of disease in different component of the sacroiliac joint is shown in the figure (Fig. 6.1).

Quantitative analyses of sacroiliac biopsies in spondyloarthropathies: T cells and macrophages predominate in early and active sacroiliitis—cellularity correlates with the degree of enhancement detected by magnetic resonance imaging.

M Bollow, T Fischer, H Reisshauer, *et al. Ann Rheum Dis* 2000; **59**(2):135–40.

BACKGROUND. Sacroiliitis is a *sine qua non* of the SPA; indeed without sacroiliitis the diagnosis of AS cannot 'officially' be made. Therefore, this is an important site to investigate for immunopathology. The Berlin group took advantage of the therapeutic efficacy of injection of active sacroiliac joints with corticosteroid to obtain biopsies from the joint guided by computed tomography (CT). At the same time they used magnetic resonance imaging (MRI) with intravenous administration of gadolinium-DTPA to determine quantitatively the activity of sacroiliitis assessed by calculating the enhancement produced in the joint by gadolinium, and to examine the structural changes ('chronicity') present in the joint. Both activity and chronicity were graded so that comparisons could be made between immunohistology and imaging. The identity and numbers of inflammatory cells in biopsy specimens were measured following immunostaining. Thirty-two patients with SPA were examined (AS, 18; undifferentiated SPA, 12; and psoriatic arthritis, two) and in 22 adequate tissue samples were obtained at biopsy for immunohistology. All patients scored >5 cm for back pain on a 10 cm visual analogue scale. MRI showed a low level of structural change in nine patients and a higher level in 13; as expected those with greater structural change had longer disease duration (mean 7.3 years versus 2.5 years). Twelve patients showed high levels (>70%) enhancement with gadolinium, indicating active disease and eight lesser levels; two refused gadolinium and could not be assessed. Biopsies contained cartilage, bone and proliferating connective tissue, but the proportions of these tissues did not vary according to chronicity or activity scores. However, numbers of inflammatory cells were increased in those with less structural change and those with greater levels of activity as indicated by gadolinium enhancement. Interestingly, even at this relatively acellular site where the synovial membrane is not a prominent structure, T lymphocytes were evident and slightly more frequent than macrophages. Clusters of proliferating fibroblasts were also seen in three cases and new vessel formation in seven cases.

INTERPRETATION. The inflamed sacroiliac joint contains both T lymphocytes and macrophages, suggesting that a T-cell-mediated response to (unknown) antigens is driving the inflammatory response. In later stages when structural change occurs such as erosion and ankylosis, components of the immune system are less prominent.

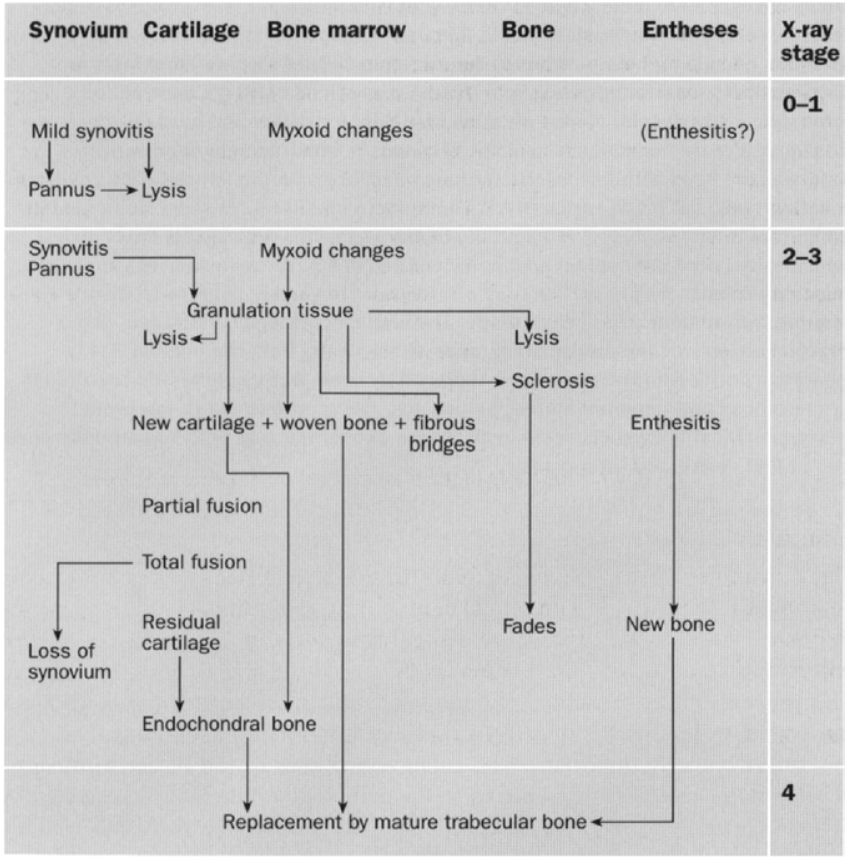

Fig. 6.1 Overview of changes in various sacroiliac tissues according to radiological stage. Source: François *et al.* (2000).

Comment

This is an impressive study which combines the power of MRI to make assessments of both structure and activity with careful immunohistology, although the latter inevitably was conducted with very small tissue fragments within which inflammatory cells comprised only a small proportion (1–4%) of the tissue. The correlated dynamic MRI images and histology displayed in the paper are particularly informative. The scoring system which this group has devised for chronicity and activity is likely to find application in future, particularly in clinical trials of novel agents to treat the SPA.

Table 6.1 MRI grading system for acute and chronic sacroiliac inflammation

1 Activity index

Grade x:	enhancement < 30°	= no sacroiliitis
Grade A:	enhancement < 30°–70°	= moderate sacroiliitis
Grade B:	enhancement > 70°	= severe sacroiliitis

In some early cases there might be no sacroiliitis detectable in the joint space but enhancement can be visible in the joint capsula or in the periarticular bone marrow. In these cases the degree of enhancement should be taken as for sacroiliitis itself.

2 Chronicity index

Grade 0:	no chronic changes
Grade I:	circumscribed subchondral sclerosis and/or ⩽ 2 erosisions
Grade II:	moderate subchondrial sclerosis and/or > 2 erosions with normal joint space width
Grade III:	severe subchondrial sclerosis and/or pseudodilatation of the joint and/or a minor degree of partial ankylosis (> 25% of the joint)
Grade IV:	definite ankylosis

The pathological changes are counted for each section separately. They only add up if clearly different regions are involved. Sacroiliitis can be diagnosed if at least an activity index A or B and/or a chronicity index >II is detected.
Source: Bollow *et al.* (2000).

Table 6.2 Comparison of the patients according to the activity and chronicity of sacroiliitis

MRI grading	Classified as group	Number	Disease duration (y) (mean (SD))	Inflammatory cells/10 mm^2 (mean (SD))	*P* Value
Activity					
enhancement 30–70%	A	8	5.6 (3.3)	6.0 (5.6)	NS
enhancement >70%	B	12	4.7 (5.8)	21.8 (17.3)	0.05*
Chronicity					
chronic changes ⩽ grade II	I	9	2.5 (2.9)†	26.7 (20.1)	0.04*
chronic changes > grade II	II	13	7.3 (4.8)†	5.3 (5.2)	NS

*For the comparison of relative proportions of inflammatory cells.
†The difference in disease duration between grade 1 and grade 2 was significant (P=0.02).
Source: Bollow *et al.* (2000).

Psoriatic arthritis and human immunodeficiency virus infection in Zambia.
P Njobvu, P McGill. *J Rheumatol* 2000; **27**(7):1699–702.

BACKGROUND. The epidemic of HIV infection in sub-Saharan Africa has had rheumatological consequences, and these authors previously drew attention to the extraordinary incidence of inflammatory arthritis in the HIV-infected population in Zambia, mostly reactive arthritis and undifferentiated SPA. These disorders were very rare prior to the arrival of HIV infection, although organisms associated with reactive

arthritis, such as *Shigella,* are very common. This report concerns psoriatic arthritis in the same population. Although this was much rarer than other forms of SPA, 28 cases were seen in 40 months (when 702 new attendees with inflammatory arthritis were seen). Twenty-seven of 28 were HIV+. The arthritis was predominantly polyarticular (22 patients), lower limb, and progressive; psoriasis was commonly an extensive guttate plaque admixture. Interestingly, psoriatic arthropathy was commoner in early stages of infection; 16 of 27 were in WHO stage 1 (no disease or lymphadenopathy alone), and only two in stage 4 (frank acquired immune deficiency syndrome [AIDS]). Indeed, the arthropathy ameliorated at the onset of AIDS in many patients, but skin disease did not.

INTERPRETATION. Psoriatic arthropathy is almost universally associated with HIV infection in black Zambians. The clinical features are similar to those described in Caucasians with HIV-associated psoriatic arthropathy.

Table 6.3 Spectrum of inflammatory arthropathies among 702 consecutive patients

Diagnostic Group	Number HIV + (%)
1. SpA, total	405 (94.0)
Undifferentiated SpA	207 (98.0)
Reactive arthritis	170 (87.0)
Psoriatic arthritis	28 (92.0)
Ankylosing spondylitis	1
2. Mono, oligo, polyarthritis (arthritis alone 'AA')	202 (64.0)
3. Rheumatoid arthritis	45 (0.0)
4. Gout	35 (26.0)
5. Other	14

Source: Njobvu *et al.* (2000).

Comment

Care was taken to exclude patients with transient psoriasiform rashes or minor changes such as onycholysis alone or nail pitting alone. In fact most patients had extensive psoriasis and in 20 the onset of psoriasis and arthropathy was simultaneous. Other features of SPA such as enthesitis and inflammatory back pain were observed. There is little doubt, therefore, that HIV infection has allowed the appearance of various forms of SPA in a population that was previously resistant to this form of arthritis. HLA-B27 is generally absent from the Zambian population, but alleles associated with psoriasis (Cw6 and B17) are present. How HIV predisposes to SPA is unclear, but the clinical observation that marked depletion of CD4+ T lymphocytes is associated with the remission in arthritis raises the possibility that the arthritogenic T cell is CD4+. This is counter-intuitive in a disease with a class I HLA antigen association, but there is evidence in animal models consistent with this notion and CD4+ T cells are abundant in SPA synovium. In contrast CD8+ T cells predominate in the psoriatic plaque, which does not remit at low levels of CD4+ T cells. These

observations would be consistent with the loss of a regulatory CD4+ T-cell subset early in HIV infection so that inflammatory responses are uncontrolled. Clearly, under normal circumstances the Zambian population deals with environmental pathogens such as Shigella without developing arthritis, while even without the influence of B27, HIV infection is able to produce the same disease phenotype seen in immunocompetent B27+Caucasian populations. Although the stimulus for the development of psoriasis±arthritis is unknown, bacteria such as skin commensals have been suggested as likely agents. Again HIV infection overcomes the natural resistance of the Zambian population to either psoriasis or psoriatic arthropathy.

The familial form of spondyloarthropathy—A clinical study of 115 multiplex families.

R Said-Nahal, C Miceli-Richard, J M Berthelot, *et al.,* on behalf of the Groupe français d'étude génétique des spondyloarthropathies. *Arthritis Rheum* 2000; **43**(6):1356–65.

BACKGROUND. Family history is often positive in the SPA, and is due to more than just the influence of HLA-B27. This family study was designed to document the clinical features of 'familial' SPA in detail. One hundred and fifteen white French families were recruited, each of which had at least two members with SPA. Pedigrees were established, clinical data and pelvic radiographs collected, and the HLA-B27 status of all patients determined. Analysis was performed to determine the prevalence of SPA manifestations according to sex, disease duration and HLA-B status, and to examine clustering of specific manifestations in subsets of families. Three hundred and twenty-nine SPA patients were identified. The mean ±SD age at onset was 24±9.4 years, and the male/female ratio 186:143, or 1.3, with few sex differences in disease expression—men had significantly more radiographic sacroiliitis and oligoarthritis. Axial manifestations and HLA-B27 were each present in 97% of the patients. IBD and HLA-B35 were over-represented in the seven families containing HLA-B27- patients, but some B27− patients had B27+ relatives with SPA. The frequency of radiographic sacroiliitis increased in parallel with disease duration. Peripheral enthesitis, radiographic sacroiliitis, and psoriasis were evenly distributed in the families. Clustering independent of age was only observed for peripheral arthritis, suggesting that specific factors may predispose individuals to this manifestation.

INTERPRETATION. In this study familial SPA appeared to be homogeneous, based on the high frequencies of axial skeletal involvement and HLA-B27. The lack of clustering of most manifestations in families suggests that a predominant set of genes, including HLA-B27, predisposes individuals to all forms of familial SPA, and that ubiquitous genetic (e.g. gender) or environmental factors (e.g. infection) contribute to phenotype diversity.

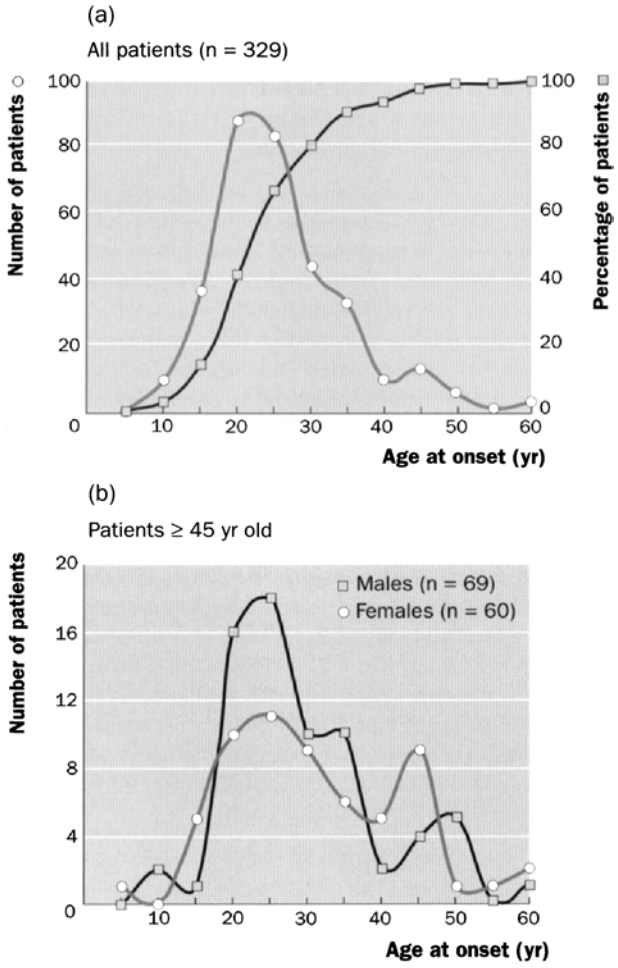

Fig. 6.2 Age at onset of the familial form of SPA. (a) Number of patients (open circles) and cumulative percentage of all patients (open squares) as functions of age at onset. (b) Numbers of male (open squares) and female (open circles) patients of age ≥45 years as functions of age at onset. Source: Said-Nahal *et al.* (2000).

Comment

This is a fascinating documentation of a large number of families in which more than one member had SPA—an average of 2.86 affected members per family, and one family with 10 affected members! Patients fulfilled either Amor or ESSG diagnostic criteria, but as expected >95% of patients fulfilled both criteria. Proband selection is likely to have influenced the findings; 97% of probands had axial symptoms, so it is less surprising that the family members were almost all

B27+ and also had axial disease. Eighty-six per cent had sacroiliitis after 20 years of disease, with 67% of the group as a whole. It has been suggested previously that AS and Reiter's syndrome 'breed true', and the clustering of peripheral arthritis in certain families may identify the minority in this cohort with susceptibility for reactive arthritis. Patients with self-limiting episodes of reactive arthritis would not be captured by this study. Few conclusions about B27 − SPA can be derived from the study as there were only nine such patients but the coexistence of B27+ and B27− AS (apparently primary) in one family is noteworthy and would suggest that in certain circumstances the a set of genes may be sufficient to produce the AS phenotype in the absence of B27, as occurs in secondary AS (associated with psoriasis or IBD). However, silent IBD is difficult to exclude and may account for cases of apparently B27− primary AS.

Clinical phenotype is related to HLA genotype in the peripheral arthropathies of inflammatory bowel disease.
T R Orchard, S Thiyagaraja, K I Welsh, *et al. Gastroenterology* 2000; **118**(2):274–78.

BACKGROUND. Peripheral arthropathies in IBD are well recognized and are classified with the HLA-B27-related SPA by the European Spondyloarthropathy Study Group. However, previous HLA studies in IBD have only shown this association with axial disease rather than peripheral arthropathy. Orchard |2| recently reported a clinical classification that describes two types of peripheral arthropathy in IBD, distinguished by their natural history and articular distribution. This paper examined these two groups for their HLA antigens and compared them with patients with other related SPA: AS with IBD and reactive arthritis triggered by enteric infection. IBD patients with type 1 (*n*=57) and type 2 (*n*=45) peripheral arthropathy were identified by case note review and questionnaire. Patients and 603 controls from Oxfordshire, were assigned HLA-A, -B, -C, -DR and -DQ genotypes by sequence-specific primer polymerase chain reaction (PCR). Patient results were compared with controls (corrected for multiple comparisons), and then with each other. The results were compared with those of a cohort of 30 patients with post-enteric reactive arthritis and 16 patients with IBD-associated AS (IBD-AS). Type 1 arthropathy was associated with HLADRB1*0103 (DR103; a rare subtype of DR1) in 33% [*P*<0.0001; relative risk (RR), 12.1], HLA-B35 in 30% (*P*=0.01; RR, 2.2) and HLA-B27 in 26% (*P*=0. 001; RR, 4.0). In contrast, type 2 was associated with HLA-B44 in 62% (*P*=0.01; RR, 2.1). Similar significant associations to type 1 arthropathy were found in reactive arthritis, except that the association with HLA-B27 was significantly stronger and an association was found with DRB1*0101 (DR1) in 43% (*P*=0.001; RR, 2.2). IBD-AS was associated only with HLA-B27 and DRB1*0101.

INTERPRETATION. These data suggest that the clinical classification into type 1 and type 2 arthropathies describes immunogenetically distinct entities and establish that in polygenic disorders, genes may determine clinical phenotype without conferring overall disease susceptibility (in this case, HLA genes). Type

1 arthropathy is clinically and immunogenetically similar to the SPA, but different HLA associations may define phenotypically distinct groups. Type 2 arthropathy has different HLA associations and may have a different aetiology. Further studies are now required to confirm these associations and to elucidate the different pathogenetic mechanisms.

Comment

The paper indicates the importance of accurately classifying patients on clinical grounds before carrying out genetic studies, particularly those involving HLA antigens—the associations described here would have been obscured if all patients with arthritis and IBD were examined as a single group. The emergence of HLA associations, which are also seen in related disorders, gives them additional plausibility. In subsequent work on a larger cohort of patients a clear association has been described between DRB1*0103 and IBD-AS; it will be of great interest to determine whether the association indicates involvement of a class II HLA gene in pathogenesis or whether the association is due to linkage to another gene in the MHC (e.g. TNF-α). Overall, the paper emphasizes that SPA is the end-result of a number of environmental factors (infection, colonic inflammation) and genetic factors of which B27 is of varying importance—nearly ubiquitous in AS but present in only a minority of patients with SPA due to psoriasis or IBD.

Evidence that HLA-B*2706 is not protective against spondyloarthropathy.
D Sudarsono, S Hadi, A Mardjuadi, *et al. J Rheumatol* 1999; **26** (7): 1534–6.

BACKGROUND. As molecular methods of HLA typing have become available, a large number of subtypes of HLA-B27 have been found (21 at the last count). As most of these differ from one another at only a few amino acids, it has long been hoped that subtypes that are not associated with disease would be found, and that this would give an insight into the pathogenesis of the disease; this is in much the same way that sequencing subtypes of HLA-DR4 showed the critical importance of certain amino acids and their conservation in DR4 subtypes associated with disease, and also in other disease-associated alleles such as DR1 and DR10. When subtypes are very rare they are not informative, but it was previously shown that whereas both HLA-B*2704 and HLA-B*2706 are common in South-east Asia only HLA-B*2704 is positively associated with SPA. Presumably, B*2704 presents pathogenetic peptides, which B*2706 is unable to. Indeed B*2706 was not found in such patients, raising the possibility that B*2706 might actually protect against the disease. To address this possibility the authors identified families in which both B*2704 and B*2706 occurred to see whether in B*2704/B*2706 heterozygotes the effect of one of the subtypes would show a dominant effect over the other. Two families of mixed Chinese/

Indonesian origin were studied. HLA-B27 subtyping was performed by PCR in combination with sequence-specific oligonucleotide probes. In one family, members with B*2704, B*2706, or both occurred. In the other family B*2704, B*2706, and B*2708 were present. In both families SPA was seen only in B*2704 positive members, while the B*2706 and B*2708 positive members were healthy. However, some B*2704/B*2706 or B*2704/B2708 heterozygotes did have disease.

INTERPRETATION. This study shows that a B27 allele, which is not positively associated with SPA, is also not protective in individuals, which also have a disease-associated allele. Thus, the pathogenic influence of B*2704 is dominant over B*2706. The same dominance is seen over B*2708, a relatively rare allele, which is also probably not associated with SPA.

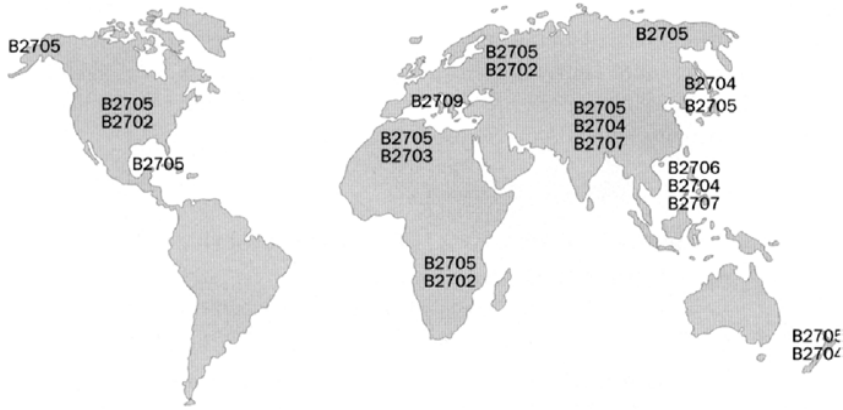

Fig. 6.3 Worldwide distribution of common HLA-B27 subtypes. Source: Zeidler *et al.* [4].

Comment

These are valuable observations which exclude some models of how certain alleles of B27 predispose to arthritis. For instance, it is not now likely that patients with e.g. B*2704 fail to delete a potentially arthritogenic set of T cells in the thymus, since in this model possession of a non-disease associated allele would allow deletion and confer dominant protection. Difference in alleles often affect the binding of antigenic peptides; indeed the existence of 21 subtypes is taken to imply selection of certain subtypes where they confer advantages in dealing with local infectious agents (Fig. 6.3). However, although work has been carried out showing the differences between peptides bound by B*2704 and B*2706, this does not readily lead to the identification of arthritogenic peptides.

Multispecific CD4$^+$ T cell response to a single 12-mer epitope of the immunodominant heat-shock protein 60 of *Yersinia enterocolitica* in *Yersinia*-triggered reactive arthritis: overlap with the B27-restricted CD8 epitope, functional properties, and epitope presentation by multiple DR alleles.

A K H Mertz, P Wu, T Sturniolo, *et al. J Immunol* 2000; **164**(3):1529–37.

BACKGROUND. When T-cell clones have been isolated from the joints of reactive arthritis patients, they commonly respond to antigens from the bacteria responsible for triggering the arthritis. Some of these clones recognize bacterial antigens that have human homologues, a major example being heat shock protein (hsp) 60. Therefore, the question can be asked whether the T cells that recognize bacterial hsp60 cross-react with self-hsp60 making an autoimmune response. This paper addresses this question with respect to *Yersinia* hsp60-specific T cells. *Yersinia* hsp60 has been found to be a dominant CD4 and CD8 T-cell antigens in *Yersinia*-triggered reactive arthritis. The nature of this response with respect to the epitopes recognized and functional characteristics of the T cells is largely unknown. CD4+T-cell clones specific for *Yersinia* hsp60 were raised from synovial fluid mononuclear cells from a patient with *Yersinia*-triggered reactive arthritis, and their specificity was determined using three recombinant *Yersinia* hsp60 fragments, overlapping 18-mer synthetic peptides, as well as truncated peptides. Functional characteristics were assessed by cytokine secretion analysis in culture supernatants after specific antigenic stimulation. Amino acid positions relevant for T-cell activation were detected by single alanine substitutions within the epitopes. The majority of clones recognized an epitope within the amino acid sequence 182–371. All these clones were specific for peptide 319–342. Th1 clones and IL-10-secreting clones occurred in parallel, sometimes with the same fine specificity. The 12-mer core epitope 322–333 is a degenerate MHC binder (i.e. it will bind to many different HLA-DR alleles) and is presented to some T-cell clones in a 'promiscuous' manner. Interestingly, this epitope is almost identical with a previously mapped B27-restricted epitope of *Yersinia* hsp60 recognized by CD8+ cytotoxic T cells. Cross-reactivity of *Yersinia* hsp60-specific T-cell clones with self-hsp60 was not observed.

INTERPRETATION. This study showed that, although hsp60 is a major target antigen of the immune response to *Yersinia* in *Yersinia*-induced reactive arthritis, the T cells involved do not cross-react with human hsp60.

Comment

There have been previous reports of bacterial hsp60-reactive T cells from reactive arthritis joints that do appear to cross-react with self-hsp60, and it is not yet clear what the general rule is. Even where T cells cross-react with the human hsp60, this does not necessarily mean that they will be pathogenic. Evidence

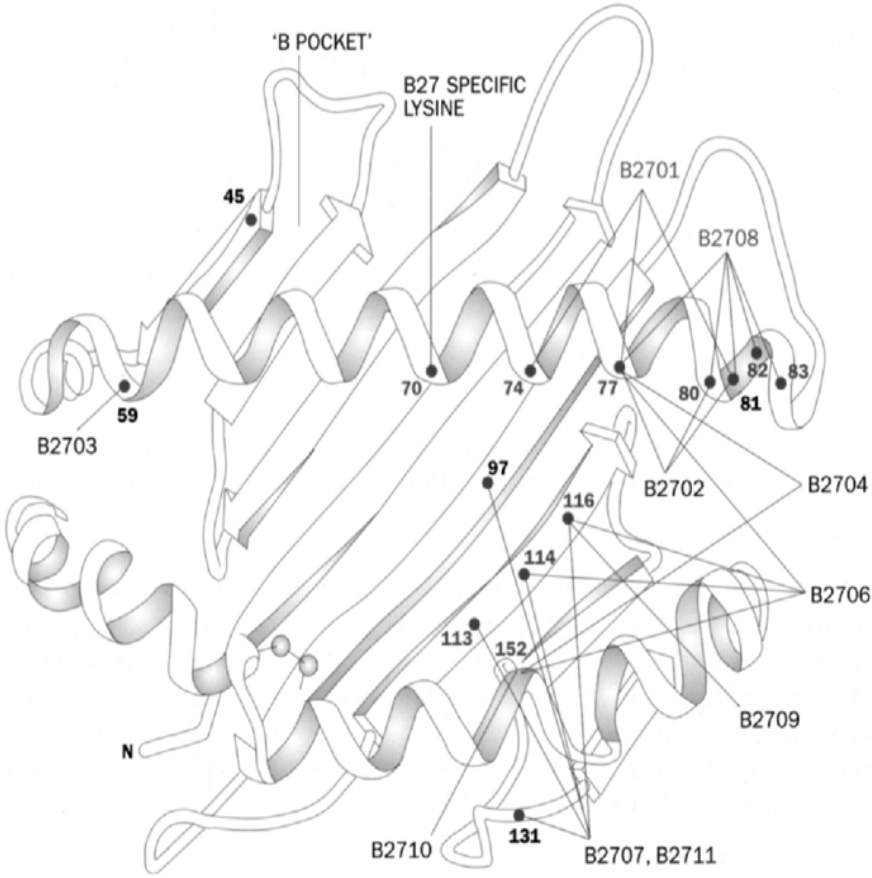

Fig. 6.4 The crystallized HLA-B27 molecule, viewed facing into the peptide-binding groove (after the model proposed by Bjorkman *et al.* (1987) |**3**| , showing the locations on the peptide of the polymorphic amino acid residues that distinguish the subtypes. Source: Zeidler *et al.* |**4**|.

from animal studies suggest that such autoreactive T cells can actually have an anti-inflammatory role and protect against the development of arthritis. The mapping of an epitope, which is presented by both class I (B27) and class II HLA antigens, is particularly interesting as this means that the same processed peptide could stimulate both cytotoxic T-cell responses and the T helper response that cytotoxic T cells usually require. However, it remains to be determined whether this epitope is also relevant in other reactive arthritis patients, although this seems likely.

CD8(+) T-cell autoreactivity to an HLA-B27-restricted self epitope correlates with ankylosing spondylitis.
M T Fiorillo, M Maragno, R Butler, *et al. J Clin Invest* 2000; **106**:47–53.

BACKGROUND. HLA-B27 is highly associated with AS, but the mechanism is unknown. The starting point of this investigation is that among the HLA-B27 alleles, B*2709, which differs by one amino acid from the susceptible B*2705, is not associated with the disease. These observations were made in the Sardinian population; recently, two B*2709+ patients with undifferentiated SPA were reported in southern Italy (abstract presented at the American College of Rheumatology, Philadelphia 2000 |5|), so protection may not be absolute. These workers had previously studied difference in the way that B*2705 and B*2709 present peptide from Epstein-Barr virus (EBV) latent membrane protein 2 (amino acids 236–244), an important target of the CD8+ T-cell recognition of EBV-infected cells. The authors looked for human peptides with similarity to the LMP peptide and selected a peptide from the vasoactive intestinal peptide receptor 1 (VIP1R). Whereas both B*2705(+) and B*2709(+) subjects possessed LMP2 236–244-specific, HLA-B27-restricted T cells, only the B*2705(+) individuals responded significantly to the homologous peptide from VIP1R (400–408). These results led to a comparison, by IFN-γ ELISPOT analysis, of the T-cell response to VIP1R 400–408 in patients with AS versus B*2705 healthy controls. The data showed that reactivity to VIP1R 400–408 was much more prominent in the patients with AS.

INTERPRETATION. It is argued that the findings demonstrate the target of an autoreactive response by CD8+ T cells in AS. The response was absent in individuals with a B27 subtype not associated with disease, and more prominent in B*2705+ patients than in controls, both properties that would be expected in a putative ‘arthritogenic’ peptide.

Comment

Although AS is often assumed to be autoimmune in nature, and that B27 presents an arthritogenic peptide, no target of the autoimmune response has been identified and arthritogenic peptides have not been defined. Hence the interest in this paper, but there are a number of questions that can be raised. B*2709 differs from B*2705 by one amino acid, which influences the binding of the C-terminal amino acids in an antigenic peptide. The LMP and VIP1R peptides mainly vary at the C-terminus and, therefore, might be expected to bind differently to B*2705 and B*2709. This proved to be the case as the VIP1R peptide bound much more strongly to B*2709. This in turn means that a failure to detect responses to the VIP1R peptide in B*27– 9+ individuals is not surprising, as cells that could recognize the peptide are much more likely to be deleted in the thymus during T-cell development. The main observation therefore rests on the differences seen between B*2705+ AS patients and controls and this is based on small numbers (8

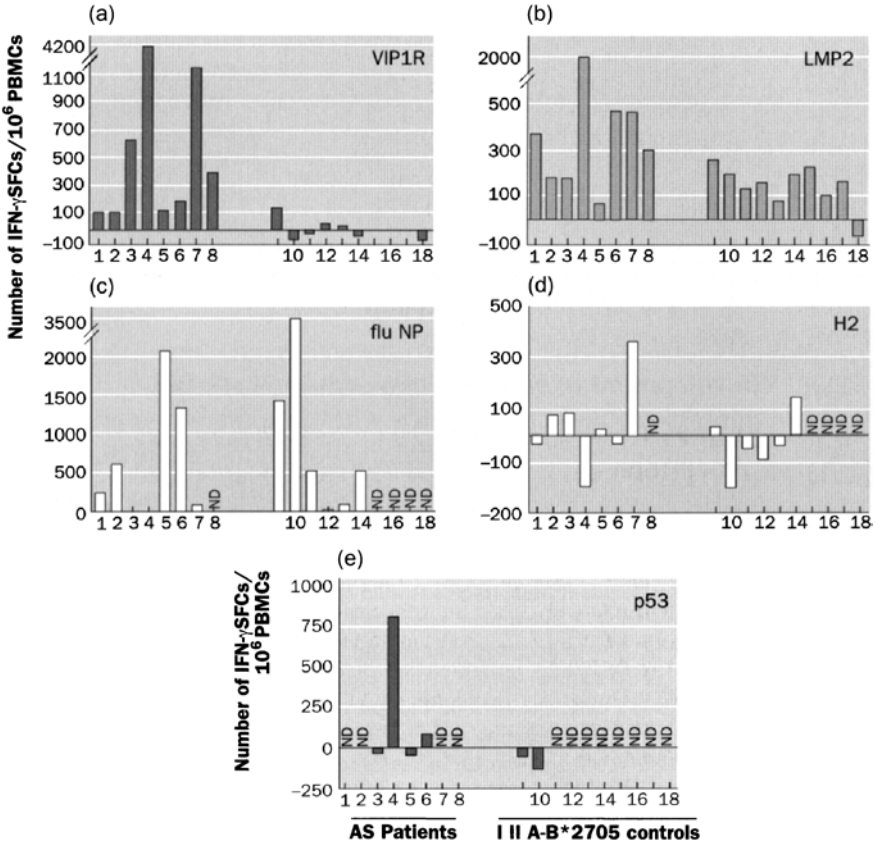

Fig. 6.5 Frequency of VIP1R and LMP2 T-cell responders evaluated by IFN-γ ELISPOT assay. Enumeration of IFN-γ spot-forming cells (SFCs) in a 10-day ELISPOT assay performed with peptide-restimulated PBMCs from eight patients with AS and in 10 B*2705* healthy subjects. (a) VIP1R400–408; (b) LMP2 236–244; (c) NP 383–391 from influenza nucleoprotein; (d) H2 325–333 from TIS 11B; and (e) p53 266–274. For some individuals, the reactivity to control peptides (flu NP, H2, and p53) was not tested because of limited number of cells. ND, not done. Values are reported as the number of IFN-γ SFCs from peptide-restimulated PBMCs minus the number of IFN-γ SFCs from medium-grown PBMCs per 10^6 PBMCs. Comparison of the frequency of VIP1R- and LMP2-responder T cells between patients with AS and healthy controls was performed using the two-tailed non-parametric Mann-Whitney test. VIP1R 400–408: P=0.0003; LMP2 236–244: P=0.04. Source: Fiorillo *et al.* (2000).

and 10) and a very wide range of responses as judged by the ELISPOT technique, and only after an initial *in vitro* priming step. Therefore, the results are interesting and provocative, but require confirmation. It would be especially useful to determine whether B*2705 transgenic rodents have responses to VIP1R.

It is also unclear why responses to this receptor would produce the pattern of organ involvement typical of SPA.

Development of spontaneous arthritis in beta2-microglobulin-deficient mice without expression of HLA-B27: association with deficiency of endogenous major histocompatibility complex class I expression.

D J Kingsbury, J P Mear, D P Witte, *et al. Arthritis Rheum* 2000; **43**(10): 2290–6.

BACKGROUND. Mice deficient in β_2-microglobulin (β2m), but expressing the human MHC class I molecule HLA-B27, have been reported to develop spontaneous inflammatory arthritis |2|. These investigators sought to determine whether, under certain conditions, β_2m deficiency alone was sufficient to cause arthritis, and if this might be a result of class I deficiency. To do this they generated several strains of mice with different genetic backgrounds all genetically deficient in β_2m. They also examined mice deficient in the transporter associated with antigen processing (TAP1), which are also unable to express class I MHC antigens normally, and B27 transgenic, β_2m-deficient mice on the same background as one of the β_2m-deficient strains (B6). In the original report of spontaneous arthritis in B27 transgenic mice, arthritis only occurred when mice were removed from a specific pathogen-free (SPF) environment. Therefore, cohorts of all these mice were also transferred from SPF to conventional animal rooms, and evaluated clinically and histologically for the development of arthritis. Arthritis occurred in TAP1-deficient and β2m-deficient mice (i.e. both class I-deficient) mice at a frequency of 30–50% in one genetic background, and at 10–15% of β_2m-deficient mice on other genetic backgrounds. One strain was resistant to developing arthritis despite β_2m deficiency. On the same genetic background expression of B27 in β_2m-deficient mice did not increase the frequency of arthritis above that seen in mice that were only β_2m deficient.

INTERPRETATION. These experiments suggest that deficiency in surface expression of class I MHC antigens is sufficient to cause spontaneous arthritis in mice, but the frequency of disease is modified by other genes.

Comment

These are provocative findings as it had been assumed that the arthritis seen in β_2m-deficient mice made transgenic for HLA-B27 was due to unique properties of B27. The strain used by Khare *et al.* |6| in making the original observation differs from those used in this paper (B10 and B6, respectively, both substrains of C57BL mice). It might be that B10 genes prevent the occurrence of arthritis due to a deficiency of β2m, but that this is overcome by the additional presence of transgenic B27. In any case the work points to the arthritogenic potential of a failure to express class I MHC antigens correctly, although arthritis only becomes evident after colonization by bacteria, which occurs when SPF

Table 6.4 Lack of HLA-B27-induced arthritis in β2-microglobulin (β_2m^0)-deficient mice

Cohort (B6)	Male, no. with arthritis/total (%)	Female, no. with arthritis/total (%)	Total %
B*2705 + β_2m^0	2/29 (7)	0/30 (0)	3
B*2705 + β_2m^{0*}	2/21 (0)	0/14 (0)	0
B*2703 + β_2m^{0*}	2/19 (11)	1/14 (7)	9
β_2m^{0*}	5/39 (13)	3/13 (23)	15
C57BL/6J	2/42 (5)	3/27 (11)	7

*Cohort housed at the University of Texas Southwestern Medical Center.
Source: Kingsbury *et al.* (2000).

conditions are removed. Later observations by the same group (see Mear *et al.,* below) indicate that physical properties of B27 may mean that B27+ cells have some similarity with β2m-deficient cells in that both have difficulty in expressing surface class I MHC antigens: in the β2m-deficient cells because there is no partner for the class I heavy chain, and in the B27+ cells because B27 is unusually slow to fold into a correct conformation. How these abnormalities lead to arthritis remains unclear.

Misfolding of HLA-B27 as a result of its B pocket suggests a novel mechanism for its role in susceptibility to spondyloarthropathies.
J P Mear, K L Schreiber, C Munz, *et al. J Immunol* 1999; **163**(12): 6665–70.

BACKGROUND. The MHC class I protein HLA-B27 is strongly associated with susceptibility to SPA and can cause arthritis when expressed in rats and mice, implying a direct role in disease pathogenesis. A prominent hypothesis to explain this role suggests that the unique peptide binding specificity of HLA-B27 confers an ability to present arthritogenic peptides. The B pocket, a region of the peptide binding groove that is an important determinant of allele-specific peptide binding, is thought to be critical for arthritogenicity. However, this hypothesis remains unproven. This paper addresses a different aspect of the B pocket of B27 and shows that in addition to its role in peptide selection, the B pocket causes a portion of the pool of assembling HLA-B27 heavy chains in the endoplasmic reticulum to misfold, resulting in their degradation in the cytosol. The misfolding phenotype is corrected by replacing the HLA-B27 B pocket with one from HLA-A2. Our results suggest an alternative to the arthritogenic peptide hypothesis. Misfolding and its consequences, rather than allele-specific peptide presentation, may underlie the strong link between the HLA-B27 B pocket and susceptibility to SPA.

INTERPRETATION. Careful biochemical analysis showed that HLA-B27 has an unusual property of folding rather slowly within the cell to achieve its final conformation. This abnormality was abolished by altering one part of the B27 heavy chain, the ‘B’ pocket which is responsible for anchoring amino acid

side chains from antigenic peptides. Replacing this with the 'B' pocket from HLA-A2, which involves only 6 amino acid changes, produced a normal rate of folding.

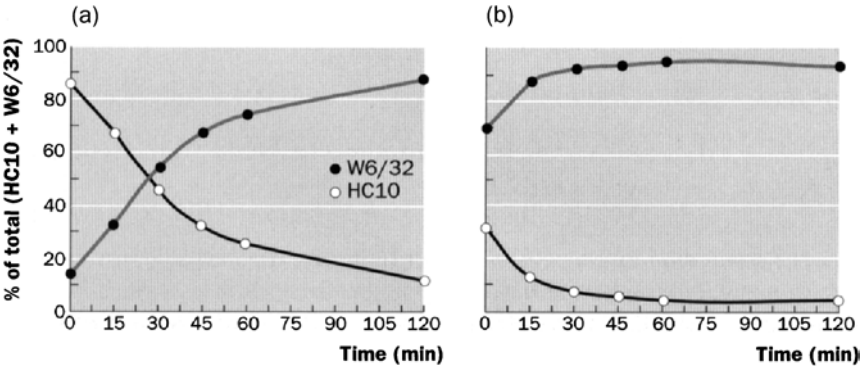

Fig. 6.6 Inefficient HLA-B*2705 folding. Cells were pulsed for 5 min with radiolabelled methionine and cysteine (which is incorporated into HLA-B27 and other proteins) and chased for up to 2 h in the presence of an excess of non-radioactive methionine and cysteine. At each time point sequential immunoprecipitations were carried out with W6/32 which binds to fully folded HLA molecules followed by HC10 which binds free heavy chains. The left hand panel shows the results for HLA-B*2705 and the right hand panel results for HLA-B*2705 in which the 'B pocket' has been replaced by one from HLA-A2. Note that at 30 min ~50% of the B*2705 has failed to fold correctly (still precipitates with HC10), whereas this proportion is <10% for the modified B*2705 molecule. Source: Mear *et al.* (1999).

Comment

What is abnormal about HLA-B27? This study demonstrates a novel way in which B27 differs from other class I HLA molecules. Previous work has concentrated on the fact that B27 binds a different set of antigenic peptides from any other class I allele, and therefore presents a unique set of peptides to the immune system, one or more of which might be important in causing SPA. However, previous workers have commented on other 'peculiarities' of the B27 molecule—a tendency for there to be B27 molecules on cell surfaces which do not have antigenic peptides bound to them, and an ability to be relatively independent of peptide for their assembly and expression. How these properties translate into disease is currently unknown. It is difficult to regard HLA-B27 as a 'faulty' HLA allele, since it is excellent at presenting viral peptides to the immune system, and B27+ HIV-infected patients do better than average. However, biochemical properties which allow it to present certain viral peptides efficiently (and confer a survival advantage to B27+ individuals) may have other consequences which result in the development of SPA.

Modification of disease outcome in Salmonella-infected patients by HLA-B27.
P Ekman, J Kirveskari, K Granfors. *Arthritis Rheum* 2000; **43** (7):1527–34.

BACKGROUND. This study was designed to determine whether HLA-B27 modifies the outcome of *Salmonella* infection *in vivo,* and took advantage of a large outbreak of *Salmonella* food poisoning in which the frequencies of joint symptoms and excretion of organisms could be accurately determined. The frequency of HLA-B27 was determined in 198 Salmonella-infected patients and 100 healthy controls by immunofluorescence and PCR. The excretion of *Salmonella* was monitored at monthly intervals. The symptoms of acute infection and possible joint involvement were evaluated using questionnaires. Thirty-eight of 198 Salmonella-infected patients (19.2%) and 13 of 100 healthy controls (13.0%) were HLA-B27 positive. The excretion of *Salmonella* did not differ significantly between HLA-B27-positive and -negative patients, or for patients with and without joint symptoms. Thirty-five patients (17.7%) reported *Salmonella*-triggered joint symptoms. The duration and severity of joint symptoms directly correlated with the presence of HLA-B27; B27 was present in three of 14 patients (21.4%) with arthralgia, five of 13 patients (38.5%) with probable reactive arthritis, and six of eight patients (75%) with confirmed reactive arthritis. Women reported *Salmonella*-induced pain and swelling of joints more frequently than men (*P*=0.07 and *P*=0.03, respectively). Patients with *Salmonella*-triggered joint symptoms reported abdominal pain and headache more frequently than patients without joint symptoms (*P*=0.05 and *P*=0.004, respectively).

INTERPRETATION. HLA-B27 did not have any major effect on either susceptibility to *Salmonella* infection *per se,* or the ability to clear the organism from the gastrointestinal tract. However, B27 did have a strong effect on the likelihood of developing significant joint and tendon symptoms.

Comment

Outbreaks of food poisoning provide a useful opportunity of assessing the incidence and duration of musculoskeletal symptoms in a cohort of patients, and allows documentation of patients with low-grade symptoms, which would not otherwise present to a rheumatologist. The study shows that such symptoms are surprisingly frequent (~18% of patients), of whom less than 25% had definite reactive arthritis. Less than half of the patients were HLA-B27 positive, which shows that the reactive arthritis syndrome does not require the presence of B27. However, symptom severity and duration are substantially increased by HLA-B27, and this explains why the proportion of B27+ patients in hospital-based series of reactive arthritis patients is often 70–80% as they are more likely to require medical attention. Although difficulty in clearing the infection would be an obvious explanation for the findings in B27+ subjects, there was no difference

Table 6.5 *Salmonella*-triggered joint symptoms in HLA-B27-positive and -negative *Salmonella* patients

	All patients n = 198)	**HLA-B27+**	**HLA-B27−**
No. (%) without joint symptoms	163 (82.3)	24 (14.7)*	139 (85.3)
No. women/no. men	83/80	9/15	74/65
No. (%) with joint symptoms	35 (17.7)	14 (40.0)	21 (60.0)
Joint swelling, no. (%)	21 (10.6)	9 (42.9)	12 (57.1)
No. women/no. men	22/13	6/8	16/5
Mean (range) duration of symptoms, days	68.5 (1–360)	128.4 (1–360)†	26.0 (1–120)
Mean (range) no. of affected joints	3.5 (1–8)	3.2 (1–8)	3.6 (1–8)
Arthralgia	14 (7.1)	3 (21.4)*	11 (78.6)
No. women/no. men	7/7	1/2	6/5
Mean (range) duration of symptoms, days	38.6 (1–360)	150.3 (1–360)	8.2 (1–8)
Mean (range) no. of affected joints	3.2 (1–8)	4.3 (1–8)	2.9 (1–7)
Probable ReA‡	13 (6.6)	5 (38.5)*	8 (61.5)
No. women/no. men	11/2	3/2	8/0
Mean (range) duration of symptoms, days	63.8 (7–180)	89.6 (14–180)	42.3 (7–90)
Mean (range) no. of affected joints	4.0 (1–8)	3.0 (2–4)	4.6 (1–8)
Confirmed ReA	8 (4.0)	6 (75)*	2/8 (25)
No. women/no. men	4/4	2/4	2/0
Mean (range) duration of symptoms, days	131.0 (28–330)	149.7 (28–330)	75.0 (30–120)
Mean (range) no. of affected joints	2.9 (1–5)	2.8 (2–4)	3.0 (1–5)

*P=0.0001 for comparison of HLA-B27 frequency in different diagnostic groups by cumulative logistic regression analysis.
†P<0.002 for comparison of HLA-B27-positive and -negative patients by Wilcoxon's 2-sample test.
‡ReA=reactive arthritis.
Source: Ekman *et al.* (2000).

in the time during which stools were *Salmonella*-positive in B27+ and B27− patients. Thus any effect of B27 on the host's ability to deal with *Salmonella* must be acting at a site other than the gastrointestinal mucosa.

Detection of *Salmonella infantis* in synovial fluid cells of a patient with reactive arthritis.
P Ekman, S Nikkari, A Putto-Laurila, *et al. J Rheumatol* 1999; **26**(11): 2485–8.

BACKGROUND. The first reports that reactive arthritis-associated organisms might traffic to affected joints was obtained by detecting organism-specific antigens in synovial fluid and synovium, using specific antibodies. In the case of *Chlamydia trachomatis,* atypical organisms were also detected by electron microscopy, and in keeping with the idea that intact organisms were getting to the joint, PCR studies were also positive, and have

demonstrated both *Chlamydia* DNA and RNA. However, in contrast enteric organisms could not be detected in PCR studies, although their antigens were readily detected. This suggested a fundamental difference between the two kinds of triggering organism in reactive arthritis. However, a study in 1999 used reverse transcription (RT) -PCR to amplify *Y. pseudotuberculosis* ribosomal RNA from synovial fluid of a reactive arthritis case caused by this organism. As rRNA is unstable, this result was also taken to indicate that the organism reached the joint in a viable state |7|. This study reports the observation by immunofluorescence of abundant 5. infantis-specific antigens in synovial fluid cells of the patient with *S. infantis* triggered reactive arthritis during acute joint inflammation. Salmonella-specific DNA was detected by Southern blotting of the amplified PCR product on one occasion, but the result could not be repeated.

INTERPRETATION. Whereas *Salmonella* antigens are relatively abundant in synovial fluid in some cases of reactive arthritis triggered by the organism, the presence of *Salmonella* is much harder to demonstrate implying that it is present in extremely low amounts.

Comment

The previous report |7| of *Yersinia* nucleic acids in a reactive arthritis joint is not necessarily in conflict with this report. The previous paper used a nested technique (non-specific amplification of bacterial rRNA and nested amplification of the product using *Yersinia*-specific primers. This involves a large amount of amplification, and the RT-PCR technique also exploits the fact that there may be up to 10^5 copies of ribosomal RNA per organism. Thus results in both cases are consistent with very low amounts of intact bacteria reaching the joint. How long these bacteria remain viable in the joint is unknown, but it may be a very short time. Nevertheless, continuous replenishment with bacteria from a site of persistent infection could play an important part in the pathogenesis of reactive arthritis.

Evolution of chronic recurrent multifocal osteitis toward spondyloarthropathy over the long term.
O Vittecoq, L A Said, C Michot, *et al. Arthritis Rheum* 2000; **43**: 109–19.

BACKGROUND. There has been much discussion about the nature of chronic recurrent multifocal osteitis, also sometimes classified as a form of SAPHO (synovitis, acne, pustulosis, hyperostosis, osteitis) and its relationship to SPA. This paper reports the outcome in 15 patients with prolonged follow-up (mean 11.6 years; median 9 years). There were eight children/adolescents and seven adults with no family history of rheumatic disease who had been diagnosed as having chronic recurrent multifocal osteitis between 1979 and 1995. Ten patients had undergone at least one bone biopsy of the lesions, with histological examination and multiple

cultures. In 1996, in addition to an in-depth interview, 12 patients underwent an extensive physical examination, laboratory evaluation, HLA-A, -B, -C and -DR typing, bone radiography and scintigraphy, and CT scan of the sternoclavicular and sacroiliac joints. Remission was observed in three patients. The other 12 patients developed various other features: 11 had involvement of the anterior thorax (sternoclavicular, sternocostal, manubriosternal and acromioclavicular joints); 11 had vertebral involvement; seven had sacroiliitis, four had peripheral enthesopathy and two peripheral arthritis. There was skin involvement in five (three palmoplantar pustulosis and two psoriasis). Spine involvement was the most common and occurred the earliest (median time to appearance after the onset of osteitis 5.6 years). Clinical sacroiliitis was clinically unilateral in all cases, but radiologically bilateral in three. No patients were HLA-B27 positive. Twelve patients met the European Spondyloarthropathy Study Group (ESSG) criteria for SPA.

INTERPRETATION. Over a 10-year period of follow-up, chronic recurrent multifocal osteitis tended to evolve to SPA, as defined by the ESSG criteria.

Table 6.6 Extent of involvement observed in the 12 patients re-examined in 1996, as assessed clinically, scintigraphically and radiologically

Localization	Clinical		Scintigraphic		Radiologic*	
	No.	%	No.	%	No.	%
Anterior thoracic syndrome	11	92	8	67	9	75
Sternoclavicular articulation*	8	67	8	67	5	42
Sternocostal articulation*	5	42	0	0	6	50
Manubrium–sternum articulation	3	25	1	8	3	25
Acromioclavicular articulation	4	33	2	17	1	8
Vertebral involvement	11	92	3	25	7	58
Cervical	2	17	0	0	1	8
Thoracic	6	50	2	17	6	50
Lumbar	7	58	1	8	4	33
Sacroiliac region involvement	7	58	4	33	7	58
Unilateral	7	58	3	25	4	33
Bilateral	0	0	1	8	3	25
Peripheral enthesopathy	4	33	2	17	5	42
Peripheral joint involvement	2	17	0	0	2	17

*Sternoclavicular and sternocostal articulations were examined by thin-cut, high-resolution computed tomography scans.
Source: Vittecoq *et al.* (2000).

Comment

The ESSG criteria for SPA are fairly wide, but the gradual accumulation of new clinical and radiological features typical of SPA suggest the classification is valid. As with other forms of SPA there is speculation about the involvement of bacteria particularly *Propionibacterium acnes,* but this organism was cultured from the initial lesions in only one case in this series. The lack of involvement of

B27 parallels that seen in psoriatic arthritis, although B27 is increased when axial disease is present in psoriatic arthritis. The series was too small to identify other HLA associations such as those associated with psoriasis.

Effect of a three month course of ciprofloxacin on the outcome of reactive arthritis.
T Yli-Kerttula, R Luukkainen, U Yli-Kerttula, *et al. Ann Rheum Dis* 2000; **59**(7):565–70.

BACKGROUND. Treatment of reactive arthritis with antibiotics has so far remained controversial. Eradication of the causative microbe appears logical, but short-term antibiotic treatment has no beneficial effect on the outcome of reactive arthritis. This study set out to evaluate the effect of a 3-month course of ciprofloxacin on reactive arthritis in a randomized, double-blind, placebo-controlled trial. Over a 3-year period, 71 patients with acute reactive arthritis triggered by a gastrointestinal or a urogenital infection were randomly assigned to receive ciprofloxacin 500 mg or placebo twice daily for 3 months. Patients were assessed at study entry, at 6 weeks, 3 months, 6 months and 12 months. Sixty-two patients were valid for the efficacy analysis. The primary outcome measures were erythrocyte sedimentation rate (ESR), number of swollen joints, patient's self-assessment and proportion of patients achieving complete recovery. Secondary outcome measures included Ritchie index, swelling index, C-reactive protein, haemoglobin, white cell count and doctor's assessment of improvement by visual analogue scale. There were no statistically significant differences in any of the primary or secondary efficacy variables between the study groups at baseline or during the 12-month follow up. All primary outcome measures indicated that the condition of the patients improved during the study.

INTERPRETATION. Both groups tended to recover. Ciprofloxacin, given as a 3-month course, had no advantage over placebo treatment.

Comment

A useful study of a substantial cohort of reactive arthritis patients. Duration of disease was generally quite short (means of 39 and 52 days in treatment and placebo groups), but experimental studies by the same workers on a rat model of *Yersinia*-induced arthritis suggest that very early treatment after onset of infection is required to abrogate arthritis. Thus antibiotics may be efficacious in preventing the onset of reactive arthritis, but not useful in a practical time-frame, i.e. when the patient presents with established arthritis. Patients in both arms of the study received intra-articular steroids rather frequently (96 and 62 injections, respectively) and it is possible that this might mask a treatment effect, although the antibiotic-treated patients required more intra-articular steroid. It is unlikely, therefore, that the study failed to detect a major benefit of ciprofloxacin in reactive arthritis.

Table 6.7 Triggering microbes

	Ciprofloxacin group No. (%)	Placebo group No. (%)
Enteroarthritis	30 (83)	30 (86)
Salmonella	11 (31)	11 (31)
Yersinia	10 (28)	10 (29)
Campylobacter	5 (14)	7 (20)
Clostridium difficile	0	1 (3)
Undefined	4 (11)	1 (3)
Uroarthritis	6 (17)	5 (14)
Chlamydia	2 (6)	1 (3)
Gonorrhoea	1 (3)	0
Undefined	3 (8)	4 (11)

Source: Yli-Kerttula *et al.* (2000).

Efficacy of sacroiliac corticosteroid injections in patients with inflammatory spondyloarthropathy: results of a 6-month controlled study.

J G Hanly, M Mitchell, L MacMillan, *et al. J Rheumatol* 2000; **27**(3): 719–22.

BACKGROUND. Injection of corticosteroid has been reported to be effective in active sacroiliitis, but controlled trials are lacking. This study evaluated changes in articular symptoms, spinal mobility and global function over 6 months after intra-articular injections of long-acting corticosteroid into the sacroiliac joints of patients with inflammatory low back pain. Inflammatory back pain was defined as four of five of the following features: onset of low back pain at <40 years; insidious onset; duration >3 months; low back early morning stiffness of >30 min, improvement in symptoms with exercise. Nineteen patients fulfilled these criteria and were investigated radiographically. Thirteen (68%) had radiographic evidence of sacroiliitis. The remaining six patients (32%) had normal imaging studies (radiographs, MRI scan or SPECT scan) and thus were considered to have mechanical low back pain. All patients received bilateral sacroiliac joint injections of triamcinolone hexacetonide (40 mg/joint) under CT guidance. Outcome variables included the duration of low back morning stiffness, back pain (by visual analogue scale, McGill Pain Questionnaire), spinal mobility (chest expansion, Schober test, 10 cm segments test, finger-fibula distance) and self-report health status (SF-36). Both groups of patients showed a transient improvement in stiffness and pain, spinal mobility and general health status that was most pronounced at 1–3 months after intra-articular therapy. This did not reach statistical significance (P>0.05) and by 6 months' follow-up, all outcome variables had reverted to pre-therapy levels in both groups. Previous studies had suggested that the duration of effect of sacroiliac joint injection is 6–9 months.

INTERPRETATION. These observations were interpreted to suggest that delivering corticosteroid injections to the sacroiliac joint is ineffective in the management of patients with inflammatory back pain.

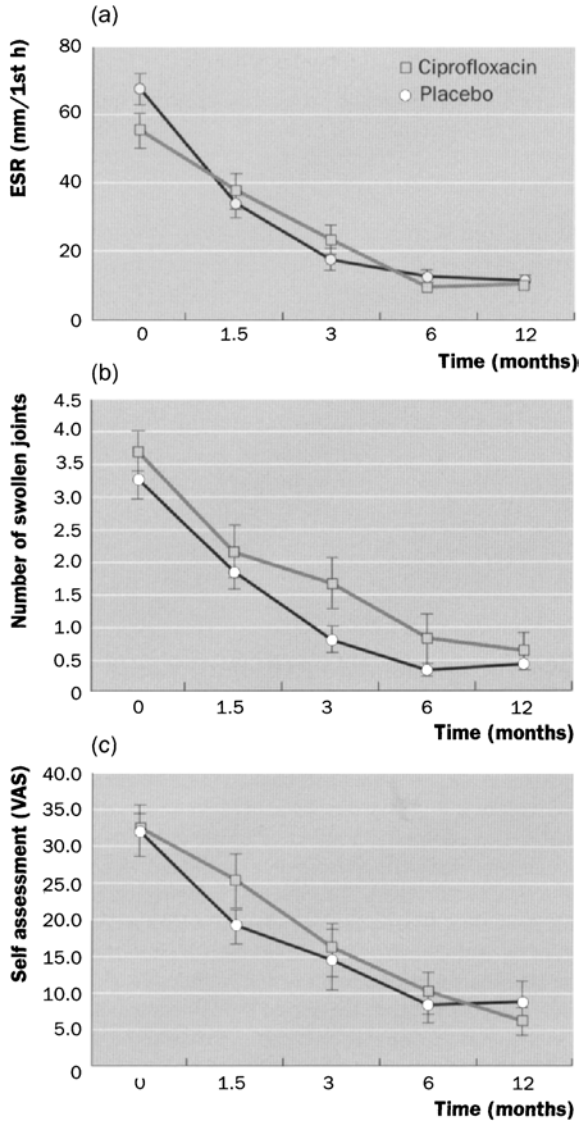

Fig. 6.7 Changes in (a) ESR, (b) number of swollen joints and (c) patient global assessment in patients treated with ciprofloxacin (n=30) or placebo (n=32). Values are mean (SEM). Differences between groups overtime were not significant. Source: Yli-Kerttula *et al.* (2000).

Comment

This is an interesting and somewhat unusual study. While it might be feasible to compare intra-articular steroid and placebo in the treatment of sacroiliitis, this is not an attractive option given the difficulty of needle placement and the need for visualization with CT or MRI to do this successfully. The authors, therefore, opted for an alternative strategy by identifying a group of patients with inflammatory back pain by history but radiologically normal sacroiliac joints. However, it is still possible that these patients had early SPA, and that their pain was truly inflammatory but did not arise in inflamed sacroiliac joints. Local injection with steroid might still have an effect on adjacent structures so that groups might both improve and be indistinguishable. The study also suffers from low numbers and a relatively high drop-out rate, but is perhaps a useful counter to open studies where the majority of patients (~80%) are reported to receive significant benefit.

Treatment of spondyloarthropathy with 5-aminosalicylic acid (mesalazine): an open trial.
B J Dekker-Saeys, B A Dijkmans, G N Tytgat. *J Rheumatol* 2000; **27**(3):723–6.

BACKGROUND. Sulphasalazine is well established to be effective in the treatment of IBD, and the active moiety is 5-acetylsalicylic acid (5-ASA, mesalazine), which is now commonly used instead. In contrast, for effective treatment of rheumatoid arthritis (RA), the other moiety of sulphasalazine, sulphapyridine, is the active compound and treatment with 5-ASA is ineffective. In SPA there is conflicting evidence on the effectiveness of sulphasalazine, but Dougados |8| reported useful results with a dose of 3 g/ day. Since several investigators have emphasized the importance of underlying IBD in SPA, it is attractive to argue that the same treatment which is effective in IBD might work in SPA. However, a previous study by Taggart suggested that sulphapyridine was the effective moiety in SPA as in rheumatoid disease. This paper revisited the question in two open trials. In the first, 20 patients with SPA were switched to 5-ASA (Pentasa), having been treated with sulphasalazine; they had failed to tolerate the drug (7 patients) or received no benefit. In the second, 19 patients with active SPA were treated with 5-ASA without previous administration of sulphasalazine. Two positive outcomes were recorded; in the first group 17 of 20 patients responded as assessed by the physician global clinical assessment compared with the previous treatment period with sulphasalazine; in the second patient group a statistically significant improvement was obtained in ESR.

INTERPRETATION. The results were held to support the hypothesis that 5-ASA might be the active moiety of sulphasalazine in the treatment of SPA. However, this conclusion must be treated with some scepticism.

Comment

Although positive results were obtained, these were not impressive. As an objective measure ESR improved, but only from 35 to 27. There was a striking divergence between the patients' global assessment that did not detect improvement and that of their physicians who suggested that~80% of the patients were improved; in the context of an open trial these results are not persuasive. Until a formal controlled trial is carried out it remains entirely possible that the mode of action of sulphasalazine is the same in RA and SPA, or indeed that the drug has such limited efficacy in SPA that only a very large study would have the power to show clear differences between 5-ASA and sulphasalazine.

Interferon alpha-2b, colchicine, and benzathine penicillin versus colchicine and benzathine penicillin in Behçet's disease.

H Demiroglu, O I Özcebe, I Barista, *et al. Lancet* 2000; **355**: 605–9.

BACKGROUND. Development of eye involvement in Behçet's disease may be potentially sight threatening. In this study the role of IFN-α2b, in combination with colchicine and benzathine penicillin, was investigated in a randomized trial.

INTERPRETATION. Treatment with IFN-α2b, colchicine and benzathine penicillin was effective in the prevention of recurrent uveitis, and protected vision. There were no serious side-effects. Arthritis, mucocutaneous lesions and thrombosis were also less common in the IFN-α2b group.

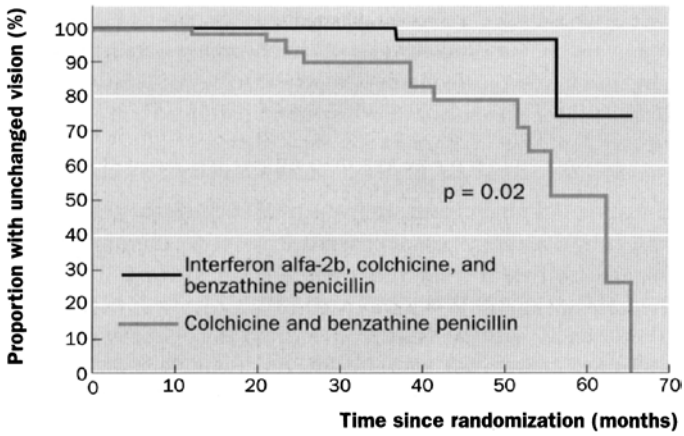

Fig. 6.8 Kaplan-Meier estimates of probability of preservation of baseline visual acuity during the course of disease. Source: Demiroglu *et al.* (2000).

Comment

Colchicine and benzathine penicillin have previously been shown to be effective therapy for arthritis and mucocutaneous lesions, and but not for ophthalmological involvement. Sixty-five patients were randomized to each group; patients had no previous history of Behçet's disease related eye involvement. IFN-α2b was given subcutaneously at a dose of 3 million units on alternate days for 6 months. Colchicine was given at 50 μg three times daily and benzathine penicillin 1.2 million units intramuscularly every third week. Visual acuity (the primary end-point) deteriorated in two patients in the IFN-α2b group and in 13 patients in the control group. The severity of visual loss was mild (2 line drop) in the IFN-α2b group, mild/moderate (2–5 line drop) in 10 of the control group, and severe (>5 line drop) in three patients in the control group. In nine patients anterior uveitis was observed (three in the IFN-α2b group) and in 26 patients (five IFN-α2b group) the posterior segment was involved. Attacks were treated with both topical and local immunuosuppressants. Visual acuity was preserved during follow up. IFN-α2b is associated with a retinopathy distinct from that seen in Behçet's disease, but this was not seen in this study.

Conclusion

What are the main messages that emerge from current research on SPA? Increasingly, the disease can be seen as a failure in normal relationship between bacteria and the human host, acted on by certain host genes of which HLA-B27 is the most obvious. In reactive arthritis the relationship with gut or genitourinary pathogens, which usually results in local self-limiting disease (gastroenteritis, urethritis) and resolution of infection, is disordered so that prolonged joint inflammation and extra-articular pathology result, with some evidence that infection persists for abnormally long periods. In arthritis associated with IBD, the disordered relationship seems to be with normal gut flora rather than with specific pathogens, and the same may hold true for AS.

How can this breakdown in relationships with bacteria be treated more effectively in future? Antibiotics might eliminate pathogenic organisms in reactive arthritis, but this is not an option for commensal bacteria. Therefore, attention turns to altering the host response, with anti-inflammatory measures such as anti-TNF therapies —interestingly these are useful in both SPA and IBD. Ideally, these kinds of treatment might allow the re-establishment of the normal peaceful coexistence between host and commensal bacteria, and allow specific therapy to be withdrawn. It will be interesting, as experience with anti-TNF and related treatment accumulates, to see whether a proportion of patients achieve some form of remission, or whether a permanent change in relationship has occurred—such as the induction of autoimmune responses, which no longer require triggering bacterial antigens.

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7
Juvenile arthritis

Introduction

Juvenile idiopathic arthritis (JIA) encompasses a heterogeneous mixture of conditions, which are distinct from the adult inflammatory arthritides.

To improve communication and interpretation of research, the International League of Associations of Rheumatologists (ILAR) proposed new criteria in 1998, to try to classify categories of childhood arthritis that are clinically homogeneous |**1**|. Clinical homogeneity may also reflect common aetiology and pathology, and enable insight into disease mechanisms and development of new therapies.

No clinical classification is ideal, especially when aetiology and pathogenesis are unknown and, therefore, these criteria will continue to be reviewed and modified. The first stage involves evaluation of the criteria in clinical practice, in a variety of populations.

Incidence and prevalence of JIA, and also the ratios of the subtypes, vary across different populations, which may be explained by genetic differences. Genetic risk for JIA is likely to relate to the interaction of multiple genes and the clinical subtypes also differ immunogenetically. Previous work has focused on major histocompatibility complex (MHC) genes and there are many papers reporting associations between different MHC alleles and haplotypes and subgroups of JIA |**2**|. Other non-MHC genes (e.g. T-cell receptor, cytokine or cytokine receptor genes) may be additional risk factors for development of JIA and may also improve our understanding of pathogenesis in JIA.

Advances in molecular biology have enabled studies of cytokines, cytokine receptors and T-cell subsets, and we may have some initial insights into the pathophysiology of JIA. Inflamed synovium contains a dense infiltrate of highly activated T cells, which differ phenotypically from peripheral blood cells. In JIA there is a skew in cytokine production towards T-helper (Th) 1 cytokines such as interferon (IFN) -γ, and also in chemokine receptor expression, implying selective recruitment of specific T cells into the joint |**3**|.

Tumour necrosis factor (TNF)-α is important in several mechanisms involved in inflammatory arthritis, and its activity is inhibited by its soluble receptors. The

relative proportions of TNF-α and the soluble receptors may vary according to subtype and reflect different clinical courses. TNF-α is, therefore, a logical target for therapy in JIA and etanercept, the first anti-TNF-α therapy to be used in JIA, shows significant improvements in children with active polyarticular disease.

Low-dose oral methotrexate (MTX) was previously shown to be effective in poly-articular JIA by the USA/USSR collaborative study group |4|, but two of the most disabling subgroups of JIA, systemic and extended oligoarticular, were under-represented by that study. A further study to assess the efficacy of MTX in these two subgroups has, therefore, been conducted.

Steroid therapy is well established in JIA, but toxicity is a major problem, particularly growth impairment and osteoporosis. Alternative regimens to daily oral treatment are worth considering, provided they are effective and also have reduced toxicity.

The use of new treatment regimens and new therapies may highlight the shortcomings of current assessments of disease activity, and prompt the search for new markers. Myeloid-related protein 8 and 14 are expressed by infiltrating neutrophils and monocytes, but not by resting macrophages or lymphocytes. Their release is triggered by the interaction of monocytes with activated inflammatory endothelium, and serum concentrations may be useful sensitive markers of local inflammation.

Iron deficiency is difficult to assess in children with active JIA and associated anaemia of chronic disease. Serum transferrin receptor concentration reflects iron requirements at a cellular level, and unlike ferritin and transferrin levels, is not affected by inflammation. It may be a useful marker of iron status in these patients.

Previous outcome studies have assessed heterogeneous groups of patients, but the new classification criteria, with more homogeneous groups, may enable more accurate outcome studies. High-risk patient subgroups may be identified who warrant a more aggressive therapeutic approach. Long-term outcome studies are useful to assess other consequences of chronic inflammatory disease. Osteoporosis and growth delay can be a result of the effects of disease activity on bone metabolism, and also treatment with steroids. Intervention with growth hormone may be useful for these children.

Children with JIA may be expected to have increased rates of emotional, psychological and behavioural problems, and studies have been undertaken to examine this and also areas of reproductive behaviour and sexual health in young adults with JIA. The evidence that 30–50% of patients with JIA continue to have active disease into adulthood has prompted the need to examine transition from paediatric to adult rheumatology services and to develop adolescent services.

Comparison of criteria for the classification of childhood arthritis.
S E Ramsey, R K Bolaria, D A Cabral, *et al. J Rheumatol* 2000; **27**(5): 1283–6.

BACKGROUND. This study evaluated the applicability of the ILAR criteria for the classification of childhood arthritis in an outpatient paediatric rheumatology clinic population. They determined the proportion of children who met standard classification criteria, but failed to meet ILAR criteria for specific arthritides, and therefore became unclassifiable.

INTERPRETATION. The ILAR classification criteria applied to a group of children with chronic arthritis classified by traditional criteria results in reassignment of 11.6% of the patients, predominantly in the oligoarticular group. It will be important to determine the role of the presence of a family history of psoriasis in classifying these patients.

Comment

A North American Paediatric Rheumatology clinic applied both their traditional classification (amalgamation of: American College of Rheumatology [ACR] criteria for juvenile rheumatoid arthritis [JRA], European Spondyloarthropathy Study Group [ESSG] criteria for spondyloarthropathy [SPA], and Vancouver criteria for juvenile psoriatic arthritis [JPsA]) and the new ILAR classification to 69 children who had arthritis for at least 6 months. ILAR classification criteria are more demanding than the previously used criteria and aim to improve the homogeneity within each diagnostic category.

Sixty-one children (88.4%) were classifiable by ILAR criteria for JIA and eight failed to fulfil criteria for any specific category and were assigned to the 'other arthritis' category. This was mainly due to a family history of psoriasis, which is common in the general population (Table 7.1).

Most reclassification was seen in the 29 children with oligoarticular JRA: 23 had persistent or extended oligoarthritis by ILAR criteria; six were unclassified; four had first- or second-degree relatives with psoriasis, but were either ineligible or did not fulfil criteria for psoriatic JIA; one boy fulfilled both categories for persistent oligoarticular JIA and enthesitis-related arthritis (ERA) and one girl was rheumatoid factor (RF) positive and therefore ineligible for classification as oligoarticular JIA. All children with systemic onset JRA were classified in the corresponding category in the ILAR system. All children with polyarticular JRA were classified as polyarticular RF negative or polyarticular RF positive JIA. Eight of nine children with SPA met criteria for ERA, one being excluded because of a family history of psoriasis in a first-degree relative. All six children with definite JPsA and only two of four with probable JPsA (Vancouver) met ILAR criteria for psoriatic JIA. One was classified as RF-negative polyarthritis and the other as 'other arthritis' because the family history of psoriasis was only in a second-degree relative.

This was a small study, with patients from only one centre. It is important to validate the new ILAR criteria, but using a larger patient population, from different centres.

Validation of the proposed ILAR classification criteria for juvenile idiopathic arthritis.

I Foeldvari, M Bidde. *J Rheumatol* 2000; **27**(4):1069–72.

BACKGROUND. The new ILAR classification criteria were proposed to facilitate communication among paediatric rheumatologists. Before they are applied in daily practice they should be clinically validated.

INTERPRETATION. Using the ILAR proposed classification criteria, 88% of patients could be classified. In patients classified as 'other,' the psoriatic trait caused the most difficulty in classification.

Table 7.1 Comparison of classification of patients by ILAR and current classification systems

Current Classification	Proposed ILAR Classification System								
	Oligo Persistent	Oligo Extended	Poly RF+	Poly RF−	Systemic	Enthesitis Related	Psoriatic	Other	Total
Oligo-JRA	14	9	0	0	0	0	0	6	29
Poly-JRA	0	0	4	14	0	0	0	0	18
Systemic	0	0	0	0	3	0	0	0	3
JSpA	0	0	0	0	0	8	0	1	9
JPsA	0	0	0	0	0	0	6	0	6
Probable JPsA	0	0	0	1	0	0	2	1	4
Total	14	9	4	15	3	8	8	8	69

Source: Ramsey *et al.* (2000).

Table 7.2 Results of patient classification according to the proposed Durban classification criteria in comparison with the classification criteria currently used

Current classification	Durban Classification Criteria						
	Oligoarthritis n = 56	Polyarthritis (RF–) n = 4	Systemic Arthritis n = 8	Psoriatic Arthritis n = 4	Enthesitis Related Arthritis n = 11	Other Arthritis n = 12
Oligoarticular JRA, n = 67	56	—	—	2	1	8
Polyarticular JRA, n = 6	—	4	—	1	—	1
Systemic JRA, n = 8	—	—	8	—	—	—
Psoriatic arthritis, n = 5	—	—	—	3	—	2
Spondyloarthritis, n = 11	—	—	—	—	10	1

Source: Foeldvari *et al.* (2000).

Comment

A European Paediatric Rheumatology clinic also re-classified their patients using the new ILAR classification criteria for JIA. They had previously used criteria from the ACR for JRA, rather than the European League of Associations for Rheumatology (EULAR) criteria for juvenile chronic arthritis (JCA), because the ACR criteria excluded SPA, including psoriatic arthritis, from JRA. Eighty-five children (88%) were classifiable by ILAR criteria, 12 (12%) were classified as 'other arthritis' (Table 7.2). The children with systemic JRA remained in the systemic JIA category.

The main difficulties arose with psoriasis. Eight patients with oligoarticular JRA were excluded from the oligoarthritis category, due to a family history of psoriasis, but did not fulfil criteria for psoriatic JIA. Two children with psoriasis, but no family history of psoriasis, fulfilled ILAR criteria for both oligoarthritis and psoriatic JIA. The results were similar to those from Vancouver and again highlight the problem with psoriasis, which is common in the general population, when using the ILAR criteria. Modifications to the criteria were suggested, which would enable 10 further children to be classified, but still left two children who fulfilled the criteria for two categories and could not be classified.

Further studies are needed to validate the new ILAR criteria, and modifications may be necessary, particularly if consistent problems (as with psoriasis) are found.

Subtyping of juvenile idiopathic arthritis using latent class analysis.

E Thomas, J H Barrett, R P Donn, *et al.* British Paediatric Rheumatology Group. *Arthritis Rheum* 2000; **43**(7):1496–503.

BACKGROUND. Latent class analysis (LCA) is a statistical technique which is used to identify underlying subtypes of JIA that best explain the observed relationships of clinical and laboratory variables. These subtypes are compared with those defined by the ILAR criteria and examined for HLA associations.

INTERPRETATION. LCA is a novel approach to the task of identifying homogeneous subtypes within JIA. The latent classes identified will be examined further for associations with other candidate genes and differences in outcome.

Comment

JIA is a heterogeneous group of diseases, and in order to understand these conditions further it is essential to identify homogeneous groups of patients. The new ILAR criteria and previous classification systems are based on clinical disease patterns, but may not define biologically unique groups. Thirty clinical and laboratory variables were assessed in a large cohort of UK children with JIA. Latent class models were fitted to the 10 variables perceived as most important in

disease development (age at onset, large joint involvement, small joint involvement, polyarthritis, symmetric arthritis, spinal pain, fever, psoriasis, antinuclear antibodies [ANA] and RF).

LCA assumes that the frequencies with which different symptom profiles (each subject's characteristics) occur can be explained by a small number of mutually exclusive classes, with each class having a distinct set of item probabilities that is constant for all members of that particular class. The probability of membership of each latent class was calculated for each patient and the patient assigned to a specific class if the probability was ≥0.7. Seven classes gave the best fit for the data, with 81% of patients assigned to a particular class with a probability≥0.7. Patterns of joint involvement and the presence of ANA were most influential in determining latent classes and may be useful in future classification criteria. Fever, spinal pain, psoriasis and RF were variables included in the analysis but in contrast with the ILAR classification, these were not defining features of any latent classes. There was some correspondence between the latent classes and ILAR categories, but they did not coincide completely.

Haplotypes were available for three HLA loci (DRB1, DQA1 and DQB1), for 369 patients and 296 of these could be assigned to a particular latent class. Significant differences between the latent classes were found for haplotypes examined. There was a very strong association between DRB1*08-DQA1*0401-DQB1*0402 and latent class 2 (odds ratio 9.6, $P<0.005$). LCA may have identified a genetically homogeneous group, as most children had ANA-positive oligoarthritis.

This was an exploratory study to assess the usefulness of the LCA model in JIA. It is an objective technique but relies on the subjective choice of clinical features. Further studies are planned using different populations of patients, which may give classes with similar characteristics, but possibly different frequencies.

Age-specific effects of juvenile rheumatoid arthritis-associated HLA alleles.
K J Murray, M B Moroldo, P Donnelly, *et al. Arthritis Rheum* 1999; **42**(9): 1843–53.

BACKGROUND. This study aims to define the onset and duration of effect of the HLA alleles that are associated with susceptibility and protection in JRA and two of its subtypes, using a clinically and immunologically characterized cohort of patients.

INTERPRETATION. These data define at what age and for how long various HLA alleles influence susceptibility and protection (window-of-effect) in patients with JRA. In addition, these data establish more clearly the boundaries of ages-at-onset for two of the subtypes of the disease.

Table 7.3 Frequency of gene combinations in the JRA groups and control population*

HLA allele	% of control subjects	% of JRA patients			
		All patients	Pauci	Poly	Systemic
A2 and DR5	9	19†	23†	12	13
DR1 or DR4	40	43	19‡	54‡	40
DR1/1, 1/4, or 4/4	10	8	4‡	12.5†	17
DPB1*0201 and A2	10	23†	27†	10	10
DPB1*0201 and DR5	3	5	10†	4	5
DQA1*0401/0501/0601	63	54‡	66	55‡	57

*For the A and DR alleles, n=254 control subjects and 680 patients (377 with pauciarticular [Pauci] juvenile rheumatoid arthritis [JRA], 184 with polyarticular [Poly] JRA, and 119 with systemic JRA). For the DPB plus A and the DPB plus DR alleles, n=210 control subjects and 464 patients (268 with Pauci JRA, 126 with Poly JRA, and 70 with systemic JRA). For the DQA alleles, n=186 control subjects and 452 patients (264 with Pauci JRA, 121 with Poly JRA, and 67 with systemic JRA). DPB1*0201, DR5, A2 were also tested and were not significantly different at the 0.05 level (data not shown).
†Significant at P=0.001, by chi-square test with 1 degree of freedom (df; unadjusted values).
‡Significant for the absence at P<0.001, by chi-square analysis with 1 df (adjusted values).
Source: Murray *et al.* (1999).

Comment

JRA appears to be a group of diseases and classification is currently based on clinical features. This study took place before the ILAR classification for JIA was published and, therefore, uses the ACR criteria for JRA. Previous studies have shown HLA associations with different subtypes of JRA, particularly pauciarticular (<5 joints involved at onset) or polyarticular, and imply strong genetic contributions to JRA predisposition and pathogenesis.

A cohort of 680 children with JRA, seen in Cincinnati and Boston over the past 15 years had HLA typing performed and the HLA allele frequencies were compared with an ethnically matched control group. During the long time period taken to collect a cohort of sufficient size, HLA typing technology had progressed significantly, with DNA-based techniques replacing serology. However, the associations of individual HLA alleles were comparable with those reported by previous studies.

Combinations of certain alleles were associated with a risk of developing JRA, and specific subtypes of disease. This is thought to be due to interactions between MHC genes and not through linkage disequilibrium (Table 7.3). Life table analyses, with onset of arthritis (rather than death) were used to calculate the median survival times, $S_t0.5$ and $S_t0.2$, which were the ages when 50% and 80% of children with a specific allele had developed JRA. This was also more clearly illustrated graphically in the original paper (Table 7.4).

Table 7.4 Age (in years) at which 50% ($S_t0.5$) and 80% ($S_t0.2$) of children with various HLA alleles developed JRA

			JRA subgrooup					JRA subgroup				
	All JRA		All pauci		All poly			Pauci females		Pauci males		
HLA allele	$S_t0.5$	$S_t0.2$	$S_t0.5$	$S_t0.2$	$S_t0.5$	$S_t0.2$	*P*†	$S_t0.5$	$S_t0.2$	$S_t0.5$	$S_t0.2$	*P*†
A2	4.0	10	2.9	7.4	8.4	12.7	< 0.001	2.7	4.9	8.2	11.6	< 0.001
B27	8.0	11	7.3	11.9	10.2	12.0	NS	3.0	8.8	10.6	12.4	< 0.001
DR1	4.8	10	3.5	10.0	7.0	10.9	0.01	3.0	7.0	9.5	11.3	0.004
DR2	5.5	11	2.9	7.9	9.9	13.1	< 0.001	2.7	6.0	10.0	11.7	0.017
DR3	3.8	11	2.9	8.0	8.5	14.8	< 0.001	2.6	4.8	7.5	10.5	< 0.001
DR4	9.3	13	6.6	11.0	10.7	13.5	< 0.001	4.2	9.0	10.7	12.0	< 0.001
DR5(11)	3.3	8	2.8	6.1	6.6	10.2	< 0.001	2.9	5.3	2.7	11.6	NS
DR6(13)	4	8	3.2	7.2	6.4	8.5	< 0.001	2.8	5.2	7.5	11.4	< 0.001
DR7	10.4	13	7.9	11.7	12.3	14.2	< 0.001	3.8	11.2	10.8	12.2	0.0015
DR8	3.1	8	2.8	5.2	8.0	11.1	< 0.001	2.7	4.7	4.5	10.7	0.031
DPB1												
*0201	3	7	2.6	5.0	7.3	12.2	< 0.001	2.4	4.2	4.5	11.6	0.01
*0301	4.8	10	3.5	5.6	8.0	11.0	< 0.001	3.5	4.9	4.0	11.8	NS
*1104	2.7	4.5	2.4	4.5	9.0	10.7	< 0.001	2.7	3.4	2.3	2.7	NS

*$S_t0.5$ and $S_t0.2$ based on the life-table analysis function in SPSS version 7.5, in which the terminal event is onset of arthritis. Pauci=pauciarticular; poly=polyarticular=not significant.
†*P* values based upon the generalized Wilcoxon (Gehan) test comparing the overall survival functions.
Source: Murray *et al.* (1999).

HLA-A2 had $S_t0.5$ and $S_t0.2$ values of 4.0 and 10 years for all JRA, and was mostly an increased risk in females for pauciarticular JRA. HLA-DR/DQ: DR5 and DR8 were associated with increased susceptibility to pauciarticular JRA, with 80% developing JRA by 6 years. DRB1*1104 was particularly associated with a risk of iridocyclitis, and gave $S_t0.5$ and $S_t0.2$ values of 2.7 and 4.5 years for all JRA, again in females with pauciarticular JRA. HLA-DP: HLA-DPB1*0201 had been shown by several series to associate with JRA. The $S_t0.5$ and $S_t0.2$ values of 2.4 and 4.2 years for females with pauciarticular JRA suggest an interaction of HLA-DP with DR and DQ.

There appeared to be a window of susceptibility for developing pauciarticular JRA, with most risk occurring up to age 5 years. Risk of pauciarticular JRA is likely to involve combinations of interacting MHC alleles. For all the combinations assessed, the $S_t0.5$ and $S_t0.2$ values were lower, indicating greater susceptibility to pauciarticular JRA in early life. HLA-DPB1*0301 was associated with a risk of polyarticular JRA, at an intermediate age range of 3–12 years. HLA-DR1 appeared to be associated with early onset pauciarticular JRA (especially extended pauci JRA) and a later association with polyarticular JRA, with $S_t0.5$ and $S_t0.2$ values of 7 and 10.9 years. HLA-DR4 and DR7 appeared to be protective in young children, but associated with polyarticular JRA in older children.

Defining the windows of susceptibility for the specific subtypes of JRA may help to understand pathogenesis of these diseases. Infections that occur in the young may interact with genetic susceptibility, but have less influence at an older age. Endocrine factors may be important, interacting with alleles that contribute to susceptibility to JRA in older children. These data may also allow a more homogeneous classification of the patient subtypes, useful for clinical trials and clinical outcome analysis.

Identification of a genetic risk factor for systemic juvenile rheumatoid arthritis in the 5′-flanking region of the TNFalpha gene and HLA genes.

Y Date, N Seki, S Kamizono, *et al. Arthritis Rheum* 1999; **42** (12):2577–82.

BACKGROUND. This study examines polymorphisms in the 5′-flanking promoter/enhancer region of the TNF-α gene and in the coding regions of HLA class I and class II genes, in order to understand better the genetic background of JRA.

INTERPRETATION. The −1,031C/−863A allele and the −857T allele of the TNF-α gene, both of which are related to high production of TNF-α, are associated with systemic JRA. The -857T allele may enhance the effect of the DRB1*0405/DQB1*0401 haplotype in predisposing to the development of systemic JRA.

Comment

JIA is a heterogeneous condition, and each subtype appears to be genetically different. Several previous reports have shown oligoarticular JIA to be associated with HLA-A2, DR5, DR6, DR8, DQA*0101, DQA*0401, DQA*0501, DQA*0601 and DPB1*0201. HLA genes are unlikely to be the only genes involved in genetic risk, clinical course, or severity of JIA.

Two polymorphisms in the 5′ flanking region of the TNF-α gene have been found in association with susceptibility to, or severity of, infections and autoimmune diseases, and as TNF-α is a cytokine central to the pathogenesis of JIA, polymorphisms of the TNF-α gene may be important. This group had previously identified three further polymorphisms in the 5′ flanking region of TNF-α, and therefore all five polymorphisms were studied in children with JIA. One hundred and eleven Japanese patients with JIA (50 systemic, 29 oligoarticular and 32 polyarticular) were compared with 525 healthy, unrelated controls. HLA genes and the 5′ flanking region of TNF-α were amplified from genomic DNA and the HLA alleles and polymorphisms of TNF-α identified using sequence-specific oligonucleotide probes.

The frequencies of TNF-α−1,031C,−863A, and −857T alleles in the patients with systemic JIA, but not oligoarticular or polyarticular JIA, were significantly higher than in controls. Previous studies had shown linkage disequilibrium between −1,031C and −863A, and to a lesser extent with −238A, but not with the other polymorphic sites. Functional studies have shown that the −1,031C/−863A and −857T alleles result in higher levels of TNF-α, in response to various stimuli, and imply that they may be important in systemic JIA, via increased promoter activity of the TNF-α gene.

HLA-DRB1*0405 and DQB1*0401 were significantly more frequent in systemic ($P<0.02$ and $P<0.007$, respectively), but not the other subtypes of JIA. No other alleles showed significantly increased frequencies. DRB1*0405 and DQB1*0401 are strongly linked in Japanese people.

Linkage disequilibrium has been reported between some HLA alleles and the TNF-α alleles. A high odds ratio (OR) of 3.84 ($P<0.0001$) was found in systemic JIA, for patients with both TNF-α −857T and DRB1*0405, but not in patients with only one of these alleles (TNF-α −857T 0.87 and DRB1*0405 1.58). The combination of the two alleles may be a genetic risk factor for systemic JIA, but either allele alone may not be sufficient to predispose to disease. In contrast, TNF-α −1,031C/ –863A allele was associated with systemic JIA, but there was no increased frequency of DRB1*0901 with which it is in linkage disequilibrium, implying that −1,031C/ −863A allele predisposes to systemic JIA.

DRB1*0405 is not commonly associated with systemic JIA in Caucasians, and this may suggest a different aetiology in Japanese patients. However, this allele is the most frequent DR4 subtype in the Japanese population, and is increased compared with Caucasians. It is also strongly associated with rheumatoid

arthritis (RA) in Japanese adults, but DRB1*0401, which is a well-known predisposing factor for RA in Caucasians, is only weakly associated.

This study makes interesting observations of HLA associations with JIA in Japan-ese patients, which vary from those in other populations studied. The TNF-α polymorphisms may be additional predisposing factors for systemic JIA, in combination with certain HLA alleles. It would be interesting to examine these in other populations.

Polymorphism at NRAMP1 and D2S1471 loci associated with juvenile rheumatoid arthritis.
C B Sanjeevi, E N Miller, P Dabadghao, *et al. Arthritis Rheum* 2000; **43**(6): 1397–404.

BACKGROUND. NRAMP1 is examined as a candidate gene for susceptibility to JRA.

INTERPRETATION. The NRAMP1 allele conferring susceptibility to JRA drives high levels of NRAMP1 expression, while the allele associated with protection drives low levels. These two alleles are inversely associated with susceptibility to infectious disease, consistent with their maintenance in populations through balancing selection.

Comment

The NRAMP1 gene encodes the natural resistance-associated macrophage protein and has been shown to be a non-HLA component of susceptibility to adult RA. It is involved in macrophage activation and cytokine regulation and therefore a potential candidate gene for susceptibility to JIA. DNA from 119 Latvian patients with JIA (72 oligoarticular and 47 polyarticular) and 111 healthy controls was genotyped for a functional repeat polymorphism in the promoter of NRAMP1 and a linked microsatellite D2S1471. Three of four previously identified polymorphisms in the NRAMP1 promoter were present in the Latvian population. Functional studies with reporter genes had shown allele 3 to increase, and alleles 1, 2 and 4 to reduce, NRAMP1 expression.

Allele 3 of the NRAMP1 promoter conferred increased risk for JIA (P=0. 0006) and for the oligoarticular (P=0.003) and polyarticular (P=0.019) subtypes, while presence of allele 2 was protective. Homozygosity for allele 3 was associated with an increased risk of disease (OR 2.32, 2.28 and 2.38; P=0.004, 0. 018 and 0.036 for combined JIA, and oligoarticular and polyarticular groups) and risk was high even for heterozygotes, although only significant for the combined JIA group. Allele 2 homozygotes had a reduced risk for JIA (OR 0.33), and even in heterozygotes significant protection was seen for all three groups of disease (combined JIA OR 0.43, P=0.004, oligoarticular OR 0.44, P=0.018 and polyarticular OR 0.42, P=0.036), suggesting that the presence of a single copy of allele 2 may be protective.

Sixteen alleles of the microsatellite marker D2S1471 were identified in this population. Stratification by subtype of JIA was not possible due the high number of alleles at this locus. Alleles 6 (OR 6.6; *P*=0.009) and 12 (OR 4.62; *P*=0.005) were associated with an increased risk of JIA, particularly when they occurred with allele 3 of NRAMP1, while allele 11 was protective (OR 0.48; *P*=0.002) when on a haplotype with allele 2 of NRAMP1. These patients had previously been HLA typed for DR and DQ, and allele 3 of NRAMP1 was found to be additive with HLA-DQ7 for JIA susceptibility, and allele 2 additive with HLA-DQ5 for protection.

These findings for Latvian JIA patients suggest that chronic macrophage activation, associated with high levels of NRAMP1 expression via allele 3, is linked to disease susceptibility, and that low levels of expression, promoted by allele 2, contribute to protection. Infections with intra-macrophage pathogens (e.g. tuberculosis) require macrophage activation and in these circumstances allele 3 is protective, whereas allele 2 is associated with susceptibility to infection.

Tumour necrosis factor alpha and its soluble receptors in juvenile chronic arthritis.
M Rooney, H Varsani, K Martin, *et al. Rheumatology* 2000; **39** (4):432–8.

BACKGROUND. TNF-α is important in several mechanisms involved in inflammatory arthritis. The activity of TNF-α is inhibited by its soluble receptors, TNFRI and TNFRII. The relative proportions of TNF-α and its soluble receptors may be different in the synovial fluid (SF) of various subgroups of JIA, reflecting their contrasting clinical courses.

INTERPRETATION. These results suggest that the increased joint destruction observed in polyarticular disease compared with the other two subtypes (oligoarticular and SPA) may be related to the lower TNFR/TNF-α ratios observed.

Comment

Serum and SF from 45 children with JIA (25 oligoarticular, 13 polyarticular and seven SPA) were examined. TNF-α levels were assayed by an enzyme amplified sensitivity assay (EASIA), and as 60% had levels below the limit of the assay (<20 pg/ml) these samples were re-assayed with a more sensitive enzyme-linked immunosorbent assay (ELISA). TNFRI and TNFRII were measured by ELISA.

TNF-α was assayed in 33 serum samples, and there was no significant difference between the three groups. Only 16 samples had TNFRI and TNFRII levels measured. The mean TNFRII level was 3.5 ng/ml, twice that of TNFRI, mean 1.25 ng/ml. This contrasts with sepsis, where TNFRII levels are at least three or four times higher than TNFRI. These variations in TNFR ratio in

different diseases may reflect different mechanisms of shedding the receptors in response to TNF-α, but may also reflect different standards used in the assays.

TNF-α levels in SF were significantly lower in the SPA group compared with oligoarticular JIA (P=0.01) and polyarticular JIA (P=0.002). Significantly higher levels of TNFRI were found in polyarticular JIA patients compared with oligoarticular JIA (P=0.004), and similarly for TNFRII (P=0.03). There were no differences between the SPA group and the other two subgroups for either receptor.

Biological activity of TNF-α appears to be related to the ratio of TNF-α to its soluble receptors and, therefore, these were calculated for the SF results. TNFRI/TNF-α ratio was much greater in the SPA group than either the oligoarticular or polyarticular groups (P=0.003, for each). A similar trend was found for TNFRII/TNF-α, but the differences were not statistically significant. The soluble receptor levels were combined to represent a total TNF-α inhibitory function, and expressed as a ratio to TNF-α. Again this was greatest in the SPA group, compared with oligoarticular JIA (P=0.01) and polyarticular JIA (P=0.05).

The different ratios may reflect the differing clinical courses, as a low ratio of TNFR/TNF-α, as seen in polyarticular JIA, would be expected to result in increased bioactivity of TNF-α. This may lead to more erosive changes this group, via the resorptive effects of TNF-α on bone and cartilage. However, TNF-α is only one of many cytokines, and the mechanisms are complex.

Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis.

M Frosch, A Strey, T Vogl, *et al. Arthritis Rheum* 2000; **43**(3):628–37.

BACKGROUND. To analyse which physiological stimuli induce secretion of myeloid-related protein 8 (MRP8) and MRP14, two S100 proteins expressed in neutrophils and monocytes, and to determine whether serum concentrations of these proteins are reliable parameters for monitoring inflammatory activity in pauciarticular JRA.

INTERPRETATION. MRP8 and MRP14 are specifically released during the interaction of monocytes with inflammatory activated endothelium, probably at sites of local inflammation. Their serum concentrations represent a useful marker for monitoring local inflammation in JRA.

Comment

MRP8 and -14 are expressed by infiltrating neutrophils and monocytes in inflammatory conditions, but not by resting macrophages or lymphocytes, and serum levels correlate with disease activity (e.g. in RA, inflammatory bowel disease, inflammatory airways disease and transplant rejection). Stimuli triggering their release via protein kinase C activation had not previously been

identified. Secretion of MRP8 and MRP14 was measured using a co-culture system of monocytes and endothelial cells. Initial experiments showed an increased release of MRP8 and -14 during interaction of monocytes and TNF-activated endothelial cells, but not with unstimulated endothelial cells. Serum MRP8 and -14 concentrations were assessed in 35 patients with oligoarticular JIA and 30 controls, by ELISA, and were five times higher in patients with active versus inactive disease (Fig. 7.1).

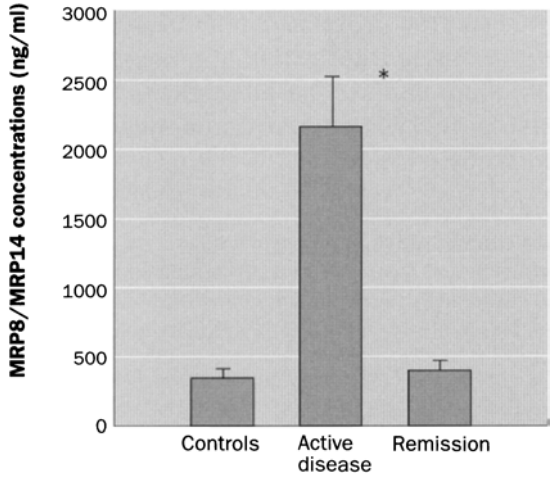

Fig. 7.1 Serum concentrations of MRP8/MRP14 in JRA patients. Data on 35 JRA patients with active disease (90 samples from 35 patients) or during remission (17 samples from 13 patients) according to the criteria for remission of the ACR and on 30 healthy controls are presented as the mean and SEM. The asterisk indicates statistically significant differences compared with the control group ($P \leq 0.05$). Source: Frosch *et al.* (2000).

Table 7.5 Differences in values for MRP8/MRP14 concentrations and laboratory parameters before and after local therapy with triamcinolone*

	MRP8/MRP14 (ng/ml)	ESR (mm/hour)	CRP (mg/dl)
Responder			
ΔIAT (n = 8)	−1,430	−63	−1.90
Non-responder			
ΔIAT (n = 9)	1,295	10	0.83
P	≤ 0.001	NS	≤ 0.05

*MRP8=myeloid-related protein 8; ESR=erythrocyte sedimentation rate; CRP =C-reactive protein; ΔIAT=after intra articular triamcinolone therapy minus prior intra articular therapy; NS=not significant.
Source: Frosch *et al.* (2000).

High levels of MRP8 and -14 were expressed by monocytes and neutrophils from synovial fluid (SF) and also demonstrated in infiltrating monocytes, by immuno-histochemical staining of synovial biopsy specimens. MRP8 and-4 are

probably released at the site of inflammation, as the SF concentrations were 20 times higher than serum, and there was a strong correlation between SF and serum for individual patients (Fig. 7.2).

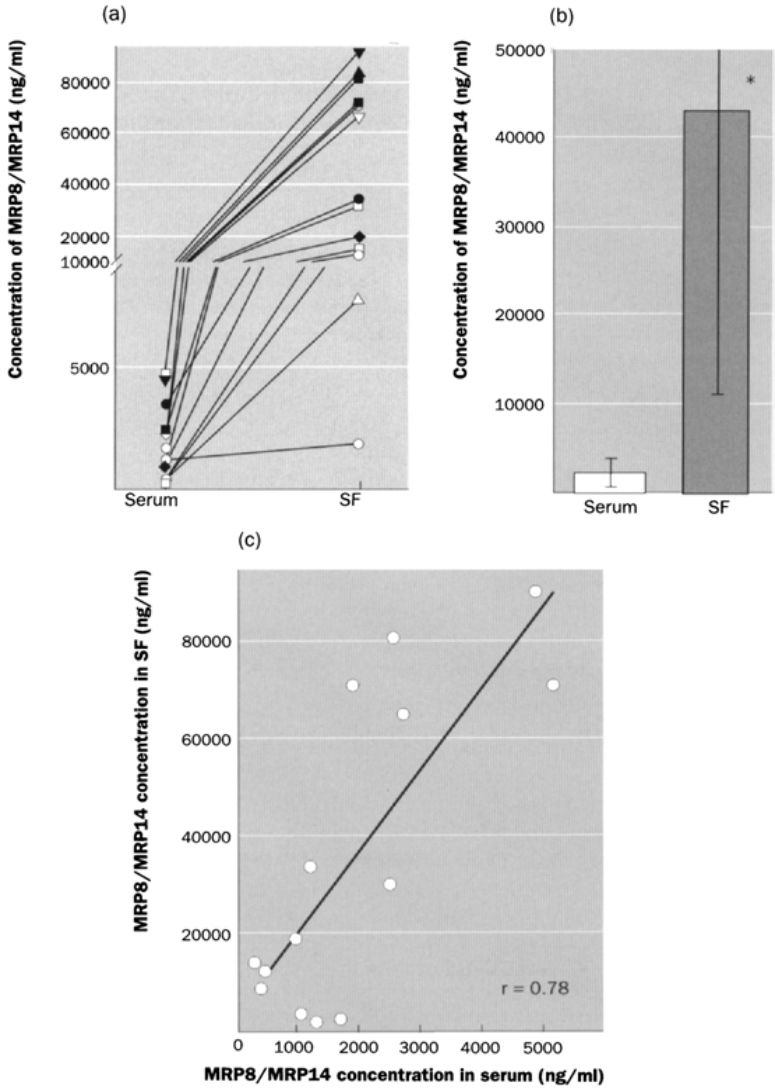

Fig. 7.2 Correlation of MRP8/MRP14 concentrations in the SF and serum of JRA patients. (a) Linked data on serum and SF concentrations are shown. Each symbol represents an individual patient. Note the break in the *y*-axis. (b) Serum and SF concentrations in 15 JRA patients are presented as the mean ±SD. The asterisk indicates statistically significant differences between serum and SF concentrations ($P \leq 0.05$). (c) Correlation of MRP8/MRP14 serum (*x*-axis) and SF (*y*-axis) concentrations in individual patients ($P \leq 0.01$). Source: Frosch *et al.* (2000).

A subgroup of patients had serum MRP8 and -14 concentrations measured before and after intra-articular triamcinolone injection, to assess whether these proteins may be helpful in monitoring response to treatment. Eight of 17 children improved following treatment, and the levels fell to the normal range, whereas the non-responders showed no significant change (Table 7.5).

Serum MRP8 and -14 showed correlation with clinical markers of disease activity, especially indicators of local inflammation (active and limited joint counts), which was not seen with erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP).

This was a small study, but MRP8 and–14 may be additional, sensitive markers for local disease activity, and may complement ESR and CRP, which reflect systemic inflammation. They may be particularly useful in patients with low ESR/ CRP, who have occult clinical disease and may be at risk of ongoing joint damage, or who may relapse as treatment is reduced.

Selective recruitment of polarized T cells expressing CCR5 and CXCR3 to the inflamed joints of children with juvenile idiopathic arthritis.

L R Wedderburn, N Robinson, A Patel, *et al. Arthritis Rheum* 2000; **43**(4): 765–74.

BACKGROUND. T cells infiltrating inflamed joints of children with JIA may have a skewed cytokine phenotype and may be recruited into the joint through expression of specific chemokine receptors. The expression of chemokine receptors CCR5 and CXCR3 and the Th1/Th2 cytokine balance in children with oligoarticular or polyarticular JIA is examined.

INTERPRETATION. The high expression of CCR5 and CXCR3 and high IFN-γ/interleukin (IL) -4 ratios suggest a type 1 phenotype of SF T cells in JIA. The difference between CD45RO+ T cells from SF and from peripheral blood suggests that specific activation events have occurred in synovial T cells. It is suggested that the highly activated, Th1-type phenotype of T cells within the chronically inflamed joints of children with JIA may reflect specific recruitment events that contribute to the polarization of these cells.

Comment

Three-colour immunofluorescence was used to measure expression of CCR5 and CXCR3 on paired samples of peripheral blood and SF T cells from 20 patients with oligoarticular or polyarticular onset JIA. SF T cells expressed higher levels of CCR5 than peripheral blood; the expression of CXR3 was more variable (Fig. 7.3).

Most SF T cells expressed high levels of both CCR5 and CXCR3, but four patients had strong CCR5+ expression and lower CXCR3, compared with peripheral blood cells. One patient with extended oligoarticular JIA, did not express CCR5 on T cells from either peripheral blood or SF, but expressed very

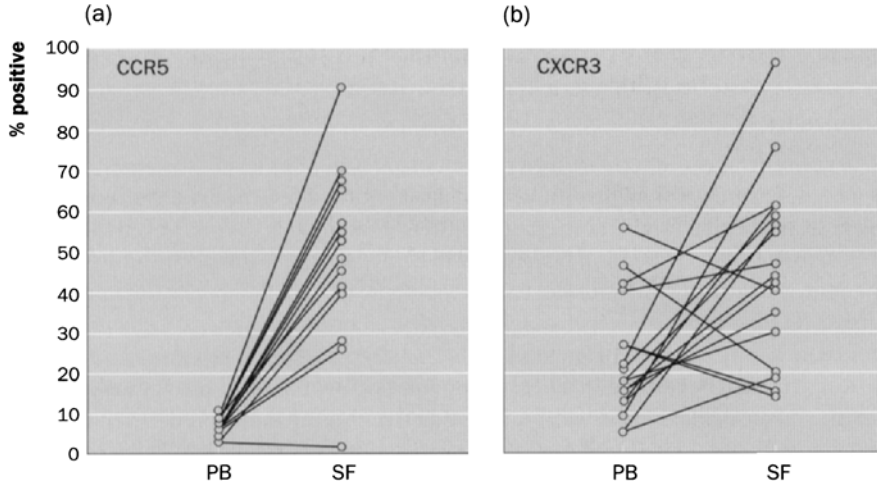

Fig. 7.3 Levels of expression of (a) CCR5 and (b) CXCR3 on CD3+peripheral blood (PB) and SF T cells from patients with JIA. After immunofluorescence staining for CD3, CCR5 and CXCR3, cells were gated on a live lymphocyte gate and 20 000–50 000 events collected per condition. Source: Wedderburn *et al.* (2000).

high levels of CXCR3 on SF T cells. This patient was homozygous for the Δ32 mutant allele of CCR5. This is a 32-bp deletion, which creates a frameshift and inability to express CCR5, and suggests that both chemokine receptors are not required for persistent synovitis.

An *in vitro* assay that did not alter the pre-existing balance of T cells was used to compare the production of IFN-γ and IL-4, representative of Th1 and Th2 phenotypes, respectively. Ratios of IFNγ/IL-4 were significantly higher for SF, demonstrating a strong Th1 bias for SF T cells compared with peripheral blood T cells. The four patients with reduced CXCR3 had IFNγ/IL-4 ratios below the mean for the whole group, and there may be some children with less Th1 polarization. These patients were clinically similar to the rest of the group, but the numbers were too small for subgroup analysis.

It was important to examine CD45 status of the T cells because they express different isoforms of the CD45 surface marker as they change from naïve to memory cells (naïve, CD45RA+; memory, CD45RO+) and differ in their cytokine expression. The SF T cells showed greater expression of CD45RO+than peripheral blood T cells and there was a significant Th1 bias with higher IFNγ/IL-4 ratios. Similarly,the expression of CCR5 was also increased in the SF CD45RO +population, compared with peripheral blood.

These results suggest that the Th1 polarization within the joint may occur with selective recruitment via chemokine receptor (CCR5 and CXCR3) expression. Chemokine expression and functional cytokine assays also demonstrated that the SF T cells were more polarized than the equivalent CD45RO+ population from

peripheral blood, indicating that there is not simply non-specific recruitment of activated T cells into the joint. These recruited T cells may contribute to the patho-physiology of disease.

Elevated serum transferrin receptor concentration in children with juvenile chronic arthritis as evidence of iron deficiency.

S M Kivivuori, P Pelkonen, H Ylijoki, *et al. Rheumatology* 2000; **39**(2): 193–7.

BACKGROUND. Active JCA is accompanied by anaemia of chronic disease, which may be indistinguishable from anaemia due to iron deficiency. Elevation of serum transferrin receptor (sTfR), which should not be influenced by inflammation, may be useful for assessing iron status in anaemic children with JCA.

INTERPRETATION. In 13 of the 30 patients with JCA, the sTfR concentration, which is an indicator of iron status and erythropoiesis, was elevated. The results raise the possibility that sTfR is able to distinguish iron-deficiency anaemia from anaemia of chronic disease. It should be further explored as a candidate marker for iron deficiency.

Comment

sTfR concentration reflects iron requirements at a cellular level. It is elevated early in iron deficiency, reflecting reduced availability of tissue iron. Iron status, including sTfR concentrations, was measured in 30 children with JIA (16 polyarthritis, 13 oligoarthritis, one systemic). Twenty-two had active inflammation and 15 were anaemic. The use of conventional criteria to diagnose iron deficiency (anaemia, ferritin <10 μg/l and transferrin >3.5 g/l) detected only one patient. Only four children had low ferritin and four had high transferrin levels. The normal range for sTfR had been determined in a healthy population as 2.2–6.3 mg/l. Ratios of sTfR/ ferritin were calculated as an additional sensitive indicator of iron deficiency (normal value <200). The median sTfR concentration was 6.1 (range 3.4–13.0 mg/l) and 13 (43%) children had levels greater than the upper limit of normal. The median ratio sTfR/ ferritin was 285 (range 75–1150), and for 20 children the ratio was>200.

Eight of 13 patients with high sTfR had both ferritin and transferrin levels within the normal range. The sTfR level may be more useful to detect iron deficiency in these patients, where inflammation affects ferritin and transferrin levels. A previous study in children with systemic JIA found similar results, with sTfR levels elevated threefold in patients compared with healthy controls. sTfR levels had a negative correlation with haemoglobin in both studies. sTfR also correlated with ESR, but not to ferritin or transferrin. However, anaemia can elevate ESR and, therefore, CRP would have been a more accurate measure for

disease activity. A study in adults with RA showed that sTfR was not associated with CRP.

sTfR may be a useful marker in the difficult clinical problem of iron deficiency with anaemia of chronic disease, but larger studies are needed.

High dose, alternate day corticosteroids for systemic onset juvenile rheumatoid arthritis.
Y Kimura, E Fieldston, B Devries-Vandervlugt, *et al. J Rheumatol* 2000; **27**(8):2018–24.

BACKGROUND. This study determines the safety and efficacy of high-dose alternate day prednisone as therapy in acute systemic onset JRA (SOJRA).

INTERPRETATION. High-dose alternate day prednisone appears to be effective in controlling the systemic features of SOJRA and was well tolerated. Side-effects attributable to corticosteroids, including growth suppression, were minimal.

Comment

Alternate day dosing of steroids has fewer side-effects than daily, but is generally used when disease activity is low. Previous studies in patients with active inflam mation using twice the daily dose on alternate days were not able to gain control of systemic JIA and higher doses were required.

Twenty patients with active systemic JIA, treated with prednisone alternate day (in addition to other disease modifying drugs for their arthritis), and followed for at least a year, were reviewed retrospectively. Disease activity (fever, rash, active joint count, complete blood count and ESR), and possible side-effects of treatment, were assessed at each visit. Within a mean of 2.1 months, all systemic features in all patients had resolved and there were significant improvements in all laboratory indices of disease activity ($P<0.001$ to $P<0.0001$). The mean prednisone dose was 3.2 mg/kg alternate day (range 1–5.8 mg/kg). Steroid doses were tapered as disease activity improved and four patients were able to stop prednisone at 12 months; unfortunately, two of these had relapse of systemic symptoms after the study period and required further steroid treatment. Seventy-five per cent of those who continued steroids had chronic arthritis and required other medications (non-steroidal anti-inflammatory drugs [NSAIDs] [11], MTX [nine], hydroxychloroquine [one], sulphasalazine [one] and auranofin [one]).

There were few side-effects reported: one patient developed a subcapsular cataract and one patient, already Cushingoid due to previous daily steroids, remained Cushingoid. Two other Cushingoid patients improved with alternate day prednisone. Three patients had weight gain >15% expected for their height. Long-term treatment resulted in cataract and a vertebral crush fracture in one patient (6.6 years), and cataract and possible avascular necrosis in another (8

years). There were no serious infections reported in either the study period or with long-term treatment.

Growth is poor in patients with active systemic JIA, but is also impaired by daily steroid treatment. At 1 year, height standard deviation scores (SDS) remained in the normal range for all but two patients. The mean change in height SDS did not change over 3 years and there was no significant difference between patients who continued prednisone compared with those who had stopped steroids.

This is a small retrospective review, and lacks a control group. The results suggest that this regimen may be effective and have less toxicity than treatment with daily steroids. Patients were only assessed for osteopenia, osteoporosis, vertebral compression fractures, peripheral fractures and avascular necrosis if symptomatic, and some of these complications may have been missed. Further controlled, prospective studies are needed to validate these findings.

Prevention of leg length discrepancy in young children with pauciarticular juvenile rheumatoid arthritis by treatment with intraarticular steroids.

DD Sherry, LD Stein, AM Reed, *et al. Arthritis Rheum* 1999; **42** (11):2330–4.

BACKGROUND. This study examines whether intra-articular injection of triamcinolone hexacetonide (steroids) used early in the course of pauciarticular JRA is associated with less leg length discrepancy (LLD) or thigh circumference discrepancy (TCD).

INTERPRETATION. Early and continued use of intra-articular steroids may be associated with less LLD in young children with pauciarticular JRA. This may indicate decreased duration of synovitis.

Comment

Most children with oligoarticular JIA have a good outcome. Long-term sequelae besides ocular damage from uveitis include LLD and TCD. Persistent synovitis may cause hyperaemia of the growth plates and is thought to lead to overgrowth in young children; however, in adolescents, it may lead to premature fusion and shortening. TCD may be due to quadriceps muscle disuse or decreased tone, secondary to knee joint distension. Treatment with intra-articular steroids in children with oligo-articular JIA is safe and rapidly reduces inflammation. Optimal timing is unknown and no previous studies have determined long-term benefit.

Children aged <7 years from two different centres, with asymmetric lower limb arthritis were studied. It was felt that they had more leg growth potential and may be at increased risk of LLD. Sixteen children from Washington (WA) were treated with intra-articular steroids within 2 months of diagnosis, and repeated if necessary. They were compared with 14 children from North Carolina (NC), who had not been treated with intra-articular steroids. The groups were

otherwise comparable. A single observer examined the children, LLD was assessed by standard means: anterior superior iliac spine to medial malleolus and verified by three non-quantitative means. X-rays were not clinically justified. TCD was measured 10 cm above the patella.

No child from WA had measurable LLD, compared with seven from NC, P=0.002. This was reflected by the difference in numbers of children with shoe lifts (none from WA, seven from NC). Similar findings were obtained when children with monoarticular knee arthritis were analysed. There was no significant difference in TCD between the two groups.

Early treatment with intra-articular steroids in young children with oligoarticular JIA involving the lower limb may be associated with less LLD, presumably by decreasing the duration of synovitis. These results are also applicable to other children with lower limb inflammatory arthritis. The disparity between LLD and TCD probably reflects their different mechanisms, and intra-articular steroid treatment may be required much earlier to prevent muscle atrophy. A large number of children studied prospectively and treated very early would need to be studied to address this.

Randomized, placebo-controlled, crossover trial of low-dose oral methotrexate in children with extended oligoarticular or systemic arthritis.

P Woo, T R Southwood, A M Prieur, *et al. Arthritis Rheum* 2000; **43**(8): 1849–57.

BACKGROUND. JIA can persist through adolescence and adulthood, resulting in significant disability. The use of low-dose oral MTX for persistent polyarthritis has been shown to be effective by the USA/USSR collaborative study group. However, two of the most disabling subgroups of JIA, systemic and extended oligoarthritis, were under-represented in that study. The present study was, therefore, conducted to investigate the efficacy of MTX in these two subgroups.

INTERPRETATION. MTX 15–20 mg/m^2 given orally once a week was found to be an effective treatment for both extended oligoarticular and systemic JIA in this short-term trial. Long-term efficacy needs to be addressed in future studies.

Comment

Forty-five children with systemic JIA and 43 with extended oligoarticular JIA were enrolled, and as numbers were small, the crossover design maximized power. The study comprised: 4 months MTX/placebo treatment, 2 months wash-out, 4 months placebo/MTX treatment and 2 months wash-out. Seven patients withdrew from the systemic JIA group: six with disease exacerbation (two during placebo and four during MTX treatment), one due to hepatitis A infection. Two withdrew from the extended oligoarticular JIA group: one had raised liver enzymes

and one developed an arthritis flare during MTX treatment. Even though folic acid was not given, MTX was well tolerated, with no difference in side-effect rates between MTX and placebo.

MTX was effective in extended oligoarticular JIA, with overall improvement by standard criteria, P=0.002. There were improvements of three of five core variables (ESR, physician's global assessment and parent's global assessment); $P \leq 0.005$. This was also reflected in the improvement of laboratory measures of the acute phase response.

No significant overall improvement was seen in the systemic JIA group and only two of five core variables improved (physician's and parent's global assessment). There was no significant difference in systemic feature scores between MTX and placebo treatment, and less improvement in acute phase response. MTX may improve some measures in systemic JIA, but the treatment effect may be smaller than that seen in extended oligoarticular JIA. This study may not have had adequate power to detect treatment effect in the systemic JIA group.

65.9% of extended oligoarticular JIA group received the higher dose (20 mg/m^2) during MTX treatment compared with 25.6% of systemic JIA. These dose differences may have influenced the significant improvements seen in the extended oligoarticular group, compared with the smaller improvements in the systemic group. Serum levels of MTX, 1 h after ingestion, varied widely (≤0.02–1 nmol/l) and parenteral MTX may have produced more reliable levels and therapeutic effects. When data were combined from both groups, MTX produced significant clinical improvement, P=0.006, but in view of the different clinical pictures and apparently different treatment effects, the combination of results may be misleading.

Etanercept in children with polyarticular juvenile rheumatoid arthritis.

D J Lovell, E H Giannini, A Reiff, *et al.* Pediatric Rheumatology Collaborative Study Group. *N Engl J Med* 2000; **342**(11):763–9.

BACKGROUND. This study evaluated the safety and efficacy of etanercept, a soluble TNF (p75): Fc fusion protein, in children with polyarticular JRA who did not tolerate or had an inadequate response to MTX.

INTERPRETATION. Treatment with etanercept leads to significant improvement in patients with active polyarticular JRA. Etanercept is well tolerated by paediatric patients.

Comment

Sixty-nine children with polyarticular JIA entered the study. All had active arthritis, defined as: ≥5 swollen joints and ≥3 joints with limited range of motion

and pain and/or tenderness, despite treatment with NSAID and MTX. Although they all had a polyarticular course, they may not be a homogeneous group, as 10% had pauciarticular arthritis, 58% polyarticular (37% RF positive) and 32% systemic arthritis, during the first 6 months. For ethical reasons, all patients were initially treated and received 0.4 mg etanercept per kg subcutaneously twice weekly for 3 months. The mean age was 10.5 years, and they had a relatively long disease duration of 5.9 years, which suggests that their disease had been difficult to treat.

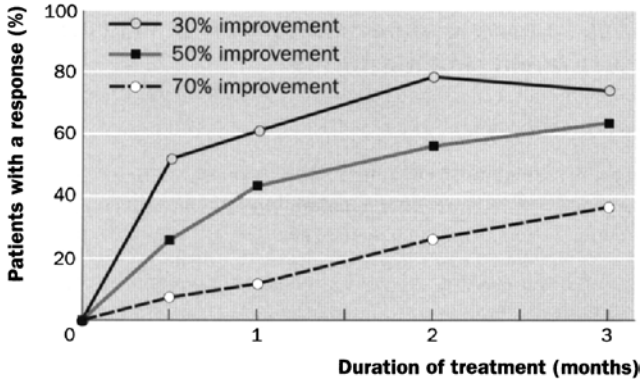

Fig. 7.4 Incidence of 30, 50 and 70% improvement in the 69 patients who received etanercept in the open-label study. At the end of the open-label study, 51 (74%) of the patients had a 30% improvement, 44 (64%) had a 50% improvement and 25 (36%) had a 70% improvement, as compared with baseline. Source: Lovell *et al.* (2000).

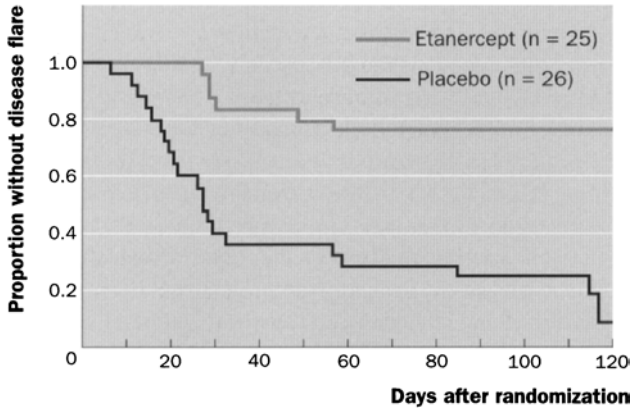

Fig. 7.5 Kaplan-Meier analysis of the time to disease flare. The median time to disease flare was significantly shorter among the patients who received placebo (28 days) than among those who received etanercept (>116 days, $P<0.001$) in the double-blind study. Source: Lovell *et al.* (2000).

Ninety-three per cent completed treatment and 74% met the criteria for improvement (30% improvement from baseline in three of six and deterioration of 30% in no more than one of six of the standard core set of outcome variables). Considerable improvements were seen in all measures of disease activity, some as early as 2 weeks after starting treatment (Fig. 7.4). These responders entered a double-blind randomized study, and either continued etanercept or received placebo until disease flared or 4 months elapsed. Flare was defined as worsening 30% in three of six variables and a minimum of two active joints with improvement of 30% in no more than one of six variables, from time of randomization. Global assessment had to decrease by ≥2 units (scale 0–10). Twenty-one of twenty-six (81%) who received placebo withdrew due to disease flare, compared with seven of 25 (28%) who received etanercept, P=0.003 (Fig. 7.5). The different patterns of arthritis in the first 6 months, showed similar differences in flare rates between placebo and etanercept groups. One patient withdrew due to urticaria after the first dose of etanercept. Other adverse events were: injection-site reaction 39%, upper respiratory tract infection 35%, headache 20%, rhinitis 16%, abdominal pain 16%, vomiting 14%, pharyngitis 14%, nausea 12%, gastrointestinal infection 12%, and rash 10%, and all were self-limiting. No significant differences in the frequency of adverse events were reported between the two treatment groups. At the end of the study, 80% of patients who received etanercept for 7 months still met the definition of improvement compared with 35% of those who received etanercept for 3 months and placebo for 4 months, P<0.001. Etanercept leads to significant improvements in active polyarticular JIA, but treatment needs to be continued long term.

Long-term outcome and prognosis in oligoarticular-onset juvenile idiopathic arthritis.
S Guillaume, A M Prieur, J Coste, C Job-Deslandre. *Arthritis Rheum* 2000; **43**(8):1858–65.

BACKGROUND. No previous studies have assessed long-term outcome and determined predictors of severity among patients with oligoarticular onset JIA.

INTERPRETATION. Oligoarticular-onset JIA is a severe disease with frequent complications. Factors predictive of severity in oligoarticular onset JIA were identified. This could allow early identification of high-risk patient subgroups, warranting a more aggressive therapeutic approach.

Comment

Oligoarticular onset JIA has previously been considered benign compared with poly-articular and systemic onset JIA. Two hundred and seven consecutive patients, who fulfilled criteria for oligoarticular onset JIA, were studied longitudinally (including children with a family history of psoriasis).

There were high rates of joint and eye complications within 6 years (50% polyarticular extension, 35% erosive and 30% uveitis) and a low rate of remission (36%). Polyarticular extension occurred mostly in the first 2 years. There was a steady rate of erosive damage, but this was strongly associated with extension ($P<0.0001$). Uveitis risk was greatest in the first year and reached a plateau at 4 years. There was no association between uveitis and polyarticular extension, in contrast with previous studies.

Involvement of >1 joint, upper limb involvement or a high ESR at onset predicted polyarticular extension. A high ESR also predicted a destructive course and a family history of psoriasis predicted uveitis. As most complications appear early in disease course it is important to look for features at onset that may predict complications. Early identification of high-risk patients would enable early intervention with more aggressive treatment. ANA did not predict patients at risk of uveitis, in contrast to previous studies, and all children with oligoarticular JIA should therefore have regular ophthalmology assessment. There were no features that predicted remission.

Previous studies may have examined heterogeneous patient populations, with oligoarticular, polyarticular and systemic onset patients. The new ILAR classification attempts to define more clinically homogeneous groups, and comparison of results is difficult. Children with a family history of psoriasis do not fulfil ILAR criteria for oligoarticular JIA, but were deliberately included, because this is a major area of difficulty with the new classification.

Systemic onset juvenile idiopathic arthritis: a retrospective study of 80 consecutive patients followed for 10 years.
C Lomater, V Gerloni, M Gattinara, *et al. J Rheumatol* 2000; **27** (2):491–6.

BACKGROUND. An investigation into the relationships between disease activity, course of the disease and functional class according to Steinbrocker |5|, in systemic onset JIA.

INTERPRETATION. Systemic onset JA may present with different clinical courses; the functional outcome is always good in subtype I (monocyclic), but can be poor in subtypes II and III. The severity of disability evaluated according to Steinbrocker classes is dependent on the cumulative duration of the active periods of the disease.

Comment

Previous outcome studies have combined all categories of JIA, which makes interpretation difficult. Even within systemic JIA there is a variable course.

Eighty consecutive patients enrolled since 1971, with systemic onset JRA by ACR criteria, were reviewed retrospectively. Disease activity was categorized by EULAR criteria: active, controlled, inactive or remission. Disease course was designated into three subtypes: I=monocyclic, II=intermittent, III=persistent

Table 7.6 Disease course, disease activity and functional outcome (Steinbrocker) at the last clinic visit of 80 patients with systemic onset JIA

Disease course	Activity status				No. of patients	Status	Class I	Class II	Class III	Class IV
	A	C	I	R						
Subtype I				9	9		9	0	0	0
Subtype II	4	7	6	10	27	A/C	4	3	4	0
						R/I	14	0	2	0
Subtype III	8	15	11	10	44	A/C	6	6	10	1
						R/I	10	5	4	2
					80		43	14	20	3

A: active; C: controlled; I: inactive; R: remission.
Source: Lomater *et al.* (2000).

(Table 7.6): 71.2% patients were in Steinbrocker class I or II, and 57.5% were in remission or had inactive disease. Functional outcome was influenced by disease activity and cumulative duration of active disease correlated with a worse functional class, with a highly significant statistical correlation (correlation coefficient r=0.586; P<0.0001). Seventy patients were treated with disease modifying drugs, which were generally effective in reducing duration of active disease. MTX as monotherapy or in com bination was effective for systemic features and polyarthritis, with few side-effects. Gold salts were used by 50 patients, reflecting the era of the review, and felt to be effective for polyarthritis. Other studies have shown high levels of toxicity and no benefit, and safer and more effective drugs have now superseded gold salts. Cyclosporin A was not well tolerated, but high doses (5 mg/kg) were used.

This study supports previous studies, suggesting that reducing periods of active disease may result in a better functional outcome, and justifying an aggressive therapeutic approach. It would be interesting to see if this is the case with the introduction of biological therapies.

Juvenile chronic arthritis into adulthood: a long-term follow-up study.
M Zak, F K Pedersen. *Rheumatology* 2000; **39**(2):198–204.
BACKGROUND. A review of 65 adults with a history of, or persistent JCA. Patients were assessed on average 26.4 years after disease onset, to describe the course of disease and disease outcome, and to identify disease-related parameters associated with poor disease outcome.
INTERPRETATION. Although the study group was biased towards the more severe cases, the data suggest that the long-term functional outcome in JCA is, in more than one-third, associated with active disease persisting into adulthood, increasing disability and the need for surgery.

Table 7.7 Retrospective and current disease characteristics of the JCA study group

Parameter	Pauciarticular *n* (%)	Extended pauciarticular *n* (%)	Polyarticular *n* (%)	Systemic *n* (%)	Total (%)
JCA subtype at disease onset	43 (66.2)	—	17 (26.1)	5 (7.7)	65 (100)
Course of the disease	21 (32.3)	22 (33.8)	17 (26.2)	5 (7.7)	65 (100)
Active disease at 10-yr follow-up	7 (10.8)	11 (16.9)	8 (12.3)	1 (1.5)	27 (41.5)
Active disease at 26-yr follow-up	4 (6.2)	11 (16.9)	8 (12.3)	1 (1.5)	24 (36.9)
JCA surgery prior to the 10-yr follow-up study	0	2 (3.1)	1 (1.5)	0	3 (4.6)
JCA surgery after the 10-yr follow-up study	1 (1.5)	8 (12.3)	2 (3.1)	2 (3.1)	13 (20)

Two participants included in the polyarticular group, one with juvenile spondylarthropathy and one with juvenile psoriatic arthropathy had active disease at the time of the study.
Source: Zak *et al.* (2000).

Comment

This is a further study of a group of patients who took part in a 10-year follow-up study in 1979–80. All patients were admitted to one Danish hospital between 1965 and 1977 and represented approximately 15% of Danish children with JCA. Sixty-five of the original 93 patients were reviewed in this study. The mean age of onset was 5.7 years with mean disease duration of 12.4 years. Twenty-four (37%) had active disease, of which 80% had either extended pauciarticular, or polyarticular disease and only 6.2% of those in the pauciarticular group had active disease (Table 7.7). The pain visual analogue scale, health assessment questionnaire, ESR and CRP were all significantly increased in patients with active disease and there was reduced perceived ability to cope. Patients had been treated with drugs of questionable efficacy and few received MTX. Physiotherapy treatments have also changed considerably and these results may represent the natural course of the disease. Eleven patients were in Steinbrocker class III or IV and 14 patients (22%) had had JCA-related surgery. Long disease duration, polyarticular course and steroid treatment, were associated with poorer outcome and reflected the patients with the most severe disease. These results are similar to the previous studies that report ongoing active disease in 30–50% of patients and this proportion remains constant beyond 10 years.

Total-body bone mineral content in non-corticosteroid-treated postpubertal females with juvenile rheumatoid arthritis: frequency of osteopenia and contributing factors.
C J Henderson, B L Specker, R I Sierra, *et al. Arthritis Rheum* 2000; **43**(3): 531–40.

BACKGROUND. A study to determine the extent of low total-body bone mineral content (BMC) in non-corticosteroid-treated white post-pubertal females with JRA compared with healthy age- and race-matched female controls, and to identify variables that significantly contribute to total-body BMC.

INTERPRETATION. In this study, ~30% of the subjects in a sample of post-pubertal female patients with mild-to-moderate, non-corticosteroid-treated JRA had low bone mass. The predictor variable that significantly contributed to total-body BMC was lean mass, which demonstrated a protective effect of 0.56 risk reduction for low total-body BMC.

Comment

Previous studies have shown low BMC in children with JIA, but these studies included children treated with corticosteroids. This study was carefully designed to minimize confounding effects: all girls were at least 2 years post-menarche and would have achieved 90–95% of peak bone mass. They had no history of smoking, pregnancy, use of oestrogen-containing contraceptive pills or steroids (oral, inhaled or injected) and no chronic illness except for JIA. These stringent

selection criteria would have excluded patients with more severe JIA, and may have underestimated the frequency of osteopenia and osteoporosis.

BMC was used as a primary outcome variable, rather than bone mineral density (BMD). BMD is an areal measurement (g/cm^2) and in growing individuals BMC (controlling for bone area, height and age) is more useful as it is not related to bone area. BMC was 4.5% lower in JIA patients than in controls, but no there were no significant differences in anthropometric, physical development, or bone mineralization measurements; 11 of 36 (30.6%) of JIA patients were classified by BMC as 'low' (Z-score>1 SD below mean) and 25 of 36 (69.4%) as 'normal'. All anthropometric variables were intercorrelated and also correlated with BMC (r=0.37– 0.85). The final logistic regression model was significant (P<0.001) and contained only lean mass (P=0.01), which accounted for 76.3% of the variation in total-body BMC.

Markers of bone resorption and bone formation were elevated in the low BMC group, which suggests an increase in bone turnover and is expected in patients with osteopenia. There were no significant differences in BMC associated with drug treatments, and MTX was positively related to total-body BMC, perhaps due to its disease-modifying effect.

It was surprising to find a higher nutrient intake in the low BMC group, as previous studies have found high rates of protein malnutrition among adolescents with JIA. Patients with low BMC had more involved joints, and this may imply more severe disease. Inflammation can shift preferential use of energy substrate from fat to lean body mass, but the mechanisms are not well understood.

Young adults with juvenile arthritis in remission attain normal peak bone mass at the lumbar spine and forearm.
M Haugen, G Lien, B Flato, *et al. Arthritis Rheum* 2000; **43**(7): 1504–10.

BACKGROUND. Disease activity in JIA may influence bone metabolism and, therefore, this study assessed acquired peak bone mass and bone turnover in young adult patients with either persistent JIA or a history of JIA (JIA in remission).

INTERPRETATION. The findings imply that most young adults with JIA attain the same BMD as healthy subjects if the disease goes into remission, while young adults with active disease have increased risk of osteopenia and osteoporosis.

Comment

One hundred and forty-five women and 84 men with JIA were studied (mean age 24.9 years for women and 25.2 years for men); 41 healthy women (mean age 27. 4 years) and 55 healthy men (mean age 25.7 years) were recruited as a reference group. Eighty-seven women (60%) and 51 men (61%) were in remission for a mean of 8.0 and 10.2 years, respectively. Men and women with persistent JIA

had similar disease duration, 15.7 years for women and 15.1 years for men. The duration of active JIA for those in remission was 7.6 years for women and 4.8 years for men.

BMD was measured in the whole body, femoral neck, lumbar spine and distal one-third radius using dual X-ray absorptiometry.

Patients with persistent JIA had lower BMD at all measured sites, compared with healthy subjects ($P<0.001$ for women and men at femoral neck and total body, $P<0.05$ for men at radius and lumbar spine). There was no significant difference in BMD at any site for men with a history of JIA, compared with healthy subjects. Women with a history of JIA had only lower total body and femoral neck BMD than healthy subjects ($P<0.05$). Patients with persistent JIA had significantly more osteopenia at all sites, except lumbar spine ($P<0.001$) and more osteoporosis at all sites, except femoral neck, compared with healthy subjects. Four patients could not have femoral neck BMD measurement due to bilateral total hip replacement. Patients in remission were similar to healthy subjects, except for lower total BMD ($P<0.05$).

Multiple linear regression analysis was used to evaluate impact of disease and its severity on BMD. Weight, urinary concentration of deoxypyridium (D-Pyd) and a diagnosis of JIA significantly affected BMD at all sites, in all subjects. For patients, only the length of steroid use influenced BMD at all sites. The impact of other variables differed between sites, and may indicate that different sites are affected by different mechanisms.

Women with active JIA had increased urinary D-Pyd, indicating increased bone resorption. Serum osteocalcin (marker for bone formation) was elevated in both patient groups. Studies in children with active JIA found low osteocalcin levels; therefore, perhaps bone formation continues into adulthood with disease remission and contributes to the normal peak bone mass achieved in lumbar spine and radius by adults with a history of JIA, despite active disease during adolescence.

Bone mineral content and bone mineral metabolism: changes after growth hormone treatment in juvenile chronic arthritis.

M Rooney, U M Davies, J Reeve, *et al. J Rheumatol* 2000; **27** (4):1073–81.

BACKGROUND. A study to determine whether recombinant human growth hormone (rhGH) affects bone mineral metabolism and BMC (g/cm) in a therapeutic trial of recombinant growth hormone in growth retarded children with JCA treated with steroid.

INTERPRETATION. Steroid treated children with both JCA and severe growth retardation have reduced vitamin D, parathyroid hormone (PTH), and osteocalcin levels. After treatment with rhGH, height velocity increased, as did BMC. Growth hormone might be a useful adjunct in the treatment of severe growth

retardation and osteoporosis in children with JCA. The long-term benefits of rhGH in the treatment of osteoporosis remain unclear.

Comment

Growth retardation and osteoporosis are complications of JIA as a result of disease activity, poor nutrition and therapy.

Twenty prepubertal children with JIA and growth retardation>1 year (mean height, −3.0 SDS and height velocity, −3.1 SDS) were randomized and treated with two different doses of rhGH (12 versus 24 IU/m^2 per week) and compared with an untreated control group with JIA. BMC increased with treatment and correlated with increasing height (r=0.67, P<0.002). There was no correlation between disease type, duration, steroid dose or CRP and BMC. Vitamin D and PTH were significantly low in these patients. A 3-day dietary assessment showed that dietary intake was grossly normal, but the study was not designed to examine this more precisely. Osteocalcin levels (markers of bone formation) increased with rhGH treatment and correlated with height velocity, for both treatment groups combined and when the two groups were analysed separately. Osteocalcin levels correlated negatively with CRP before treatment (r=0.39, P=0.03) and after treatment, and with both groups separately: low dose was r=−0.43 and P=0.001 and high dose r=−0.46 and P<0.005.

This study has previously reported that these children with JIA had normal GH secretory profiles but low insulin-like growth factor 1 (IGF-1) and osteocalcin levels. Studies using rhGH in children with GH deficiency showed a delayed but sustained rise in osteocalcin level. The osteocalcin level in the low-dose group peaked by 3 months then declined, and peaked by 6 months in the high-dose group. There was only a 30% increase in osteocalcin level, compared with 200–400% seen in GH deficiency, when treated using similar rhGH doses. The reduced response to rhGH may imply a relative GH resistance, leading to low IGF-1 levels. As CRP correlates negatively with osteocalcin levels, impaired GH response may be partly due to disease activity. Further study of the interactions of GH, GH receptors, IGF-1 and its binding proteins is required.

Effects on bone metabolism of one year recombinant human growth hormone administration to children with juvenile chronic arthritis undergoing chronic steroid therapy.
G Touati, J C Ruiz, D Porquet, *et al. J Rheumatol* 2000; **27**(5): 1287–93.

BACKGROUND. To study the effects on bone metabolism of treatment with rhGH in children with JCA who are undergoing treatment with glucocorticoids (GC) and have severe bone lesions.

INTERPRETATION. The results reflect an increase in bone turnover in these patients. Despite these biochemical changes no improvement of bone

density was observed during the 1-year treatment. Treatment of longer duration is necessary to evaluate the curative effects of GH.

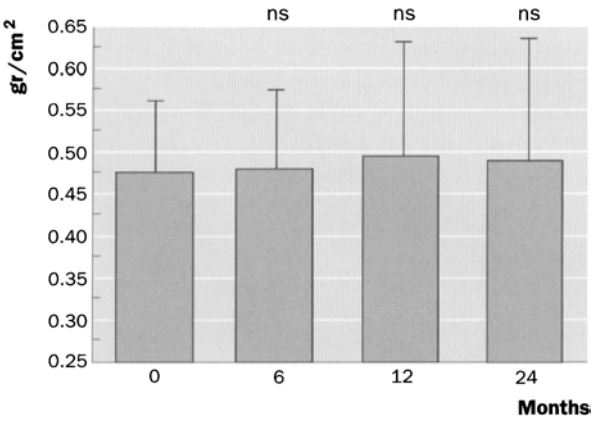

Fig. 7.6 Effect of rhGH treatment on bone density measured by X-ray absorptiometry at the lumbar vertebrae (trabecular bone). Effect of 1-year treatment for 1 year of no treatment. Median values and 90th percentile values (vertical bars) are shown. Comparisons with pre-treatment values by Student's t-test. ns: not significant. Source: Touati *et al.* (2000).

Comment

Fourteen prepubertal children with systemic JIA and severe growth delay (mean height, −4.3 SDS for chronological age (CA) and mean growth velocity (GV) −5. 1 SDS for CA, were treated for 1 year with rhGH 1.4 U/kg per week. Bone formation markers, osteocalcin and C-terminal propeptide of type 1 procollagen (PICP) and bone resorption markers, urinary hydroxyproline, pyridinoline and deoxypyridinoline increased significantly during treatment and returned to baseline levels 1 year after treatment ended. Levels of the bone formation markers all showed positive correlation with GV during treatment; osteocalcin level at 1 month was the best predictive variable of the growth response to rhGH (r=0.9, P<0.001). Pretreatment levels of osteocalcin were low-normal and increased by 80% during treatment. There was no correlation between the bone resorption markers and GV. There were no significant changes in bone age or BMD (Fig. 7.6). IGF-1 levels were low-normal, with normal GH profiles. This may reflect GH resistance, but may also reflect poor nutritional status, which was not assessed by this study.

JIA is associated with reduced bone formation and resorption and, therefore, reduced bone turnover, although disease severity correlates with bone formation markers only. Treatment with rhGH may increase bone turnover, with a predominance of bone formation.

These results are similar to those reported by Rooney *et al.* (preceding paper), although this was a more homogeneous group (all systemic JIA) and the growth delay was more severe. Both groups found a positive correlation between osteocalcin level and GV. BMD did not increase during this study; Rooney *et al.* measured BMC, as they had previously found this measure to correlate better than BMD with body surface area. Other studies using rhGH showed delayed response of BMD by 6–30 months in children with GH deficiency and 24 months in GH-deficient adults. Longer studies are necessary to determine if rhGH improves BMD in systemic JIA and a 3-year study is underway, including children from this study.

Psychological, behavioural, and social adjustment in children and adolescents with juvenile chronic arthritis.
A C Huygen, W Kuis, G Sinnema. *Ann Rheum Dis* 2000; **59**(4): 276–82.

BACKGROUND. The psychological, behavioural and social adjustment of children (7–11 years) and adolescents (12–16 years) with JCA were assessed. Higher rates of maladjustment were expected to be found in these patients.

INTERPRETATION. In contrast with expectations, children and adolescents with JCA seemed to cope quite well with the psychological and social consequences of their long-term condition. For future studies, it is hypothesized that the high levels of adaptation might imply an enduring psychological strain, which is reflected in an altered function of the autonomic nervous system.

Comment

Forty-seven patients (23 children and 24 adolescents) with JIA and 52 healthy peers (25 children and 27 adolescents) and their parents were recruited. Standard self-report questionnaires, which encompassed areas of competence and self-image, psychopathology, family functioning and social support, were used.

Overall, there were few differences between children and adolescents with JIA and their healthy peers. Children with JIA had lower self-perceived competence in athletic skills (P=0.03), reflecting a realistic assessment of physical capabilities, but with no loss of self-esteem. Children felt more positive towards their body with longer illness duration, suggesting a growing acceptance of disease and its consequences. There were no signs of psychopathology or depression, in patients or healthy peers. Patients perceived themselves socially as competent as theirs peers, although the range of activities was more limited. This was perceived more by adolescents than their parents. Children with JIA (especially systemic JIA) showed a greater tendency than their peers to focus on positive answers about social adjustment (P=0.01) and other studies have also reported high rates of active coping among children with severe JIA. Parents of children with JIA, reported cohesive but less adaptable families compared with

peers, but families of adolescents with JIA were no different to those of healthy adolescents. Adolescents with JIA reported their parents as more overprotective than their peers, reflected by increased social and emotional support. This may have implications for the adolescent with JIA achieving independence. It was surprising to see no variation in psychological or social function between the subgroups of JIA; patients with more severe disease may have had more difficulties. A greater visibility of disease correlated with more social support for parents (r=0.60, P=0.01)

This was a cross-sectional study and, therefore, may miss transient problems. A longitudinal study may have been more representative.

Social, emotional, and behavioural functioning of children with juvenile rheumatoid arthritis.
R B Noll, K Kozlowski, C Gerhardt, *et al. Arthritis Rheum* 2000; **43**(6): 1387–96.

BACKGROUND. Children with JRA may have more social and emotional problems than case-control classmates.

INTERPRETATION. Children with JRA were remarkably similar to case-control children on measures of social functioning, emotional well-being and behaviour. These findings are not supportive of disability/stress models of chronic illness in childhood and suggest considerable psychological hardiness among children with JRA.

Comment

Previous studies have shown that children with JIA may experience psychological difficulties (difficulties socializing with peers, anxiety and depression). This study attempted to avoid confounding factors and limitations, present in earlier studies, by using case-controls.

Seventy-four children with JIA, seen at one centre and 74 same sex/race, similar age classmate case-controls were recruited. Data were collected from peers, teachers, mothers and fathers, and by child self-report, using standardized measures.

1. Social functioning: there were no differences between patients and controls for any of the assessments.
2. Behaviour: no differences between patients and controls.
3. Emotional well-being: patients had higher self-perceived scholastic competence (P=0.03), but all other measures (social acceptance, athletic competence, physical appearance, behaviour and global self-worth) were not different to controls.

Mothers reported children with JIA as less adaptable and with less positive affect on the Dimensions of Temperament Survey—revised (P=0.01) and higher rates

of internalizing problems on the Child Behaviour Checklist (P=0.03). Fathers' reported scores had no significant differences. No differences were found between patients with active or inactive JIA. Previous studies, which had reported increased emotional difficulties among children with JIA, had used data from mothers only. It is important to use data from multiple sources, to evaluate these children.

The study had sufficient power to detect moderate effect sizes (0.50), but was insufficient to detect small effect sizes (0.20) and may have missed a few children who were having difficulties. A larger study could allow assessment of children with different subtypes of JIA, as children with systemic or polyarticular disease may function differently to children with oligoarticular JIA. It would be interesting to compare with other centres, to see if different centres and types of service have any impact.

Sex, reproduction, and gynecological disease in young adults with a history of juvenile chronic arthritis.
M Ostensen, K Almberg, H S Koksvik. *J Rheumatol* 2000; **27** (7):1783–7.

BACKGROUND. A study to examine the effect of chronic disease of childhood on aspects of reproduction, in young adults with a history of JCA.

INTERPRETATION. A history of JCA in young men and women can influence several aspects of reproductive behavior and health. Health care providers counselling adolescents with JCA need to be aware of these problems.

Comment

JIA may impair functional ability and influence body image and, therefore, interfere with sexual behaviour in adulthood. Disease processes and treatments (e.g. cytotoxic and immune suppressant drugs) may also affect fertility.

One hundred and twenty-six women and 35 men with JIA and age-matched healthy controls completed questionnaires examining their reproductive health and family planning choices.

In females, 77% of patients and controls were sexually active and both groups had a mean age of menarche of 13 years, with no differences seen in patients who had received steroids or cytotoxic drugs. Patients reported difficulties with conception and gynaecological problems more frequently (Table 7.8). Both groups felt two to three children desirable and contraceptive practices were similar, although more patients were sterilized and at a younger age (33 versus 44 years). Pregnancy rates were similar in both groups, 2.3/woman, but rates of miscarriage were higher among patients (Table 7.9). Disease flared in 63.7% of patients within 6 months of delivery.

In male patients, they were more frequently single and not in a relationship, compared with controls (P=0.05) and they were less sexually active (P=0.06).

Table 7.8 Gynecological problems (in %) in 126 women with JCA and 117 healthy women

Gynecological problem	Women with JCA	Healthy women	P
Metrorrhagia	26.2	10.9	0.003
Pelvic inflammatory disease	12.7	5.9	0.07
Surgery for cystic ovaries	4.8	0	0.02
Problems to conceive*	11.1	1.7	0.003

*Unprotected intercourse for more than one year without becoming pregnant.
Source: Ostensen *et al.* (2000).

Table 7.9 Outcome of 101 pregnancies of women with JCA and 105 pregnancies of healthy women

Pregnancy outcome	Women with JCA	Healthy women
No. of pregnancies	101	105
Miscarriage (%)	21 (20.8)*	10 (9.5)
Elective abortion (%)	11 (10.9)	15 (14.3)
Stillbirth	1	1

* p=0.02.
Source: Ostensen *et al.* (2000).

Previous studies had shown fewer patients with JIA married/cohabiting and lower rates of sexual activity, compared with controls. This was only seen in males with JIA in this study, but the number of male patients was small. Disease and disability did not differ between male and female patients, to account for this difference. Psychological factors (e.g. poor body image, low self-esteem and social isolation) may be important but were not examined by this study.

Two male and five female patients felt that disease activity and disability pre vented sex, and eight females and four males did not want children, due to fear of heredity. One-third of female patients had been advised against having children (by doctors 47.1% and family 35.3%). Health professionals need to be aware of a patient's wish for a 'normal' life, including the wish for a family, rather than focusing on problems of disability. It is important that adolescents with JIA receive sexual counselling, including advice about family planning and parenthood.

Audit of rheumatology services for adolescents and young adults in the UK.
J E McDonagh, H E Foster, M A Hall, M A Chamberlain. British Paediatric Rheumatology Group. *Rheumatology* 2000; **39** (6):596–602.

BACKGROUND. JIA is associated with significant morbidity in adulthood with at least one-third of children continuing to have active inflammatory disease into their adult years and up to 60% of all patients continuing to have some limitation of their activities of daily living. A survey of service provision for these young people in the transition from paediatric to adult rheumatology care was, therefore, undertaken.

INTERPRETATION. This survey identifies a heterogeneity of provision of health care for adolescents with rheumatic disease and highlights the potential for further research and development.

Comment

Fifty-five replies to a postal questionnaire were received, representing 51 (84%) paediatric rheumatology units in the UK and Ireland. A median of 24 patients (new and follow-up, range 1–225) were seen in a median of two clinics (range 0–15) per month. Nine (18%) units had a dedicated adolescent clinic, with a median of one clinic per month. All clinics involved an adult rheumatologist, plus members of the multidisciplinary team (Table 7.10). Many had contact/access to Young Arthritis Care (UK patients' organisation) (six), careers advisors (five), disabled employment advisors (five), Lady Hoare Trust social workers (four) and psychologists (five), and access to a variety of resources (Table 7.11). In units without a dedicated clinic, current provision for adolescents was varied, and included shared care between paediatricians and adult rheumatologists, the support of a nurse specialist and the use of the local young disability team (six).

Table 7.10 Personnel involved in current adolescent rheumatology clinics

	Number of adolescent rheumatology clinics (n = 9)
Consultant rheumatologists ± consultant paediatricians	5
Other consultants	
Orthopaedic	1
Rehabilitation	1
Metabolic bone	1
Ophthalmologist	1
Specialist registrar in rheumatology or paediatrics	6
Clinical nurse specialists	7
Occupational therapists	6
Physiotherapists	5

Source: McDonagh *et al.* (2000).

A demand for specific information aimed at adolescents was highlighted, both in the areas of rheumatology and general health but also career information and advice.

Studies of long-term outcome of JIA have shown the need for continued care; 27.3% of cases of JIA develop between ages 10 and 16 years and several rheumatic diseases may present in adolescence. The adolescent clinic facilitates transfer from paediatric to adult clinics, where the patient takes more responsibility for their own health care. Patient empowerment, including education, communication and self-advocacy skills, is integral to the transition process. There is considerable variation in all aspects of maturation among adolescents

Table 7.11 Available adolescent rheumatology resources

Resource	Number of adolescent rheumatology clinics ($n = 9$)
Patient information for adolescents re: disease and treatment	4
Patient information re: generic health issues	2
Disability allowance information	6
Patient education sessions	2
Access to rehabilitation services	5
Dedicated adolescent hydrotherapy sessions	5
Dedicated adolescent aerobic sessions	1
Residential independence breaks	1
Patient–parent support groups	1
Social events	1
Educational events	1

Source: McDonagh *et al.* (2000).

and chronic arthritis can also have an impact; an individual and flexible approach is, therefore, needed.

This was the first national survey of adolescent rheumatology services in the UK and Ireland, and highlights the potential for further development. Specific areas for further development include:

1. Transition policy planning (entry and exit criteria, independent consultations for adolescents, etc.).
2. Development of educational resources.
3. Inter-agency liaison.

Conclusion

Although classification of disease may sometimes appear to be a rather arcane exercise, it is essential so that clinical observations on disease manifestations, responses to therapy and eventual outcome can be assessed accurately in different clinics and countries. When aetiology is known, classification is not controversial, but in juvenile arthritis where aetiology is unclear accurate classification may actually contribute to unravelling pathogenesis. It is encouraging to see that different disease subgroups have quite different HLA associations, suggesting that the classification has biological validity.

The search for genes that influence pathogenesis of juvenile arthritis continues; some, such as polymorphisms in TNF-α may be involved directly, affecting the relative amounts of TNF-α and soluble TNF-receptors that are produced. The latter serve as a buffering system to control the effects of TNF-α; such controls are an important aspect of powerful cytokines such as TNF-α and IL-1. Augmentation of TNF receptor by treatment with etanercept has produced impressive results. The new question that arises is the extent to which etanercept

might produce efficacy equal to that of corticosteroids, but with a smaller set of side-effects. Meanwhile, better ways of using corticosteroids have been devised, such as multiple intraarticular injections.

As important as the success in controlling inflammation in joints is the long-term outcome in juvenile arthritis—particularly as inflammation will eventually remit in a proportion of patients. Attention has been paid to bone mass, growth and social and emotional development, with generally satisfactory outcomes, particularly as far as development is concerned. While this points to the considerable resilience of children and adolescents, there is still a need to provide the correct support mechanisms at different stages in the disease, and at different ages.

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8
Juvenile dermatomyositis

A series of papers is included on the treatment, outcome and assessment of this condition. Although relatively rare, it is potentially disabling and can present difficulties in management. It is encouraging that outcome appears to be improving.

Efficacy of early treatment of severe juvenile dermatomyositis with intravenous methylprednisolone and methotrexate.
S Al-Mayouf, A Al-Mazyed, S Bahabri. *Clin Rheumatol* 2000; **19**(2):138–41.

BACKGROUND. A pilot study was conducted to assess the efficacy of early treatment of severe juvenile dermatomyositis (DM) patients with intravenous methylprednisolone (IVMP) and methotrexate (MTX).

INTERPRETATION. This study suggests that MTX and IVMP are a useful combination in the early treatment of severe juvenile DM. The sample was small, therefore, further studies in a controlled trial are necessary to confirm these findings.

Comment

Corticosteroids are the mainstay of treatment for juvenile DM and MTX is added as additional immunosuppressive therapy, if necessary. IVMP pulses may be used if patients have severe complications (e.g. respiratory muscle involvement, dysphagia, gastrointestinal (GI) or cutaneous vasculopathy) and in patients with mild disease have been shown to produce remission more quickly than oral steroids. This pilot study examines the efficacy of a protocol of intermittent pulses of IVMP and early use of MTX in severe juvenile DM.

Twelve consecutive patients with severe juvenile DM were treated with the same protocol of IVMP (30 mg/kg daily for 3 days repeated weekly for 4 weeks, fortnightly ×6, then monthly for 1 year) plus oral, weekly MTX and oral prednisolone ≤2 mg/kg per day, tapered according to response. Ten patients also received hydroxychloroquine. Six patients were treated early (<6 weeks) and six treated late (5–72 months of disease) due to delayed hospital presentation. Early

treatment resulted in complete resolution of the severe complications, normal muscle strength (grade 2–3/5 to 5/5) and normal laboratory results, with no calcinosis. The group treated late had ongoing weakness (3–4/5) and two developed diffuse calcinosis. MTX doses and oral prednisolone doses were successfully tapered without relapse of disease, and two patients discontinued oral prednisolone (Table 8.1). Treatment was well tolerated, except for one patient who stopped MTX after 12 months due to GI intolerance and growth was not impaired (data not shown).

Table 8.1 Clinical indications, duration of MIX and IVMP treatment and outcome in 12 patients with juvenile DM

Patient	Indication for MTX	Duration of MTX (months)	Mean weekly MTX dose	Current muscle strength	Calcinosis	Current prednisone dose
1	GI bleeding/dysphagia	38	15 mg	5		2.5 mg OD
2	Severe subcutaneous oedema	12	14 mg	5		2.5 mg OD
3	Dysphagia	8	12.5 mg	5		2.5 mg OD
4	Severe cutaneous vasculitis	28	12.5 mg	5		2.5 mg
5	Severe cutaneous vasculitis	26	15 mg	5		5 mg OD
6	Dysphagia	40	15 mg	3	++	5 mg OD
7	Dysphonia	30	12.5 mg	5		Off prednisone
8	Dysphagia/respiratory distress	18	12.5 mg	5		5 mg OD
9	Severe cutaneous vasculitis	48	15 mg	4	++	Off prednisone
10	Dysphonia/severe cutaneous vasculitis	13	21 mg	3–4		5 mg OD
11	Dysphonia/dysphagia	10	20 mg	5		5 mg OD
12	Dysphagia	8	12.5 mg	5		5 mg OD

MIX, methotrexate; IVMP, intravenous methylprednisolone; DM, dermatomyositis; GI, gastrointestinal; OD, once daily.
Source: Al-Mayouf *et al.* (2000).

This protocol appears logical and reflects clinical practice for these difficult cases. Early and aggressive treatment of severe juvenile DM may result in a better clinical outcome, but requires further large-scale studies.

Medium- and long-term functional outcomes in a multicenter cohort of children with juvenile dermatomyositis.
A M Huber, B Lang, C M LeBlanc, *et al. Arthritis Rheum* 2000; **43**(3): 541–9.

BACKGROUND. A retrospective review to evaluate functional outcomes in a cohort of patients with juvenile DM.

INTERPRETATION. In general, patients in this cohort had favourable outcomes. Most had Childhood Health Assessment Questionnaire (CHAQ) scores of 0, and only 8% met the definition of moderate-to-severe disability. However, many patients continued to have chronic disease, persistent rash and continued taking medications >3 years after diagnosis. Further research is needed to improve outcomes for patients with juvenile DM.

Comment

Sixty-five patients (46 female, 19 male) from four Canadian tertiary paediatric centres were reviewed. The primary outcome was function, measured using the CHAQ. This had previously been validated for use in juvenile DM, but the score corresponding to significant disability was not clear and based on clinical experience, a score ≥1.0 was used to define moderate/severe disability.

The clinical characteristics of the patients were similar, although patients from Halifax and Vancouver more frequently received oral prednisolone and IVMP as initial therapy. Treatment was not standardized, but varied according to disease severity, clinicians' experience and changing practices. Twenty-four (37%) had a monocyclic course, and the remaining 41 (63%) had a polycyclic or chronic continuous course. Forty-seven (72%) had CHAQ scores of 0, implying no or minimal disability, and only five (8%) had scores ≥1.0 (Fig. 8.1). Increased disability was associated with a chronic continuous course, pain and calcinosis, reflecting prolonged muscle inflammation. Females also had higher CHAQ scores, although numbers of patients were too small to assume that females have a poorer prognosis. Only four patients had delayed treatment >12 months after onset of disease and, therefore, no association was found between CHAQ and either delay in diagnosis or treatment (Table 8.2). Growth was not significantly impaired in most patients, with only 31%>1 SD and 16% >2 SD below their predicted height. Education did not appear to have suffered; all patients were either attending school or had completed secondary education and gone on to higher education (four university, six community college and one business degree).

Outcome appears to have improved considerably since the 1960s and 1970s, probably as a result of more aggressive therapy and better treatment in early

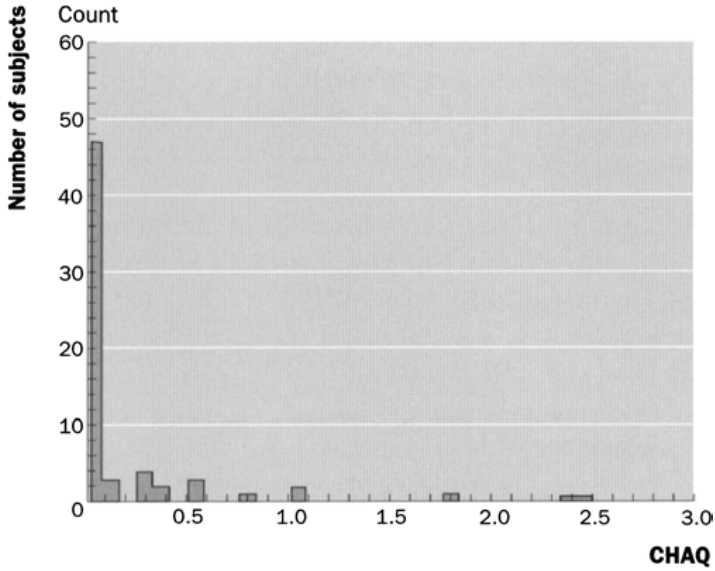

Fig. 8.1 Histogram showing the distribution of scores on the CHAQ. Source: Huber *et al.* (2000).

Table 8.2 Relationship between final CHAQ scores and potential predictor variables*

	CHAQ scores			
	Median	**Range**	**Proportion >0**	***P***
Sex				
Female	0	0–2.5	17/46	0.015†
Male	0	0–1.0	1/19	
Disease course				
Monocyclic	0	0–0.125	1/24	0.0009‡
Polycyclic	0	0–0.375	1/7	
Chronic continuous	0	0–2.5	16/34	
Calcinosis at any time				
Yes	0	0–2.5	10/22	0.01†
No	0	0–1.0	8/43	
IVMP part of initial therapy				
Yes	0	0–2.5	11/31	0.11†
No	0	0–0.5	7/34	

* Shown are the median and range of scores on the Childhood Health Assessment Questionnaire (CHAQ) for each level of the potential predictor variables of sex, disease course, calcinosis, and intravenous methylprednisolone (IVMP) therapy. *P* values test the hypothesis that there is no difference between the median CHAQ scores for the levels of each variable.
†By Mann-Whitney U test.
‡By Kruskal-Wallis test, corrected for ties.
Source: Huber *et al.* (2000).

disease. However, despite an overall good outcome there was long-term morbidity; 26 (40%) of patients had ongoing rash, although this was mostly mild and 15 (23%) had weakness. Twenty-three (35%) were still taking medication: eight MTX, one cyclosporin, one hydroxychloroquine, one prednisolone and the remaining 12 were taking combination therapy.

Juvenile amyopathic dermatomyositis: results of a case finding descriptive survey.
S Plamondon, P B Dent. *J Rheumatol* 2000; **27**(8):2031–4.

BACKGROUND. A case-finding descriptive survey was carried out using a prevalidated, peer-reviewed questionnaire sent to Paediatric Rheumatologists and Dermatologists. This requested clinical details of history, diagnostic investigations, therapy and outcome of all patients who fulfilled a diagnosis of amyopathic DM, to define an appropriate approach to its diagnosis and management.

INTERPRETATION. The classic skin changes of juvenile DM can occur in the absence of clinical muscle involvement. Physicians are not routinely performing electromyography, muscle biopsy or magnetic resonance imaging in the assessment of these patients. A significant proportion of patients with amyopathic DM will remit without systemic therapy. Optimum treatment needs to be determined through controlled trials.

Comment

Amyopathic DM (or DM sine myositis) is a recognized separate entity within the spectrum of idiopathic inflammatory myositis. The prevalence of amyopathic DM in children is unknown and management and prognosis are unclear. Euwer and Sontheimer |**1**| proposed modifications to Bohan and Peter's |**2**| classification with diagnostic criteria for amyopathic DM: (1) pathognomonic cutaneous changes of DM; (2) skin biopsy compatible with DM; (3) no evidence of muscle weakness over 2 years; (4) normal levels of creatine kinase (CK) and aldolase within the first 2 years.

Thirty-nine questionnaires were submitted for analysis. Twelve cases did not fulfil the criteria and were excluded, due to abnormal test results (two positive electromyograms, four positive muscle biopsies, eight elevated CK or aldolase). Only one case met all criteria for amyopathic DM. The remaining 26 had incomplete investigations and were, therefore, not excluded, as all their completed tests were normal. Twenty-three patients followed for >2 years had not developed myositis, suggesting that amyopathic DM is also a separate entity within juvenile DM. It remains unclear whether systemic treatment induced remission of skin disease as 10 were treated with systemic therapy, and in five cases the rash resolved, but 12 of the 17 not treated also had remission of their rash, and five had ongoing rash but no myopathy.

Some patients with apparent amyopathic DM may have subtle muscle involvement and it is unclear how intensively to evaluate subclinical muscle disease, especially using invasive tests in children. This issue is only important if early therapy induces remission and prevents long-term morbidity. Unfortunately, there is no conclusive evidence for this, even for classical juvenile DM. Multicentre studies of juvenile DM are clearly required, and would allow follow-up of these patients who have no obvious muscle involvement.

Magnetic resonance imaging detection of occult skin and subcutaneous abnormalities in juvenile dermatomyositis. Implications for diagnosis and therapy.

A B Kimball, R M Summers, M Turner, *et al. Arthritis Rheum* 2000; **43**(8): 1866–73.

BACKGROUND. A prospective study to assess skin, subcutaneous tissue and fascia in juvenile DM, using magnetic resonance imaging (MRI); its utility in evaluating disease activity and correlation with traditional measures of disease activity and development of calcinosis are evaluated.

INTERPRETATION. Oedema or inflammation in the skin, subcutaneous tissue and fascia, found on short tau inversion recovery (STIR) MRI, is common in juvenile DM patients and is often undetected by standard assessments. These MRI changes can precede the development of calcinosis. STIR MRI may be a useful adjunct for assessing disease activity and guiding the treatment of juvenile DM.

Comment

MRI is useful in assessing inflammatory conditions and in DM, STIR MRI or fat-suppressed, T2-weighted signal intensity can be used to localize muscle inflammation to improve diagnostic yield of biopsy and assess extent of disease. MRI changes in other conditions involving skin, subcutaneous tissue and fascial inflammation correlate well with histopathology, but this had not been examined in DM.

STIR MRI of thighs and buttocks were obtained on 26 children with probable or definite juvenile DM and eight controls. MRI oedema scores were calculated based on intensity and extent of tissue involvement. DM was assessed with standard measures of disease activity: skin and global disease activity scores, Childhood Myositis Assessment Scale (CMAS) and manual muscle strength testing.

At baseline, at least 50% of patients were observed to have oedema in skin, subcutaneous tissue, fascia or muscle using STIR MRI. No controls had fascial or muscle oedema, $P<0.001$ and $P=0.03$ respectively (Table 8.3). STIR MRI skin oedema scores correlated with subcutaneous oedema scores ($r=0.581$, $P=0.008$) and fascial STIR MRI scores correlated with muscle scores ($r=0.58$, $P=0.002$). There was no correlation of STIR MRI among other tissues, and this corresponds to previous observations of disparity between skin and muscle disease. Skin activity scores correlated with MRI skin oedema scores ($r=0.41$, $P=0.04$). Serum

aldolase correlated with skin and subcutaneous oedema scores. Other markers of disease activity did not predict the presence or extent of oedema in skin, subcutaneous tissue or fascia, and oedema demonstrated in subcutaneous tissue and fascia may remain clinically unrecognized; however, its clinical relevance is unclear. In subcutaneous tissue it may precede calcinosis, as five patients with thigh subcutaneous oedema developed calcinosis at that site within 9 months. However, many children with similar changes did not develop calcinosis, and it is difficult to draw conclusions.

Table 8.3 Baseline STIR MRI abnormalities in 26 patients with juvenile DM compared with a group of eight children without juvenile DM*

STIR MRI findings of the thighs and buttocks†	Juvenile DM patients with abnormal STIR MRI signal intensity, no. (%)	Distribution of positive STIR MRI scores of juvenile DM patients, median [25%, 75%]	Comparison group subjects with abnormal STIR MRI signal intensity, no. (%)	Distribution of positive STIR MRI scores of comparison group subjects, median [25%, 75%]
Skin edema	15 (58)‡	8.0 [4.9, 23.1]	0 (0)‡	—
Subcutaneous edema	22 (85)§	9.8 [4.8, 23.8]	2 (25)§	1.0 [1.0, 1.0]
Fascial edema	13 (50)¶	9.0 [8.5, 63.0]	0 (0)¶	—
Muscle edema	13 (50)	2.0 [2.0, 3.2]	3 (38)	1.0 [1.0, 1.8]

*Comparison group subjects had no defined musculoskeletal disorder (n=2), undifferentiated connective tissue disease (n=4), or juvenile polymyositis (n=1), and included 1 carrier of Duchenne's muscular dystrophy. These subjects' STIR MRI scores were compared, by Fisher's exact test, with those from the first assessment of juvenile DM patients.
†The potential range of skin, subcutaneous, and fascial edema scores was 0-56. The potential range of muscle edema scores was 0-4.
‡$P \leq .001$.
§$P \leq .01$.
¶$P \leq .03$.
Source: Kimball *et al.* (2000).

Longer studies with larger numbers are required to understand the relevance of STIR MRI oedema in skin, subcutaneous tissue and fascia in juvenile DM.

Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies. II. The Childhood Myositis Assessment Scale (CMAS): a quantitative tool for the evaluation of muscle function.

D J Lovell, C B Lindsley, R M Rennebohm, *et al.* The Juvenile Dermatomyositis Disease Activity Collaborative Study Group. *Arthritis Rheum* 1999; **42**(10):2213–19.

BACKGROUND. Muscle weakness is a major feature of the idiopathic inflammatory myopathies (IIM) and to assess clinical status and adjust treatment, it is important to measure this accurately. The CMAS is a quantitative tool for assessing the severity of muscle involvement in children with IIM and this study determines its measurement characteristics, validity and reliability.

INTERPRETATION. The CMAS demonstrated an acceptable range of observed scores, excellent convergent validity, and excellent inter- and intrarater reliability. The CMAS is validated to assess quantitatively muscle function in the areas of strength and endurance in children with IIM. It can be used in routine clinical care as well as therapeutic trials.

Comment

Various methods have been used to assess muscle strength in children but there are problems assessing young children less than 5 years old, children who are too weak to carry out the assessment, or those with mild weakness. The CMAS is quantitative, non-invasive, and designed to assess proximal muscle strength, function, and endurance from age 2 to adulthood. It was developed from two existing functional assessment tools that had been used routinely in three paediatric rheumatology departments. The 14 items are ordinal variables ranked with standard performance and scoring methods. CMAS scores vary from 0 to 51, with higher scores indicating greater muscle strength (Table 8.4).

Twelve physicians received a training video and written instructions for using the CMAS and independently assessed 10 children with IIM (nine DM and one polymyositis, aged 4–15 years) twice on the same day. The CMAS was compared with quantitative manual muscle strength testing (MMT), performed twice on each child by a paediatric physiotherapist, the Juvenile Arthritis Functional Assessment Report (JAFAR), completed by parents and standard disease activity measures.

The mean score for the 10 patients was 36.4 (median 44) and this group had a broad range of disease severity, as shown by SD 14.1 and range of scores 5–51. Correlating CMAS scores with other clinical measures assessed convergent validity. CMAS correlated with physician's global assessment Visual Analogue Scale (r^2= 0.357, P=0.05), MMT score (r^2=0.88, P=0.001), serum CK (r^2=0.40,

Table 8.4 CMAS scoring sheet

1. Head elevation (neck flexion): Item score ___
   - 0 = Unable
   - 1 = 1–9 seconds
   - 2 = 10–29 seconds
   - 3 = 30–59 seconds
   - 4 = 60–119 seconds
   - 5 = ≥ 2 minutes

   No. of seconds ____
2. Leg raise/touch object: Item score ___
   - 0 = Unable to lift leg off table
   - 1 = Able to clear table, but cannot touch object
   - 2 = Able to lift leg high enough to touch object
3. Straight leg lift/duration: Item score ___
   - 0 = Unable
   - 1 = 1–9 seconds
   - 2 = 10–29 seconds
   - 3 = 30–59 seconds
   - 4 = 60–119 seconds
   - 5 = ≥ 2 minutes

   No of seconds ____
4. Supline to prone: Item score ___
   - 0 = Unable. Has difficulty even turning onto side; able to pull arms under torso only slightly or not at all.
   - 1 = Turns onto side fairly easily, but cannot fully free arms and is not able to fully assume a prone position.
   - 2 = Easily turns onto side; has some difficulty freeing arms, but fully frees them and fully assumes a prone position.
5. Sit-ups:

   For each type of sit-up enter either '0' (unable) or '1' (able). Then enter the total subscore (maximum possible item score 6).

   Hands on thighs, with counterbalance ____
   Hands across chest, with counterbalance ____
   Hands behind head, with counterbalance ____
   Hands on thighs, without counterbalance ____
   Hands across chest, without counterbalance ____
   Hands behind head, without counterbalance ____
6. Supine to sit: Item score ___
   - 0 = Unable by self.
   - 1 = Much difficulty. Very slow, struggles greatly, barely makes it. Almost unable.
   - 2 = Some difficulty. Able, but is somewhat slow, struggles some.
   - 3 = No difficulty.
7. Arm raise/straighten: Item score ___
   - 0 = Cannot raise wrists
   - 1 = Can raise wrists at least up to the level of the acromioclavicular joint, but not above top of head
   - 2 = Can raise wrists above top of head, but cannot raise arms straight above head so that elbows are in full extension
   - 3 = Can raise arms straight above head so that elbows are in full extension

8. Arm raise/duration: Item score ___
   Can maintain wrists above top of head for:
   0 = Unable
   1 = 1–9 seconds
   2 = 10–29 seconds
   3 = 30–59 seconds
   4 = 60–119 seconds
   5 = ≥ 120 seconds
   No. of seconds ____
9. Floor sit:
   Going from a standing position to a sitting position on the floor.
   0 = Unable. Afraid to even try, even if allowed to use a chair for support. Child fears that he/she will collapse, fall into a sit, or harm self.
   1 = Much difficulty. Able, but needs to hold onto a chair for support during descent. (Unable or unwilling to try if not able to use a chair for support.)
   2 = Some difficulty. Can go from stand to sit without using a chair for support, but has at least some difficulty during descent. Descends somewhat slowly and/or apprehensively; may not have full control or balance as maneuvers into a sit.
   3 = No difficulty. Requires no compensatory maneuvering.
10. All-fours maneuver: Item score ___
   0 = Unable to go from a prone to an all-fours position.
   1 = Barely able to assume and maintain an all-fours position.
   2 = Can maintain all-fours position with straight back and head raised (so as to look straight ahead). But, cannot creep (crawl) forward.
   3 = Can maintain all-fours, look straight ahead, and creep (crawl) forward.
   4 = Maintains balance while lifting and extending leg.
11. Floor rise: Item score ___
   Going from a kneeling position on the floor to a standing position.
   0 = Unable, even if allowed to use a chair for support.
   1 = Much difficulty. Able, but needs to use a chair for support. Unable if not allowed to use a chair.
   2 = Moderate difficulty. Able to get up without using a chair for support, but needs to place one or both hands on thighs/knees or floor. Unable without using hands.
   3 = Mild difficulty. Does not need to place hands on knees, thighs or floor, but has at least some difficulty during ascent.
   4 = No difficulty.
12. Chair rise: Item score ___
   0 = Unable to rise from chair, even if allowed to place hands on sides of chair seat.
   1 = Much difficulty. Able, but needs to place hands on sides of seat. Unable if not allowed to place hands on knees/thighs.
   2 = Moderate difficulty. Able, but needs to place hands on knees/thighs. Does not need to place hands on side of seat.
   3 = Mild difficulty. Able; does not need to use hands at all, but has at least some difficulty.

13. Stool step: Item score ___
    0 = Unable
    1 = Much difficulty. Able, but needs to place one hand on exam table or examiner's hand.
    2 = Some difficulty. Able; does not need to use exam table for support, but needs to use hand(s) on knee/thigh.
    3 = Able. Does not need to use exam table or hand(s) on knee/thigh.
14. Pick up: Item score ___
    0 = Unable to bend over and pick up pencil off floor.
    1 = Much difficulty. Able, but relies heavily on support gained by placing hand(s) on knees/thighs.
    2 = Some difficulty. Needs to at least minimally and briefly place hand(s) on knees/thighs for support and is somewhat slow.
    3 = No difficulty. No compensatory maneuver necessary.

Total score (maximum possible score 51) ___

Source: Lovell *et al.* (1999).

Table 8.5 Results for individual items of the CMAS

Item	Maximum possible score	Mean	Median	SD	Kendall's W*
1. Head elevation (neck flexion)	5	2.8	3	1.7	0.99
2. Leg raise/touch object	2	1.9	2	0.3	1.0
3. Straight leg lift/duration	5	3.2	3	1.7	1.0
4. Supine to prone	2	2.4	3	1.0	0.77
5. Sit-ups	6	1.7	1.5	1.7	0.99
6. Supine to sit	3	2.4	3	0.9	0.88
7. Arm raise/straighten	3	2.7	3	0.7	1.0
8. Arm raise/duration	5	3.2	4	1.3	1.0
9. Floor sit	3	2.1	3	1.2	0.93
10. All-fours maneuver	4	3.1	4	1.6	1.0
11. Floor rise	4	2.4	3	1.5	0.96
12. Chair rise	3	2.1	3	1.4	0.97
13. Stool step	3	1.6	2	0.8	1.0
14. Pick up	3	2.6	3	0.8	0.84
Total score	51	36.4	44	14.7	0.95

*Kendall's W (Kendall's coefficient of concordance) is used to measure interrater reliability on ordinal data. For each of the 14 individual items and for the total score, $P<0.001$.

Source: Lovell *et al.* (1999).

$P= 0.004$), total JAFAR score ($r^2=0.70$, $P<0.001$) and current prednisolone dose ($r^2=0.61$, $P=0.008$), but did not correlate with age of patient, aldolase or disease duration. Intrarater reliability was good, as shown by strong correlation of the morning and afternoon CMAS scores for each patient assessed by each physician. There was also good inter-rater reliability, for individual items and overall score (Table 8.5). The CMAS was easy to use and took only 10–15 min to complete. The nine physicians with no prior experience were no different to the three physicians with previous experience of the tools that CMAS was based on.

Work continues to assess longitudinal characteristics (sensitivity to change and predictive validity), reliability when used by non-physicians, and external validity (use in other clinics or other populations).

Conclusion for paediatric rheumatology

A common theme emerging from these papers is the need for specialist management of juvenile dermatomyositis and, consequently, the requirement for rapid access to paediatric rheumatologists—of which there is still a considerable shortage in many countries (e.g. UK). While the prognosis of this disease seems to have improved in the last two decades, this is attributed to earlier and more aggressive treatment; it is striking that patients treated late with methylprednisolone and MTX seemed to do less well than those treated early. Early aggressive treatment depends in turn on accurate and complete diagnosis using modalities such as MRI. This approach is only available in specialized centres, which can obtain the necessary experience and expertise. Just as important, such centres can ensure full documentation of long-term outcome, and acquire enough patients to carry out meaningful trials.

References

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Part IV

Connective tissue diseases

9
Systemic lupus erythematosus and the antiphospholipid antibody syndrome

Introduction

Nephropathy and central nervous system (CNS) involvement are among the most severe manifestations of systemic lupus erythematosus (SLE). A long-term follow-up of patients enrolled into a trial of plasmapheresis for lupus nephritis in the early 1980s suggested that a good renal outcome was associated with the ability to gain early renal remission even in patients with severe renal disease. Neuropsychiatric SLE is often difficult to recognize and diagnose. Several studies have looked at risk factors for neuropsychiatric SLE; the condition appears to be associated with anti-phospholipid syndrome (APS) and particularly arterial thrombosis and cutaneous vasculitis lesions. The differentiation of APS from multiple sclerosis can on occasion be difficult, even with the aid of magnetic resonance imaging (MRI); anticoagulation appears to be effective in the former condition.

Over the past decade there has been increasing recognition that SLE is a risk factor for atherosclerosis. Antimalarial drugs are commonly used to treat cutaneous and musculoskeletal involvement, but in addition have a modest lipid lowering effect, suggesting that most patients with SLE should be considered for antimalarial therapy. Other therapeutic developments include investigation into the usefulness of anti-interleukin (anti-IL)-10 monoclonal antibody (MoAb) in SLE, and intravenous immunoglobulin for fetal loss in APS.

Risk factors for central nervous system involvement in systemic lupus erythematosus.
F B Karassa, J P A loannides, G Touloumi, *et al. Q J Med* 2000; **93**: 169–74.

BACKGROUND. Neuropsychiatric (NPSLE) involvement is a major cause of morbidity and mortality in SLE. The aim of this study was to determine risk factors for NPSLE using an individually matched nested case-control approach in patients derived from a large cohort of SLE patients.

INTERPRETATION. NPSLE was associated with APS in particular with arterial thrombosis and cutaneous vasculitic lesions. It was negatively associated with articular manifestations and discoid rash.

Table 9.1 Selected clinical and laboratory characteristics of 32 NPSLE patients and 96 SLE patients without CNS involvement

Variables	NPSLE patients ($n = 32$)	SLE patients ($n = 96$)	p	Odds ratio*	95%CI
Clinical characteristics					
Antiphospholipid antibody syndrome	14 (44%)	13 (14%)	< 0.001	5.1	2.32–11.6
Arthralgias/arthritis	4 (13%)	79 (82%)	< 0.001	0.03	0.01–0.12
Discoid rash	1 (3%)	28 (29%)	< 0.001	0.007	0.02–0.3
Cutaneous vasculitic lesions	14 (44%)	12 (13%)	< 0.001	8.5	3.0–24
Livedo reticularis	13 (41%)	13 (14%)	0.002	4.3	2.0–9.2
Serosis	5 (16%)	28 (29%)	0.13	0.4	0.1–1.3
Arterial thrombosis	10 (31%)	5 (5%)	< 0.001	9.3	2.8–31
Venus thrombosis	4 (13%)	14 (15%)	0.67	0.8	0.4–1.9
Pregnancy loss**	7 (22%)	6 (6%)	0.004	6.0	1.8–20
Renal disease	14 (44%)	51 (53%)	0.20	0.7	0.4–1.2
Laboratory data					
Haemolytic anaemia	4 (13%)	6 (6%)	0.12	2.5	0.8–8.0
Leukopenia (< 3500/mm^3)	10 (31%)	16 (17%)	0.08	2.4	0.9–6.2
Thrombocytopenia (< 100.000/mm^3)	7 (22%)	0	< 0.001	—	—
Positive ANA	32 (100%)	81 (84%)	0.02	—	—
Positive anti-dsDNA	16 (50%)	57 (59%)	0.35	0.7	0.3–1.5
Positive anti-Sm	1 (3%)	10 (10%)	0.20	0.3	0.03–2.3
Positive anti-SS-A/Ro	12 (38%)	34 (35%)	0.83	1.1	0.5–2.7
Positive anti-SS-B/La	7 (22%)	8 (8%)	0.01	4.3	1.4–13
Positive anti-nRNP	2 (6%)	11 (11%)	0.40	0.5	0.11–2.5
Positive aCL IgM in high titre	5 (16%)	12 (13%)	0.56	1.3	0.6–2.7
Positive aCL IgG in high titre	12 (38%)	15 (16%)	0.001	4.5	1.9–10.9
Positive LA	6 (19%)	6 (6%)	0.29	1.8	0.6–5.4
C_3 < 53 mg%	8 (25%)	7 (7%)	0.003	3.8	1.6–9.4
C_4 < 20 mg%	16 (50%)	21 (22%)	< 0.001	3.5	1.8–6.8

Data are numbers (percentages). *Derived from conditioinal logistic regression analysis. **Results were similar regardless whether the analysis included all women (main analysis) or only women with a history of pregnancies. ACL, anticardiolipin; ANA, antinuclear antibodies; Cl, confidence interval; CNS, central nervous system; LA, lupus anticoagulant; NPSLE, neuropsychiatric systemic lupus erythematosus; SLE, systemic lupus erythematosus.
Source: Karassa *et al.* (2000).

Comment

Thirty-two patients with NPSLE as classified by the American College of Rheumatologists (ACR) case definitions for NPSLE were identified from a cohort of 324 SLE patients. Ninety-six individual matched (sex, age and disease

Table 9.2 Independent prognostic factors of CNS involvement in SLE

Variable	Odds ratio	95%CI	*p*
Arthralgias/arthritis	0.015	0.00–0.17	< 0.001
Arterial thromboses	13	0.82–220	0.001
Discoid rash	0.004	0.00–0.35	0.016
Cutaneous vasculitic lesions	33	1.5–720	0.028

CNS, central nervous system.
Source: Karassa *et al.* (2000).

duration) controls were obtained from this population. The most frequent NPSLE features were cerebrovascular disease (nine patients), seizures (eight), acute confusional states (six), psychosis (three), demyelinating syndrome (three) and cranial neuropathies (three). Twelve patients presented with multiple NPSLE syndromes. Patients with poorly defined cognitive dysfunction, anxiety disorders and subtle psychiatric manifestations were excluded. Conditional logistic regression analysis (Table 9.1) revealed that NPSLE was associated with APS, cutaneous vasculitic lesions, arterial thrombosis, thrombocytopenia, IgG anticardiolipin antibodies (ACA) and low complement levels. Arthralgias and discoid lesions were protective. These factors were analysed using a stepwise multiple conditional logistic regression model (Table 9.2); discoid rash and arthralgias remained protective factors while cutaneous vasculitic lesions and arterial thrombosis were independent risk factors. The pathology of NPSLE differs from cutaneous disease. NPSLE is a small vessel non-inflammatory vasculopathy, while chronic cutaneous SLE is a perivascular mononuclear cell infiltrate. This could explain the negative association between NPSLE and discoid lesions seen in this study.

Evaluation of predictive factors for neurocognitive dysfunction in patients with inactive systemic lupus erythematosus.
D D Gladman, M B Urowitz, D Slonim, *et al. J Rheumatol* 2000; **27**: 2367–71.

BACKGROUND. Cognitive dysfunction has been noted in patients with SLE in the absence of other evidence of disease activity. In this study patients with inactive disease were assessed using a range of neuropsychological and cognitive deficits.

INTERPRETATION. Patients with inactive SLE demonstrated neurocognitive dysfunction. This was associated with more disease activity at presentation but not with specific organ damage or involvement.

Comment

One hundred and thirty-nine potential subjects with inactive SLE (SLE disease activity index [SLEDAI]=0) were identified; however, only 58 were able to take

part. There was no difference in sex, age and organ involvement between those taking part and those who declined. Forty-seven controls were recruited from hospital staff. A panel of 10 neuropsychological assessments were administered, together with a Beck Depression Inventory, General Health Questionnaire-60, Barsky's Somatic Amplification Scale and a structured interview for past and present psychiatric disorders. Twenty-five of 58 SLE patients compared with nine of 47 healthy controls demonstrated neurocognitive dysfunction ($P<0.01$). Neurocognitive dysfunction was not associated with depression, psychiatric diagnosis, use of steroids or presence of fibromyalgia. A SLEDAI >10 at first presentation and previous vasculitis were associated with neurocognitive dysfunction; however, previous CNS disease, renal disease, or atherosclerosis were not predictive of neurocognitive dysfunction. Neurocognitive dysfunction is common in SLE patients with inactive disease; this could relate to previous active disease or vasculitis. The pathophysiological significance of these abnormalities is unknown.

Is there an association of malignancy with systemic lupus erythematosus? An analysis of 276 patients under long term review.

S M Sultan, Y loannou, D A Isenberg. *Rheumatology* 2000; **39**: 1147–52.

BACKGROUND. Malignancy has been associated with autoimmune rheumatic diseases, including rheumatoid arthritis, systemic sclerosis and SLE. The aim of this study was to estimate the risk of malignancy in a large cohort of SLE patients.

INTERPRETATION. There was no overall increased risk of malignancy, but there was an increased risk of Hodgkin's lymphoma compared with the general population. There was no statistical difference in cytotoxic therapy between those who developed malignancy and those who did not.

Comment

Two hundred and seventy-six patients with SLE were identified with a median follow-up of 4.8 years, and a total of 1695 patient years. Sixteen malignancies were observed in 15 patients; five before the diagnosis of SLE were excluded from the analysis. Compared with the general population there was no increased risk in the SLE cohort (standardized incidence rate 1.16; 95% confidence interval 0.55–2.13). Hodgkin's disease (one patient only) was associated with an increased incidence (standardized incidence rate 17.82; 95% CI 0.45–99.23). Treatment with azathioprine, hydroxychloroquine and corticosteroids did not constitute an additional risk. One of 49 patients treated with cyclophosphamide developed a malignancy; this was not a statistically increased risk.

Factors predictive of outcome in severe lupus nephritis.
S M Korbet, E J Lewis, M M Schwartz, *et al.,* for the Lupus Nephritis Collaborative Study Group. *Am J Kidney Dis* 2000; **35**: 904–14.

BACKGROUND. Nephritis is one of the more severe manifestations of SLE and is associated with significant morbidity. Remission can be induced in many patients, but factors predictive of this are uncertain. This study was a long-term follow-up of patients treated with apheresis for severe lupus nephritis during 1981–88.

INTERPRETATION. Renal remission was associated with improved survival. Predictive factors for renal remission included stable serum creatinine 4 weeks after therapy, category IV lesions on renal biopsy, lower chronicity index, white ethnic origin, lower baseline serum creatinine and lower urine protein excretion. Factors predictive of end-stage renal disease were higher baseline creatinine, anti-Ro antibodies and failure to attain remission.

Comment

Eighty-six patients were originally enrolled in a trial of plasmapheresis in addition to prednisolone and cyclophosphamide, which had shown no difference between the two arms |**1**|. The follow-up was extended to a median of 120 months. Renal remission was defined as creatinine <123 μmol/l and proteinuria <330 mg/ 24 hours. This occurred in 37 of 86 (43%) patients. Survival was 95% at 10 years in the remission group and 60% at 10 years in no-remission group. Renal survival was 94% at 10 years in the remission group and 31% in the no remission group. The patients entered into the trial all had severe renal disease with proliferative changes and/or necrosis in >75% of glomeruli. Patients with diffuse proliferative glomerulonephritis (PGN) had a better outcome. Patients even with severe disease can have a good outcome provided a clinical remission can be induced.

Table 9.3 Baseline histological features

	All	**Remission**	**No remission**	***P****
No. of patients	86	37	49	
Biopsy (category)				
III (> 50%)	24	9	15	NS
IV	35	21	14	< 0.05
Vc (> 50%), Vd	26	7	19	0.06
Unclassified	1		1	NS
Activity index	12.0 ± 4.7	11.8 ± 4.2	12.1 ± 5.0	NS
≥ 12	42	16	26	NS
Chronicity index	3.4 ± 2.5	2.3 ± 1.8	4.3 ± 2.6	< 0.001
≥ 4	41	10	31	< 0.001

NOTE. Values reported as number or mean ±SD. Abbreviation: NS, not significant.
*Remission versus no remission.
Source: Korbet *et al.* (2000).

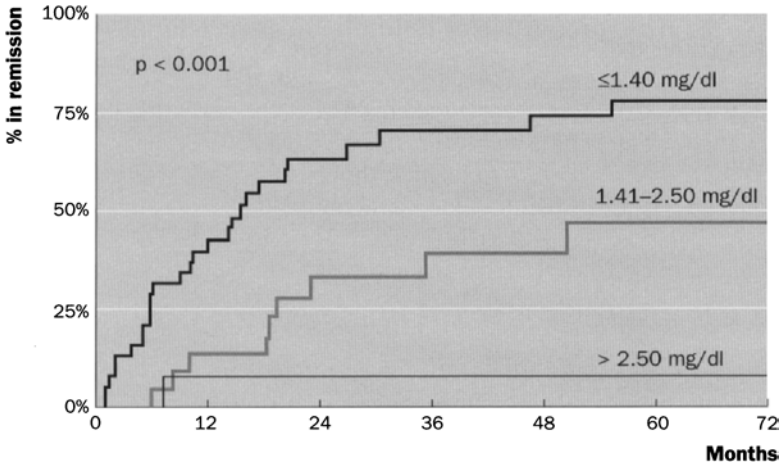

Fig. 9.1 Proportion of patients entering into remission based on serum creatinine level at baseline. At each interval, the number of patients who entered into remission is noted (patients reaching end-stage renal disease or death were censored). Serum creatinine level less than 1.4 mg/dl (*n*=40), 1.41–2.5 mg/dl (*n*=27) and greater than 2.5 mg/dl (*n*=19). Source: Korbet et al. (2000).

Effect of anti malarial agents on the fasting lipid profile in systemic lupus erythematosus.
L S Tam, D D Gladman, D C Hallett, *et al. J Rheumatol* 2000; **27**:2142–5.

BACKGROUND. Premature atherosclerosis is recognized as a major cause of mortality in SLE. Hyperlipidaemia is an important risk factor. The aim of this study was to ascertain the relative effect of antimalarial drugs (AM) on the fasting lipid profile in SLE.

INTERPRETATION. Total cholesterol, very low density lipid-cholesterol (VLDL-C), and low-density lipid-cholesterol (LDL-C) were lower in patients taking AM, including those taking prednisolone. AM may, therefore, have benefits in addition to disease suppression in patients with SLE.

Comment

One hundred and twenty-three patients were studied with a mean age of 45.3 years and disease duration of 13.4 years. Ninety patients (73.2%) were taking prednisolone, 59 patients (48.0%) were taking AM (34 hydroxychloroquine and 25 chloroquine). Over the whole group, patients taking AM had a 12.5% lower total cholesterol (5.11±1.27 versus 5.84±1.23; *P*=0.002), 22.1% lower VLDL-C (0.66±0.40 versus 0.85±0.39; *P*=0.01) and 15.7% lower LDL-C (3.01±1.14 versus 3.58±1.10; *P*=0.007). Patients (38) taking prednisolone with AM had lower total cholesterol (5.26±1.30 versus 5.99±1.29, *P*=0.01), VLDL-C (0.65±0.

39 versus 0.85±0.41, *P*=0.02) and LDL-C (3.05±1.20 versus 3.69±1.09; *P*=0.01) than those not taking AM (48 patients). Similar results were seen in patients taking low dose (<10.0 mg/day prednisolone) and AM. The study did not correct for other factors that affect lipid levels—diet, exercise, alcohol use and family history. Random total cholesterol may be sufficient routine monitoring in patients with SLE. The long-term clinical significance of the effect of AM on mortality and morbidity in SLE is unknown.

Cyclosporine for lupus membranous nephritis: experience with ten patients and review of the literature.
D Hallegua, D J Walace, A L Metzger, *et al. Lupus* 2000; **9**:241–51.

BACKGROUND. Progression to end-stage renal failure occurs in 10–25% of cases of lupus membranous nephropathy, a worse prognosis than for other forms of lupus nephritis in some studies. While this is not the first trial of cyclosporin in lupus renal disease, the study gives further insight into the potential benefits and risks of this treatment option.

INTERPRETATION. Ten patients are reported in a retrospective study, including a follow-up of 1–5 years (mean 2 years). All had clinical nephrotic syndrome and biopsy proven lupus membranous nephropathy, which was resistant to oral steroid treatment. Doses of 2.2–6 mg/kg per day were used, adjusted according to adverse effects and response. Remission off treatment was achieved in only one patient, but proteinuria, systemic markers and prednisolone dose were reduced in all patients. Serum creatinine was elevated on cyclosporin in all cases, although this could be controlled with dose adjustments. Hypertension proved problematic, requiring one or more drugs for adequate control.

Comment

A brief review of the literature sets the background for this study, showing the relatively poor outcome in this type of renal lupus and the lack of a clear treatment advantage with existing options (mainly prednisolone, azathioprine and cyclophosphamide). While cyclosporin is potentially nephrotoxic, it is also a useful immunosuppressive therapy. This study, therefore, is valuable in that it clarifies that renal disease can be treated with cyclosporin and that the nephrotoxicity (elevations in serum creatinine and blood pressure) can be managed separately without losing efficacy. It is perhaps disappointing that remission was not achieved in more cases, and that cyclosporin could only be discontinued in one patient. The reduction in prednisolone dose, from a mean of 28 mg to a mean of 7 mg is clinically important particularly in the context of accelerated atherosclerosis associated with lupus and with nephrotic syndrome. Unfortunately, no indication is given as to how soon after introducing cyclosporin prednisolone reductions began, nor how quickly doses were tapered.

Therefore, the possibility that the observed treatment effect applies only to combined cyclosporin with prednisolone must be considered. The authors' claim, that cyclosporin is 'the most effective' drug in controlling lupus membranous nephropathy, is not justified by their study, particularly as more aggressive regimens of immunosuppressive therapy might also show reduced proteinuria and allow steroid reductions. Furthermore, the treatment period analysed is retrospective, and other factors that might adversely or beneficially affect the outcome are not considered—a prospective trial does seem justified. The authors also recognize the fact that we do not yet know if controlling proteinuria will translate into a better prognosis overall, although this does appear to be the case when proteinuria in diabetic nephropathy is reduced using angiotensin-converting enzyme inhibitors.

Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis.
T M Chan, F K Li, C S Tang, *et al.,* Hong Kong-Guangzhou Nephrology Study Group. *N Engl J Med* 2000; **343**:1182–3.

BACKGROUND. Proliferative lupus nephritis remains a leading cause of morbidity and mortality in SLE. While steroids with cyclophosphamide or azathioprine are quite effective, advances that minimize the considerable treatment-associated morbidity are welcome.

INTERPRETATION. Patients (n=42) with established proliferative lupus nephritis were given a tapering dose of prednisolone (initial dose 0.8 mg/kg per day) with randomization to additional 1 g twice daily mycophenolate mofetil (group 1) for 12 months, or to 6 months cyclophosphamide (2.5 mg/kg per day) changing to azathioprine (2.5 mg/kg per day) in the second 6 months (group 2).

Complete remission was rigorously defined by renal and systemic parameters, with clinically relevant improvements scored as partial remission. There was no significant difference between treatments in the proportions achieving either outcome (81% complete and 14% partial remission, versus 76% and 14%, for groups 1 and 2, respectively). Relapse (occurring during the ninth month) affected 15% and 11% respectively. However, recorded adverse effects (infection, amenorrhoea, alopecia, leucopenia) were more common in the conventional treatment group and included two deaths.

Comment

Mycophenolate mofetil inhibits purine synthesis and is a relatively specific inhibitor of T and B cell proliferation. It has previously been reported in lupus as a steroid-sparing therapy. Potential advantages include reduced adverse effects, particularly gastrointestinal intolerance. This does appear to be the case, with only one patient having to discontinue mycophenolate because of diarrhoea. The rate of infection was also considerably lower in the mycophenolate group (19% versus 33%). This is one of the most important complications of aggressive

immunosuppressive therapy—one of the two fatalities was a result of miliary tuberculosis (despite isoniazid prophylaxis in at-risk cases).

A number of exclusion criteria applied in this study. In particular, those with serum creatinine >300 μmol/l were excluded. Furthermore, it is known that 25% with class IV proliferative lupus nephritis progress to end-stage renal failure within 5 years of diagnosis. The mean duration of nephritis in these groups was 54 and 77 months, suggesting they represent a more benign end of the spectrum. Therefore, the efficacy of mycophenolate in treating patients with more severe renal disease is still uncertain. The relapses reported occurred in the first year of therapy. The authors state this was during the maintenance phase of treatment. While it is true that the steroid dose was reduced to 10 mg during the first 6 months, and that group 2 changed to azathioprine at this point, group 1 continued the same dose of mycophenolate throughout the 12 months, before changing to azathioprine. The study was not powered to detect a statistically significant difference in relapse rates, and it will be of interest to note whether a higher rate follows the change to azathioprine at the end of the first 12 months in group 1.

Osteopenia in young hypogonadal women with systemic lupus erythematosus receiving chronic steroid therapy: a randomised controlled trial comparing calcitriol and hormonal replacement therapy.

A W C Kung, T M Chan, C S Lau, *et al. Rheumatology* 1999; **38**(12): 1239–44.

BACKGROUND. While guidelines have been published for the management of corticosteroid-induced osteoporosis, SLE is complicated by the issue of oestrogen use. This paper is of value because of the insights it offers in this regard.

INTERPRETATION. Treatment: calcitriol (0.5 mg/day or hormone replacement therapy (HRT; 6.25 mg conjugated oestrogen/day) +1 g calcium. In a cohort of Chinese women taking at least 10 mg of prednisolone randomized to one of the above treatments, lumbar spine bone mineral density (BMD) improved significantly over 2 years on HRT compared with a fall on the vitamin D preparation. Cortical bone sites (distal radius and neck of femur) did not change significantly. While adverse effects on SLE activity are not reported in detail, there was no apparent deterioration in disease over the study period.

Comment

In their introduction, the authors raise the concern about using bisphosphonate therapy in skeletally immature women. However, these women were 31–43 years of age, where the issue is clearly not relevant to their own skeleton—furthermore, they were post-menopausal, so effects on fetal skeletal development are also not relevant. It is not clear why these women had been an average of 130 months on prednisolone without bone prophylaxis. As these women had

osteopenia (rather than osteoporosis) despite such a long period on steroid therapy, this cohort has been selected as a particularly low-risk group. Extrapolation of data to other populations is also complicated by the fact that these were all Chinese women. Differences in their responsiveness to vitamin D preparations have previously been reported, and on average they have a lower BMD than their European age-matched counterparts. That said, the finding that calcitriol is of marginal value (possibly reducing the rate of bone loss, although there is no placebo group in the study) is not new, and few would argue that this is a first-line treatment for corticosteroid-induced osteoporosis in uncomplicated cases. Considering the Eastell *et al.* |**2**| guidelines for corticosteroid-induced osteoporosis (barely discussed by the authors, perhaps because of the ethnic differences), a comparison with a bisphosphonate would have been more relevant. The main issue is whether, in SLE, premature ovarian failure is a significant contributory factor making HRT a more appropriate choice over a bisphosphonate, and whether this carries significant risk of exacerbating SLE as has previously been suggested. It is a pity that the authors did not use an index such as the SLEDAI to assess objectively any impact of HRT on disease. Presumably, these women had more than minimal disease given the steroid requirement, and it is certainly reassuring that their disease did not appear to flare under the influence of HRT.

Clinical and biological effects of anti-interleukin 10 monoclonal antibody administration in SLE.
L Llorente, Y Richaud-Patin, C Garcia-Padilla, *et al. Arthritis Rheum* 2000; **43**:1790–800.

BACKGROUND. Patients with SLE produce large amounts of IL-10, and increased levels of IL-10 correlate with disease in SLE. B-N10, a neutralizing MoAb to IL-10, has been demonstrated to reduce lupus activity in the NZB/W mouse model.

INTERPRETATION. Six patients with steroid-dependent SLE (despite chloroquine and immunosuppressant therapy) received daily infusions over 3 weeks of a MoAb to IL-10. Clinical disease parameters as measured by the SLEDAI score improved considerably at the end of the treatment period in five of six patients, with further improvements or stable course over the next 6 months. Biological markers (antibodies to double-stranded DNA [dsDNA], levels of C3 and C4) did not change, although other markers (serum IL-1 receptor antagonist, serum tumour necrosis factor-receptor p75) did reduce. The only reported adverse effect was a serum-sickness-like reaction in one patient at day 16. Prednisolone requirement was reduced by at least 50% in all (mean 27.9–9.6 mg/day), with further reductions in immunosuppressive therapy in three, and increased or changed in two.

Table 9.4 Patients' clinical features at the beginning and end of B-N10 administration and at the end of the follow-up period

Patient, time point	Cutaneous involvement	Joint involvement	Vasculitis	Leuko/ lymphopenia	Fever	Serositis	Malaise
1							
Day 1	+	+	−	+	+	−	−
Day 21	−	−	−	+	−	−	−
Month 6	−	−	−	−	−	−	−
2							
Day 1	+	+	+	+	−	−	+
Day 21	−	−	−	+	−	−	−
Month 6	−	−	−	+	−	−	−
3							
Day 1	+	+	+	−	−	+	+
Day 16†	−	+	−	−	−	−	+
Month 6	−	−	−	−	−	−	−
4							
Day 1	+	+	+	+	+	−	+
Day 21	+	+	−	+	−	−	−
Month 6	+	+	−	+	−	−	−
5							
Day 1	+	+	+	+	+	−	+
Day 21	+	+	−	+	−	−	−
Month 6	−	+	−	−	−	−	−
6							
Day 1	+	+	−	+	+	−	−
Day 21	−	+	−	+	−	−	−
Month 6	−	−	−	−	−	−	−

†Day 16 was the last day of B-N10 administration for patient 3.
Source: Llorente *et al.* (2000).

Comment

This is a small, open-label study, which is to some extent inevitable when a new therapy is restricted to a cohort with resistant disease. Using objective measures together with a validated clinical assessment allows thorough evaluation even with small numbers. The study has also followed the patients for a reasonable period, which suggests that this treatment has a lasting benefit. Having said that, most of those who noted a resolution of disease in one or more categories of the SLEDAI had improved by the end of the treatment period, with five of the 12 remaining active disease parameters still present at 6 months. There was no single organ that did not respond in at least one patient, which is perhaps significant in terms of disease pathogenesis. It is interesting to note, in this respect, that the standard markers of disease activity did not change with the treatment. The authors report that disease was clinically inactive in five of the six patients—one would expect, therefore, to have seen a more substantial reduction

in the need for immunosuppressive therapy. Certainly, the reduction in steroid dose is the most important target in the first instance—it will be important to see if the clinical stability can be maintained in the face of reduced immunosuppressive therapy.

All patients formed antibodies to the B-N10 (present in five of the six patients by day 30). Whether these would attenuate the effects of the treatment if repeated, or whether the antibodies are short-lived is unclear—it is also likely that, in the absence of the other immunosuppressive drugs these patients were taking, antibodies might form more quickly (as occurs in patients treated with infliximab without concomitant methotrexate).

Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase 1 study.
A Traynor, J Schroeder, R M Rosa, *et al. Lancet* 2000; **356**:701–7.

BACKGROUND. In severe autoimmune disease, the use of high-dose (immunoablative) chemotherapy, with or without 'rescue' stem cell transplantation, is gaining prominence |3|. This continues to be a high-risk approach, and studies of the efficacy and safety are urgently required.

INTERPRETATION. Nine patients with severe, life-threatening SLE (predominantly renal, pulmonary in two, all of whom had been treated with conventional dose cyclophosphamide and who continued to require high-dose prednisolone) underwent stem cell mobilization (using cyclophosphamide and granulocyte-colony-stimulating factor). Of these, seven went on to have immunoablative doses of cyclophosphamide (200 mg/kg over 4 days) together with antithymocyte globulin and a comprehensive prophylactic antimicrobial regimen. All seven have survived to at least 1 year, with two followed for over 3 years, showing remission of disease, including those elements that were life-threatening for the individual patient. Infective complications did arise but responded to standard management, and ventilation and/or dialysis/hyperfiltration were required to manage fluid overload in three patients.

Comment

In further investigations, the authors show that the pre-treatment repertoire—restriction, hyper-responsiveness, and T_H2 dominance of circulating T-cell population—were normalized post-treatment. This, together with the prolonged remission in these patients, suggests that the approach of high-dose cyclophosphamide and stem cell transplantation may be having effects other than profound immunosuppression. Therefore, strategies of immunoablative therapy in which the high risk of infective complications are managed without stem cell transplantation are not necessarily comparable. As illustrated by these patients, the risks of this kind of treatment are high, even with the high-intensity prophylactic antibiotics and posttransplant care. However, well-carried out

studies of this nature will clarify how this apparently promising treatment can most appropriately be used in a disease that continues to carry serious morbidity and mortality in a small proportion.

The prevalence of antibodies to anionic phospholipids in patients with the primary antiphospholipid syndrome, systemic lupus erythematosus, and their relatives and spouses.

E L Radway-Bright, C T Ravirajan, D A Isenberg. *Rheumatology* 2000; **39**: 427–31.

BACKGROUND. Antiphospholipid antibodies (aPL) are associated with a characteristic primary syndrome (APS), but are also seen in SLE and in apparently normal individuals. The debate on the relative importance of genetic and environmental factors in the aetiology of SLE and APS continues. This study investigated the prevalence of various immunotypes of antiphospholipid antibody and explored the effect of genetics and environment.

INTERPRETATION. As might be anticipated, the prevalence of APL antibodies of IgG and IgM classes was highest in those with the primary syndrome, followed by those SLE patients with secondary APS (SLE/APS). However, there was an unexpectedly high level of an IgA antiphosphotidylinositol in SLE/APS patients. First degree relatives (only investigated in those with SLE/APS) had higher levels of IgG class antibodies to phosphotidylinositol and anticardiolipin, while the levels of all types of antibody in spouses' sera were comparable with the normal population studied.

Comment

The association between risk of thrombosis and presence of antibodies is most clearly established for the IgG class of antiphosphotidylinositol and ACA. The reasons for investigating the other anionic phospholipids, in the absence of clinical details with which to correlate any differences that might appear is not explained, nor is the relevance of detecting IgA antibodies. The authors, therefore, are investigating without any working hypothesis. With regard to the comparison between relatives and spouses, the risk of a type II error (falsely accepting the null hypothesis because the study population is too small) is considerable. As the ages and duration of cohabitation/marriage are not disclosed, it is possible that an environmental trigger is being overlooked, or that an environmental factor to which the person is exposed before marriage and cohabitation is being overlooked. It is perhaps too glib, therefore, to conclude that this study rules out the possibility of an environmental factor being relevant.

Can neurologic manifestations of Hughes (antiphospholipid) syndrome be distinguished from multiple sclerosis? Analysis of 27 patients and review of the literature.
M J Cuadrado, M A Khamashta, A Ballesteros, *et al. Medicine* 2000; **79**:57–68.

BACKGROUND. Neurological events are common in APS. Clinical syndromes mimicking multiple sclerosis (MS) have been reported as occurring in association with APS, SLE and Sjögren's syndrome. The aim of this study was to identify features that would distinguish the two conditions.

INTERPRETATION. Previous history of thrombosis and/or fetal loss, abnormal localization of lesions on MRI, and response to anticoagulant therapy were helpful in distinguishing the conditions.

Comment

Twenty-seven patients with a diagnosis of possible or definite MS together with possible connective tissue disease, aPL, atypical progression for MS, or atypical MRI were included in the study. They were compared with 25 patients with definite MS without aPL. There were no differences in neurological symptoms between MS and APS patients. Overall, MRI in MS patients showed more severe white matter disease, but in individual scans it was difficult to distinguish MS from APS. Laboratory findings were no different. Patients with primary APS responded better to anticoagulation.

Prognostic factors and clustering of serious clinical outcomes in antiphospholipid syndrome.
M G Tektonidou, J P A loannidis, K A Boki, *et al. Q J Med* 2000; **93**: 523–30.

BACKGROUND. The APS is associated with potentially poor outcome. The aim of this study was to correlate outcome with initial presentation in a cohort of patients under long-term follow-up.

INTERPRETATION. The risk of a second APS-related event was greatest in patients with anti-β2 glycoprotein I antibodies who had autoimmune haemolysis as the first event. Initial clinical features determine the long-term evolution of APS.

Comment

Eighty-two consecutive patients with ACA or lupus anticoagulant (LAC) were followed for 814 person-years after a first event suggestive of APS (livedo reticularis, haemolytic anaemia, thrombocytopenia, CNS manifestations). Mean age at presentation was 27.8 years; at presentation 34 patients had SLE. The median time to a second event was 3 years (mean 5.54 years). In an univariate Cox regression, the hazard ratio for progression was 1.95 (P=0.04) when the

presenting event was haemolytic anaemia and 0.54 (P=0.05) for recurrent fetal loss. Using multivariate analysis independent factors for progression were haemolytic anaemia and the presence of anti-β2 glycoprotein I antibodies. Subsequent serious events for patients with venous and arterial thromboses, recurrent fetal loss, CNS manifestations and autoimmune haemolysis were likely to be of the same type as the initial representation. Presentation with two events was associated with a worse outcome.

Risk factors for avascular necrosis of bone in patients with systemic lupus erythematosus: is there a role for antiphospholipid antibodies.

M Y Mok, V T Farewell, D A Isenberg. *Ann Rheum Dis* 2000; **59**:462–7.

BACKGROUND. Avascular necrosis (AVN) is a well-recognized complication of SLE. The aim of this study was to investigate whether aPL and/or LAC were risk factors for the development of AVN.

INTERPRETATION. No association was observed, following adjustment for corticosteroid use, between aPL and AVN.

Comment

Eleven patients with AVN were identified from a cohort of 265 SLE patients. Two control groups were used: (a) 22 SLE patients matched for disease duration, organ involvement, age, sex and ethnicity, and (b) 31 randomly selected unmatched SLE patients. Comparison was made using conditional logistical regression for both IgG and IgM aPL and LAC. Adjustment was made for corticosteroid use, although there was no difference in total cumulative steroid use between the AVN patients and the controls. The study fails to reveal a role for aPL in the development of AVN.

A multicentre, placebo controlled pilot study of intravenous immune globulin treatment of antiphospholipid syndrome during pregnancy.

D Ware Branch, A M Peaceman, M Druzin, et *al. Am J Obstet Gynecol* 2000; **182**:122–7.

BACKGROUND. APS is characterized by fetal loss, arterial and venous thrombosis, and thrombocytopenia. Conventional therapy for the prevention of fetal loss is heparin and low-dose aspirin. Despite this, there remains a high rate of pre-eclampsia, intrauterine growth retardation and poor neonatal outcome. Anecdotal evidence suggests that intravenous immunoglobulin (IVIg) may reduce these obstetric complication. This study was designed to explore the feasibility of a multicentre placebo-controlled trial.

INTERPRETATION. IVIg did not improve obstetric or neonatal outcome beyond those achieved with heparin and low-dose aspirin. There was no statistically significant reduction in fetal growth retardation.

Comment

This was a small study of 16 women; seven received IVIg and nine placebo. The negative finding might reflect the small size of the trial, but suggests that IVIg does not provide clinically significant benefit in addition to heparin and low-dose aspirin. A large multicentre trial is required to answer this question. At present IVIg cannot be recommended for the initial treatment of APS patients at high risk of fetal loss.

Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains.
L Morel, B P Croker, K R Blenman, *et al. Proc Natl Acad Sci USA* 2000; **97**: 6670–5.

BACKGROUND. Genetic predisposition is a factor in the development of SLE, although the primary immunopathogenic defects responsible for the initiation and progression of the disease remain poorly understood.

INTERPRETATION. Three critical loci, termed *Sle1, 2* and *3,* have been identified on chromosomes 1, 4 and 7, respectively. Mouse strains were developed combining each of these loci with the autoimmunity accelerating gene *Yaa,* and others with each combination of two, or all three, loci. The resulting phenotype of renal pathology, autoantibody expression and of lymphocyte activation was characterized. Briefly, *Sle1* was the key locus, resulting in fatal PGN when present in any combination, or with *Yaa*. *Sle3* appeared to result in dysregulation of T cells leading to the recognition of autoantigens, which, together with a lower activation threshold in B cells associated with *Sle2,* results in the expression of autoantibodies (including to glomerular basement membrane and dsDNA).

Comment

This study builds on previous work by these authors on congenic strains, in which a susceptibility locus is carried on a resistant background strain (C57BL/6). The loci were first identified by linkage analysis, and this is perhaps the weak link in the line of argument by which the authors propose their data satisfies a genetic form of Koch's postulates. Indeed, they acknowledge this, recognizing that the locus termed *Sle3* in fact comprises two loci (*Sle3* and *5*), and that both *Sle1* and *2* correspond to several loci with overlapping but distinct phenotypes. Their observations, therefore, do not exclude the possibility that further loci in linkage disequilibrium with the *Sle* loci are critical 'co-factors'.

PGN was only found in animals with the *Sle1* locus alone or in combination, whereas *Sle2/3* resulted in a non-fatal hyaline deposition, which is also seen in NZW mice from which the monocongenic strains were first developed. Interestingly, NZW mice have been found to have a suppressive allele, *Sles1*. While *Sle1* was required to produce PGN, there was no difference in the levels of antichromatin antibodies expressed in any of the polycongenic strains. This might again imply that other loci are involved, by undetermined mechanisms. Spleen size was used as a marker of lymphocyte activation. While the authors postulate that *Sle2* and *3* are most involved in T- and B-cell dysfunction, splenomegaly was greatest in the strains expressing *Sle1* with Yaa, with *Sle3,* or all three *Sle* loci.

These observations led the authors to conclude that *Sle1* is a critical initiation defect, perhaps breaking immune tolerance (compounded by *Yaa,* which, when expressed in B cells, leads to low-affinity T-cell/B-cell interactions). They propose a 'multistep' process, although this is not truly supported by their data, in which each genetic defect was present simultaneously or not at all. A 'two-hit' hypothesis, a paradigm from oncology, might be more consistent with the evidence, in which the accumulation of genetic defects, rather than the order in which they occur, is the essential genetic 'burden'; although some or all of the defects might be acted on by environmental triggers.

Rapid lupus autoantigen relocalisation and reactive oxygen species accumulation following ultraviolet irradiation of human keratinocytes.

W Lawley, A Doherty, S Denniss, *et al. Rheumatology* 2000; **39**: 253–61.

BACKGROUND. SLE is associated with exposure to ultraviolet (UV) radiation. In addition, the disease is characterized by the presence of autoantibodies to a number of intracellular antigens. This study further investigates the relation between these factors.

INTERPRETATION. Using keratinocytes in an *in vitro* investigation, the localization of the Ro and La antigens before and after exposure to UV radiation was determined, together with the uptake of a marker for reactive oxygen species. Under different conditions, Ro, La, Sm and U2-B translocated from the nucleus to the cytoplasm, or to the surface in apoptotic blebs. Reactive oxygen species were also consistently formed after a short UV exposure.

Comment

Though conducted *in vitro,* the UV dose used approximated to the dose received from normal sunlight exposure. The relevance of the results to the aetiology of SLE is somewhat more controversial; Sm is the antigen most strongly associated with SLE of those investigated, while anti-DNA antibodies are most useful in following the course of disease. It would be useful to follow this study with *in*

vivo UV exposure in experimental animals to determine whether expression of antigens on the cell surface in the context of apoptotic blebs is immunogenic, and whether this leads to the appearance of SLE-like disease manifestations. Similarly, the relevance of reactive oxygen species accumulation in the cell is unclear, although it does suggest the possibility of investigating further antioxidant therapeutic approaches in lupus.

The cell-cycle inhibitor p21 controls T-cell proliferation and sex-linked lupus development.
D Balomenos, J Martin-Caballero, M I Garcia, *et al. Nature Med* 2000; **6**(2): 171–6.

BACKGROUND. Various animal models exist for the study of lupus. A mouse deficient in p21, a critical cell proliferation regulator was not previously known to develop lupus. These authors have investigated the effects of p21 deficiency in more detail.

INTERPRETATION. Comparing male and female p21–/– versus wild-type mice, the authors examined various stages of T-cell proliferation, accumulation of memory T cells, and autoantibody development against dsDNA and nuclear antige ns. They demonstrate that p21 deficiency may be a critical factor in T-cell regulation and immune tolerance, and that female mice develop a number of features characteristic of human lupus, including a severe GN.

Comment

This is the first time that the p21–/– mouse was reported to develop lupus. The authors believe this is because the animals were followed for longer than previously. They have used reliable and validated methods to investigate T-cell functioning, and the aspects of self-antigen recognition and the autoantibodies studied are highly relevant to lupus. The finding of a severe GN only in the female mice is striking. This is not explored any further in this study. The authors may be criticized for their assumption in the discussion section that the gender difference is due to the ‘exacerbation of the tolerance defect’ by the female hormonal environment, particularly as there was no gender difference in production of IgG, and the increased (relative to wild-type) production of dsDNA antibodies was not restricted to females. Nonetheless, the paper offers a new insight into the pathogenesis of SLE; it is interesting that their findings converge with other studies in that the gene for p21 maps within one of the susceptibility loci recently identified in NZB×NZW mice and in humans.

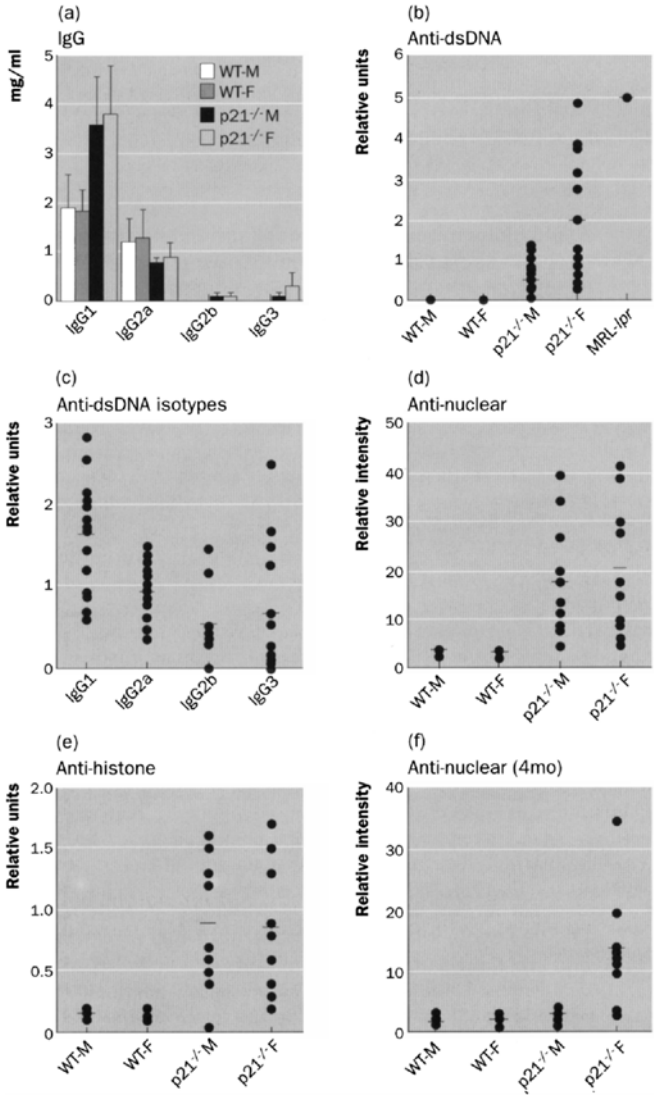

Fig. 9.2 Total IgG and autoantibody profiles. Mice were 9–12 months old unless otherwise specified. (a) Serum concentrations of IgG isotypes. Data represent the mean ± SD, *n*=15. (b) Serum concentrations of IgG antibody against dsDNA (*n*=15). For MRL-lpr mice, a serum pool from five 4-month-old mice was tested. (c) Concentrations of anti-dsDNA IgG isotypes of 15 p21–/– F mice. (d) Indirect immunofluorescence intensity of serum reactivity against antinuclear autoantibodies using fixed Hep-2 cells (*n*=10). (e) Antihistone antibody concen trations measured by enzyme-linked immunosorbent assay (*n*=10). (f) Immunofluorescence intensity of serum reactivity from 4-month-old mice against Hep-2 cells (*n*=10). Source: Balomenos *et al.* (2000).

A novel function of B-lymphocytes from normal mice to suppress autoimmunity in (NZB×NZW)F_1 mice.
S Ono, D-Z Shao, S Yameka, *et al. Immunology* 2000; **100**:99–109.

BACKGROUND. Polyclonal B-cell activation is thought to be the main cause of the immune-complex-deposition induced GN in NZB/W lupus-prone mice. GN has been transferred to irradiated normal mice by marrow cells from NZB/W mice, but could be prevented by simultaneously co-transferring normal spleen cells. This is further explored here.

INTERPRETATION. Spleen cells from normal mice were prepared, removing T cells and macrophages in a standard way. These were then transfused into first progeny NZB/W mice after the onset of autoimmune disease, on three occasions 2 weeks apart (weeks 6, 8 and 10 or 16, 18 and 20). Renal disease was ameliorated in the treated animals, associated with the reduced expression of IL-12. The effect continued for up to 30 weeks after treatment, and was more apparent in the animals treated in the delayed (weeks 16, 18 and 20) protocol.

Comment

This study confirms the previous observation that normal cells can ameliorate autoimmune disease expression, and takes the idea further by transferring the cells into inherently lupus-prone mice (rather than the previous co-transfer of pathogenic and normal cells into naïve hosts). Various possible mechanisms are discussed, the authors favouring a Fas/Fas ligand interaction. An alternative proposal is that the normal B cells act as non-activating autoantigen presenting cells, thereby enhancing immune tolerance (instead of the B cells from the lupus-prone animal that act as T-cell activating autoantigen presenting cells). With respect to the genetic study described above (Morel *et al.* 2000), the authors note that the suppressive allele *Sles1* is expressed in the normal cells, compared with expression of disease-associated *Sle1* in the B cells of NZB/W mice. It is interesting that the delayed protocol was more effective, perhaps facilitating a more complete deletion of autoreactive T-cell clones that are not fully expressed in the first 10 weeks after autoimmunity is first recognized.

The findings are perhaps of greatest interest with regard to stem cell transplantation for SLE and other autoimmune diseases, in which the relative contribution of transplanted stem cells to disease suppression, as opposed to merely reducing infective complications of immunoablative therapy, is debated. This paper would suggest that normal cells may have a definite therapeutic role.

Cutting edge—a role for CD1 in the pathogenesis of lupus in NZB/NZW mice.
D Zeng, M-K Lee, J Tung, *et al. J Immunol* 2000; **164**:5000–4.

BACKGROUND. T cells are particularly important in the transition from IgM to IgG production by B cells. CD1 is a non-major

histocompatibility complex (MHC) molecule, which is involved in the presentation of antigens to T cells, and is expressed in all murine B cells; the T-cell/B-cell interaction via CD1 is, therefore, a potentially important therapeutic target.

INTERPRETATION. Using a non-depleting MoAb to CD1 to treat lupus-prone NZB/NZW mice, the authors demonstrated a delayed onset of proteinuria and reduced expression of IgG anti-dsDNA, both of which were associated with prolonged survival compared with untreated controls. The mechanism of this effect was further explored. CD1-reactive T cells were found in the spleens of the NZB/NZW mice by showing proliferative responses to a cell line transfected with CD1; the same experiment could not be carried out in the control mice as they responded to the non-transfected cell line due to an MHC mis-match. Nonetheless, transfer of CD1-reactive T cells into syngeneic hosts was followed by the expression of autoantibodies and a lupus nephritis.

Comment

CD1 expressed at a high level in a subset of B cells accounting for approximately 20% of the splenic B-cell population. Of note, this proportion is the same in the lupus-prone NZB/NZW mice as in non-autoimmune C57BL/6 controls. However, in NZB/NZW mice, this subset was responsible for a high level of spontaneous expression of IgM autoantibodies that was further increased by interaction with CD1-reactive T cells. In a forthcoming paper these cells were also shown to be CD5+; CD5+ B cells have previously been associated with autoantibody production. Interaction with CD1-reactive T cells would also allow the CD1+/CD5+ B cells to undergo class switching and produce the IgG autoantibodies associated with lupus pathology, especially GN. As the antibody was non-depleting there was no effect on numbers of IgM+, CD1+ B cells, and levels of IgM anti-dsDNA were not affected by treatment. Thus the observations are consistent with the hypothesis put forward; however, the data in the current paper are insufficient to establish clearly this proposal, as the therapeutic effect of the anti-CD1 MoAb, which has been well demonstrated, might involve a different or additional mechanism.

Conclusion

There are real advances in our understanding of the pathogenesis of SLE, with particular contributions being made by work in mice on genes, which influence both the occurrence of SLE and whether particular organs (e.g. glomeruli) will be targets of the disease process. Genes that themselves actively suppress a genetic tendency to develop SLE are especially interesting as their identification may allow novel therapies to be devised. There is an attractive explanation for the previously unexplained formation of multiple autoantibodies to intracellular/intranuclear antigens, following the demonstration that such antigens can be

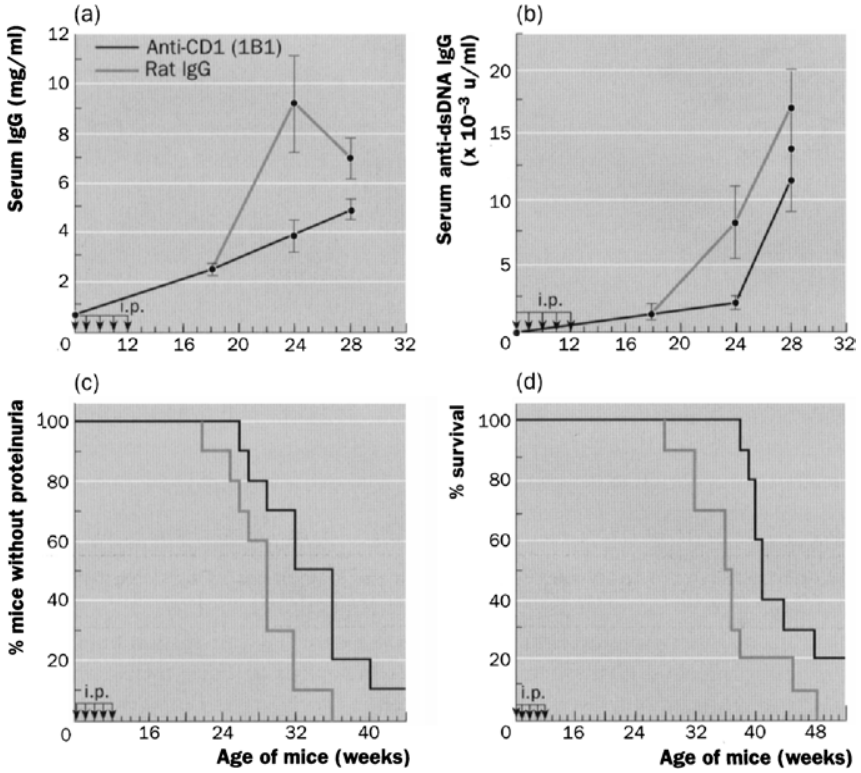

Fig. 9.3 Amelioration of lupus by *in vivo* anti-CD1 MoAb treatment. Groups of 8-week-old NZB/NZW mice were given five intraperitoneal injections of anti-CD1 MoAbs or control rat IgG at a dose of 250 μg per mouse over a period of 30 days (days 0, 3, 5, 15 and 30). Thereafter, the mice were monitored and serum levels of IgG, anti-dsDNA IgG, proteinuria and survival are shown in (a)–(d), respectively. There were 10 mice in each group. Arrows show time points of injections. Source: Zeng *et al.* (2000).

found in the apoptotic blebs formed as part of the process of programmed cell death (apoptosis). The idea that UV light could alter the contents of apoptotic blebs so that Ro, La, Sm and U-2B would be available to be presented as antigens is particularly interesting. In addition, abnormalities in initiating apoptosis, and in the clearance of apoptotic cells (which is supposed to avoid inflammatory responses) are both implicated in SLE pathogenesis by experimental models. Finally, loss of various genes that decrease and control the sensitivity of B lymphocytes predispose to SLE-like disease, but T-cell involvement in B-cell activation is also required.

None of these insights has yet led to definitive treatment, but as demonstrated in the cited papers, a variety of new approaches to SLE are under investigation, including new immunosuppressants, such as mycophenolate, and the ideas of

asking the immune system to 'start again' by means of a stem cell transplantation.

One of the most difficult forms of SLE to treat is neuropsychiatric disease, which is surprisingly common, especially manifestations such as mild cognitive dysfunction and subtle psychiatric symptoms; although even these 'minor' forms may be significantly disabling for patients. Possible links with APS have been highlighted and warrant further investigation, as does treatment of this condition whether or not it is associated with SLE.

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10
Systemic sclerosis

Introduction

Systemic sclerosis is a notoriously difficult disease to treat, in particular pulmonary disease, which is associated with increased morbidity and mortality. There have been few therapies assessed in randomized controlled trials. Recently, there have been large studies looking at the therapy of inflammatory lung disease (using cyclophosphamide) and pulmonary hypertension (using epoprostenol) suggesting that both therapies are useful. Among novel therapies are the use of recombinant human relaxin as an antifibrotic agent and the selective HT_2 serotoninergic receptor antagonist saprogrelate in Raynaud's phenomenon. Antioxidant therapy has been popular as an alternative therapy, but has recently been shown to have little effect in limited cutaneous systemic sclerosis.

Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis.

B White, W C Moore, F M Wigley, *et al. Ann Intern Med* 2000; **132**:947–54.

BACKGROUND. Inflammatory pulmonary disease (alveolitis) may cause pulmonary fibrosis in systemic sclerosis. Cyclophosphamide may be effective in maintaining lung function and improving survival in patients with systemic sclerosis. This was investigated in this retrospective cohort study.

INTERPRETATION. Pulmonary inflammation identified by bronchopulmonary lavage or biopsy was used to classify patients at risk of a poor outcome. Cyclophosphamide improved lung function outcome and survival.

Comment

One hundred and three patients with systemic sclerosis were studied using broncho-pulmonary lavage or biopsy to assess the severity of lung inflammation; 69 patients had alveolitis of whom 39 received cyclophosphamide. The decision

to treat with cyclophosphamide was made clinically; however, eight patients did not wish to receive cyclophosphamide and two had severe renal disease. Eighteen patients were judged to have mild disease only requiring prednisolone. Patients who received cyclophosphamide were more likely to have stabilization or improvement in forced vital capacity (FVC), diffusing capacity and survival. The study was retrospective and selection of patients for cyclophosphamide was biased by both physician and patient choice, such that only severely affected patients were considered for cyclophosphamide. FVC was lower in cyclophosphamide-treated patients. Prednisolone usage was similar in both groups and no clinical effect of prednisolone on outcome was detectable. The study was conducted in a tertiary care setting and some patients had bronchopulmonary lavage performed as part of a study of lung immunology in early scleroderma. Whether these results can be extrapolated to scleroderma patients in general is uncertain. Importantly, bronchopulmonary lavage is prognostically useful in predicting which patients with scleroderma will develop progressive deterioration in lung function.

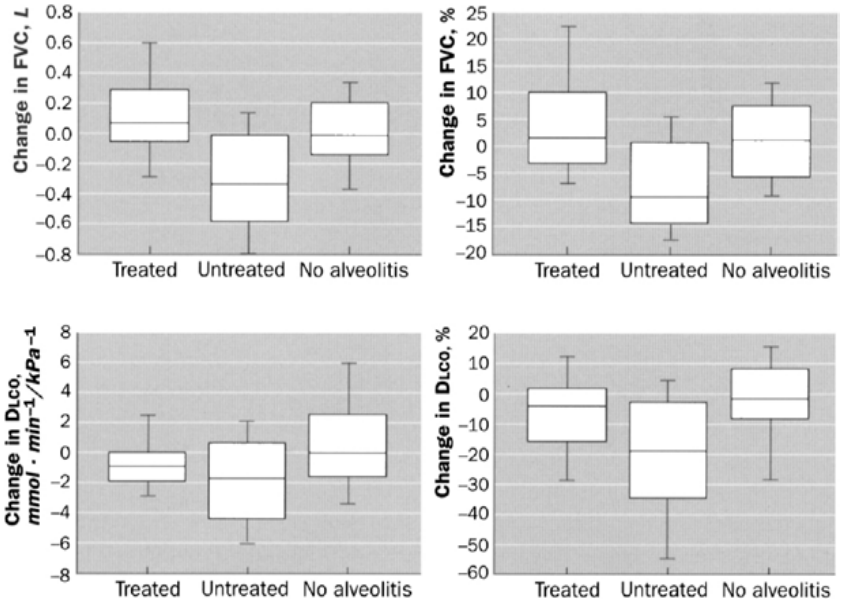

Fig. 10.1 Change in FVC (top left and right) and carbon monoxide diffusing capacity (D_{LCO}) (bottom left and right) overtime in patients with scleroderma. Results of pulmonary function tests done at the time of bronchoalveolar lavage or lung biopsy were compared with those of the last tests done in a patient. 'Treated' indicates patients with alveolitis who received cyclophosphamide, and 'untreated' indicates patients with alveolitis who did not receive cyclophosphamide. The line in the middle of each box shows the 50th percentile, the limits of the box show the 25th and 75th percentiles, and the limits of the bar show the 5th and 95th percentiles. Source: White *et al.* (2000).

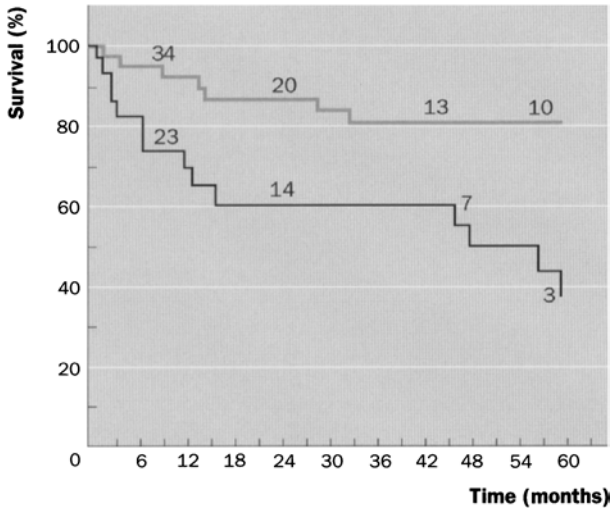

Fig. 10.2 Survival in patients with scleroderma and alveolitis. Survival was better among patients with alveolitis who received cyclophosphamide (grey line) than among patients with alveolitis who were not treated (black line) (P=0.01, Cox-Mantel log-rank test). The numbers above the lines are the number of patients at risk at the indicated time point. The median survival rate was 89% (25th, 75th percentiles, 84%, 94%) in treated patients and 71% (25th, 75th percentiles, 55%, 86%) in untreated patients. Source: White *et al.* (2000).

Continuous intravenous epoprostenol for pulmonary hypertension due to scleroderma spectrum of disease.
D B Badesch, V F Tapson, M D McGoon, *et al. Ann Intern Med* 2000; **132**: 425–34.

BACKGROUND. Pulmonary hypertension is a serious and potentially fatal complication of systemic sclerosis. This study investigated the efficacy of epoprostenol [a synthetic form of prostaglandin I_2 (prostacyclin)] in a randomized open label trial in patients with various forms of scleroderma.

INTERPRETATION. Exercise capacity (the primary outcome measure) improved in patients who received epoprostenol. Cardiovascular haemodynamics were also improved.

Comment

One hundred and eleven patients with moderate to severe pulmonary hypertension (pulmonary artery pressure >35 mmHg, pulmonary vascular resistance >3 mmHg/ 1 per min) received epoprostenol for a 12-week period. Other conventional therapywas maintained. The 6-min walk time improved in the epoprostenol group from 270 m to 316 m and decreased in the control group from 240 m to 192 m. Epoprostenol-treated patients had significant improvements in mean pulmonary artery pressure, pulmonary vascular resistance, right atrial pressure, cardiac index and mixed venous oxygen

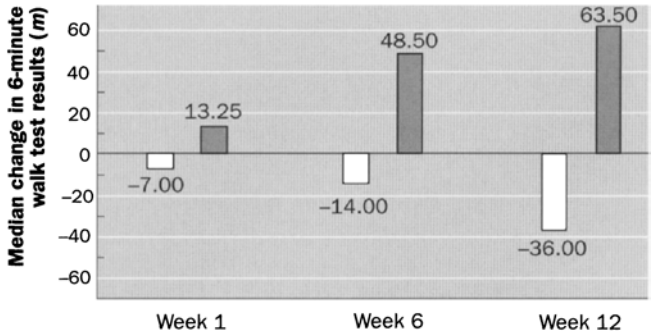

Fig. 10.3 Median change from baseline in results of the 6-min walk test at weeks 1, 6 and 12. Non-parametric analysis of covariance with adjustment for 6-min walk values and use of vasodilators at baseline showed that the median distance walked in 6 min increased in patients who received epoprostenol (solid bars) compared with patients who received conventional therapy (white bars) at weeks 6 (P=0.003) and 12 (P<0.001). Source: Badesch *et al.* (2000).

saturation; which worsened in the control group. Twenty-one patients who received epoprostenol showed an improvement in the New York functional grade, compared with no patients who only received conventional therapy. There was no significant difference in mortality—four deaths in the epoprostenol group and five in the control group. Disease-related and cardiovascular events were less common in the epoprostenol group. Epoprostenol related side-effects (flushing, diarrhoea and jaw pain) prevented blinding of physician and patient.

Systemic sclerosis-associated pulmonary hypertension. Short and long term effects of epoprostenol (prostacyclin).
E S Klings, N S Hill, M H leong, *et al. Arthritis Rheum* 1999; **42**:2638–45.

BACKGROUND. Intravenous vasodilator therapy is effective in primary pulmonary hypertension and may also be effective in pulmonary hypertension occurring secondary to systemic sclerosis. This study was an open label evaluation of epoprostenol in systemic sclerosis.

INTERPRETATION. A therapeutic response as defined by a reduction in pulmonary vascular resistance of >25% was achieved in 13 of 16 patients. Symptom and exercise tolerance improved in all patients. There was a long-term improvement in haemodynamic response in four patients.

Comment

Only 16 patients were studied. Thirteen patients received long-term (at home) epoprostenol. A haemodynamic and functional improvement, assessed using New York Heart Association class, was observed during a follow-up of 0.3–27

months. Two patients refused to continue long-term therapy and one died after 5 months. In four patients studied there was an improvement in pulmonary vascular resistance at 1 or 2 years after starting epoprostenol (two patients studied at 1 year, and two at 2 years). Epoprostenol was well tolerated, although all patients experienced headache, jaw pain, flushing and/or diarrhoea usually related to changes in dose. One patient developed pulmonary oedema. One patient had two episodes of catheter related *Micrococcus* infection. Prior to receiving epoprostenol patients received either nitric oxide or adenosine, lack of response to either agent was not predictive of response to epoprostenol. There were also no baseline haemodynamic values that predicted response to epoprostenol.

Skin thickness score as a predictor and correlate of outcome in systemic sclerosis.
P J Clements, E L Hurwitz, W K Wong, *Arthritis Rheum* 2000; **43**:2445–54.

BACKGROUND. Tightening of skin is a clinical hallmark of systemic sclerosis. The aim of this study was to investigate the prognostic value of the skin score as a predictor of outcome in systemic sclerosis.

INTERPRETATION. A baseline skin score >20 was associated with cardiac involvement at baseline and with subsequent mortality and renal crisis over a 4-year follow-up. Improvement in skin score in patients with diffuse disease was associated with an increase in functional ability.

Comment

One hundred and thirty-four patients with early (>18 months) diffuse cutaneous systemic sclerosis were entered into a multicentre drug trial (high-dose [750–1000 mg/day] versus low-dose penicillamine [125 mg alternate days]), which had shown no difference in outcome (skin score, frequency of renal crisis or mortality) between the two groups |**1**|. Patients were assessed at baseline and 2 years; however, only half the patients completed the 2-year study. Mortality and renal crisis were assessed during a mean follow-up period of 4 years. Baseline skin score of>20 was associated with baseline cardiac involvement (odds ratio 3.10; 95% confidence intervals 1.25–7.70) and was predictive of mortality (odds ratio 3.59; 1.23–10.55) and renal crisis (odds ratio 10.00; 2.21–45.91) over 4 years. Improvement in skin score was associated with improvement in hand function, joint contractures, inflammatory markers, joint count and Health Assessment Questionnaire. Thus improvement in skin score is not just associated with skin softening, but also with functional improvement.

Recombinant human relaxin in the treatment of scleroderma. A randomised, double blind, placebo controlled trial.

J R Seibold, J H Korn, R Simms, *et al. Ann Intern Med* 2000; **132**:871–9.

BACKGROUND. Relaxin is a hormone with tissue remodelling and antifibrotic effects. Scleroderma is characterized by tissue fibrosis. The aim of this randomized double-blind placebo-controlled study was to investigate the efficacy of human recombinant relaxin in systemic sclerosis.

INTERPRETATION. Therapy with human recombinant relaxin (25 μg/kg per day) for 24 weeks was associated with reduced skin thickening, improved mobility and improved hand function in patients with moderate to severe diffuse scleroderma.

Comment

Sixty-eight patients with stable moderate to severe diffuse cutaneous scleroderma for <5 years received recombinant human relaxin 25 or 100 μg/kg per day by continuous subcutaneous infusion or placebo. Patients receiving 25 μg/kg per day showed significantly reduced skin score (the primary end-point) at 24 weeks (mean change −8.7; P=0.04), however those receiving 100 μg/kg per day did not. The effect was seen after 4 weeks and maintained until 24 weeks. Similar results were observed in secondary outcome measures—FVC, oral opening and hand extension, functional status and global assessment. Side-effects included menorrhagia, reversible anaemia, and local infection or irritation at the infusion site. The study did not explain the lack of clinical benefit seen with the higher dose. It is possible that higher doses of relaxin fail to stimulate collagenase secretion and thus decrease the clinical effects that reflect reversal of fibrosis.

Suppressive effect of saprogrelate hydrochloride on Raynaud's phenomenon and respiratory failure in patients with systematic sclerosis.

S Kato, I Kishiro, N Ohnuma, *et al. Respirology* 2000; **5**:27–32.

BACKGROUND. There is evidence that the action of serotonin on vascular smooth muscule and platelets plays an important part in the pathogenesis of Raynaud's phenomenon. Saprogrelate hydrochloride is a new selective HT2-serotonergic receptor antagonist, which has been studied in an open label trial.

INTERPRETATION. Saprogelate hydrochloride decreased the frequency and duration of Raynaud's phenomenon and respiratory function.

Comment

Seven patients with Raynaud's phenomenon were treated with saprogrelate hydrochloride. Symptoms of Raynaud's (coldness, pain and numbness) were all decreased. Respiratory function also improved: both the Hugh-Jones classification and transfer factor. The effects of saprogrelate hydrochloride were mediated by reducing levels of fibrinopeptide A, β-thromboglobulin and platelet factor 4, and by reducing platelet aggregation.

A double blind placebo controlled trial of anti-oxidant therapy in limited cutaneous systemic sclerosis.
A L Herrick, S Hollis, D Schofield, *et al. Clin Exp Rheumatol* 2000; **18**: 349–56.

BACKGROUND. Oxygen-free radicals produced during repeated episodes of ischaemia reperfusion may contribute to tissue damage in systemic sclerosis. This study evaluated the effects of micronutrient antioxidants in a placebo-controlled double-blind crossover study in limited cutaneous systemic sclerosis.

INTERPRETATION. Treatment with selenium, β-carotene, vitamin C, vitamin E and methionine combined with allopurinol was not associated with any clinical benefit.

Comment

Thirty-three patients were studied with a median duration of Raynaud's phenomenon of 10 years. The patients were treated for 10 weeks with active or placebo, and then crossed over for a further 10 weeks. The study end-points were plasma concentration of von Willebrand's factor, thermographic cold challenge and the number of attacks of Raynaud's recorded. No differences were noted in the first 10 weeks in von Willebrand's factor, re-warming curve or patients' symptoms; after crossover there was also no clinical benefit. Circulating antioxidant levels did, however, increase. The treatment was well tolerated. The study may have been of too short duration or insufficiently powered to demonstrate an effect or alternatively anti-oxidant therapy might be better used in earlier disease.

Conclusion

New therapies for systemic sclerosis are urgently needed. Reports in the last year suggest potential new therapies for systemic sclerosis or some of its more serious complications such as pulmonary hypertension. It is perhaps disappointing to note that those trials showing positive effects of interventions were mainly open label and not necessarily placebo-controlled, while gold-standard double-blind, placebo-controlled studies (of relaxin and antioxidants) did not show efficacy.

Clearly full trials are required to test the preliminary reports of effectiveness of the other therapies tested in the recent literature.

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11
Vasculitis

A.
Assessment and therapy

Introduction

Wegener's granulomatosis (WG) is a systemic necrotizing vasculitis associated with a significant mortality and morbidity. The conventional therapy of WG and other antineutrophil cytoplasmic antibody (ANCA) associated vasculitides is cyclophosphamide and glucocorticoids, followed by azathioprine. Induction with cyclophosphamide and prednisolone induces remission in about 90% of patients. However, prolonged therapy with cyclophosphamide is associated with significant toxicity especially bladder malignancy. Attention has recently been focused on the use of shorter courses of cyclophosphamide before changing to less toxic drugs (conventionally azathioprine) or alternatives such as methotrexate (MTX) or intravenous immunoglobulin. Over the past year there have been trials studying MTX for maintenance therapy following induction with cyclophosphamide and corticosteroids and in patients with minimal renal disease. The use of intravenous immunoglobulin in refractory disease has been investigated. The therapy of other forms of vasculitis has been investigated with reports on the use of interferon (IFN) -α in Behçet's disease and hepatitis C virus (HCV) associated mixed cryoglobulinaemia.

Following discovery of the association between WG and ANCA several groups have studied the use of serial measurements of ANCA in predicting the relapse of WG. In a pooled analysis only 48% of rises in ANCA titres as measured by IIF were followed by relapse |**1**| and only 51% of relapses were preceded by rising titres. This issue has been addressed recently in a prospective manner.

Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibodies. A prospective study.
M M Boomsma, C A Stegeman, M J van der Leij, *et al. Arthritis*

Rheum 2000; **43**:2025–33.

BACKGROUND. The prediction of relapse in patients with WG by measurement of levels of ANCA directed against either proteinase 3 (PR3) or myeloperoxidase (MPO) is controversial. This study prospectively assessed the value of serial determinations of ANCA [by indirect immunofluorescence (IIF) and PR3/MPO by enzyme-linked immunosorbent assay (ELISA)].

INTERPRETATION. Serial measurement of ANCA is useful in predicting relapse in patients with WG, and measurement of ANCA by PR3/MPO ELISA is superior to determination by IIF for prediction of subsequent relapses.

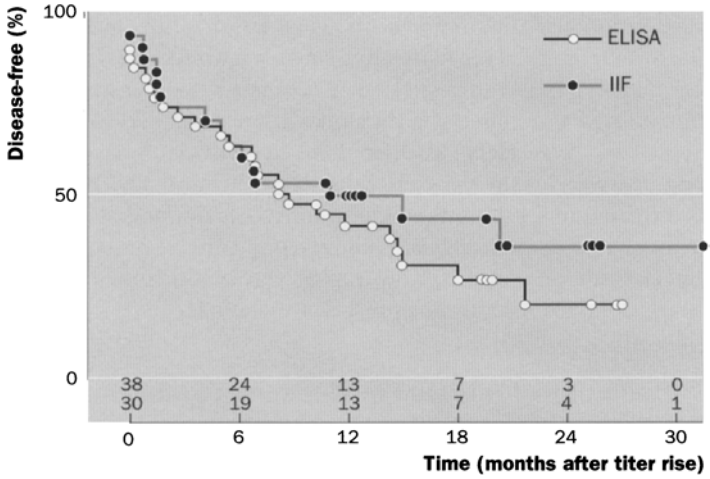

Fig. 11.1 Percentage of patients with WG who did not experience disease relapses in the indicated time period after a rise in ANCAs as measured by either IIF (n=30) or antigen-specific ELISA (n=38). The numbers above the horizontal axis indicate the number of patients who were still at risk for a relapse at 6,12,18, 24 and 30 months after the rise in antibody levels by ELISA (upper numbers) or IIF (lower numbers). Source: Boomsma *et al.* (2000).

Comment

One hundred patients with WG were studied and ANCA determined every 2 months together with disease activity using the Birmingham Vasculitis Activity Score. Thirty-seven patients relapsed during follow-up (median 1054 days). Thirty-four of 37 (92%) patients showed a rise in ANCA level preceding relapse determined by either ELISA or IIF. The predictive value of an increase in ANCA titres for relapse was 57% (17 of 30) for cytoplasmic ANCA by IIF, 71% (27 of 38) for PR3-ANCA and 100% (three of three) for MPO-ANCA. Forty-three per cent of patients who showed a rise in cytoplasmic ANCA and 29% with a rise in PR3-ANCA did not subsequently relapse. Only 39% of patients with an increase

in ANCA relapsed within 6 months of the rise in ANCA, thus a rise in PR3-ANCA is not usually an indicator of imminent relapse. It is not justified to escalate immunosuppressive therapy solely on the basis of an increase in ANCA level; such an increase should be taken as a warning and the patient observed more closely.

A staged approach to the treatment of Wegener's granulomatosis. Induction of remission with glucocorticoids and daily cyclophosphamide switching to methotrexate for remission maintenance.

C A Langford, C Talar-Williams, K S Barron, M C Sneller. *Arthritis Rheum* 1999; **42**:2666–73.

BACKGROUND. Induction therapy with corticosteroids and cyclophosphamide is effective therapy for WG, but has unacceptable toxicity when used long term. The aim of this open study was to investigate whether MTX was acceptable therapy for the maintenance of remission.

INTERPRETATION. Use of cyclophosphamide and corticosteroids for induction therapy followed by MTX is acceptable therapy even in those with severe disease at onset.

Comment

Forty-six patients with active WG were evaluated; 12 patients were enrolled in a study of open label MTX for non-severe WG. Three patients did not receive MTX, one patient was hepatitis C positive and two had received MTX unsuccessfully for endobronchial stenosis. Thirty-one patients were enrolled. Patients received daily oral cyclophosphamide at a dose of 2 mg/kg per day and prednisolone 1 mg/kg per day. Steroid reduction began after 1 month if there was clinical improvement. MTX was started once remission had been obtained and started at a dose of 0.3 mg/kg per week (maximum 15 mg/week). Remission was achieved in all 31 patients after a median of 3 months' therapy. Five patients relapsed after a median of 13 months. Comparison with other regimens is difficult as this was an open trial; however, comparison with an historical cohort from the same institution suggests that the relapse rate is similar. Two patients withdrew due to MTX pneumonitis. Cystitis was seen less frequently in this study than the historical cohort. Prophylaxis with trimethoprim/sulphamethoxazole was standard and no adverse haematological effects were seen with prophylactic dose trimethoprim/sulphamethoxazole. No *Pneumocystis* infection were observed. Staged therapy using MTX seems to be an acceptable treatment for WG.

Use of methotrexate and glucocorticoids in the treatment of Wegener's granulomatosis. Long-term renal outcome in patients with glomerulonephritis.

C A Langford, C Talar-Williams, M C Sneller. *Arthritis Rheum* 2000; **43**: 1836–40.

BACKGROUND. MTX is an alternative to cyclophosphamide/ azathioprine in patients with non-life-threatening WG. This study describes the long-term renal outcome in patients with WG and active glomerulonephritis (GN) treated in an open label trial with MTX and glucocorticoids.

INTERPRETATION. The use of MTX and prednisolone as initial therapy for patients with WG-associated GN and a normal/near normal serum creatinine was not associated with a long-term decline in renal function. The presence of GN should not preclude the use of MTX in this group of patients.

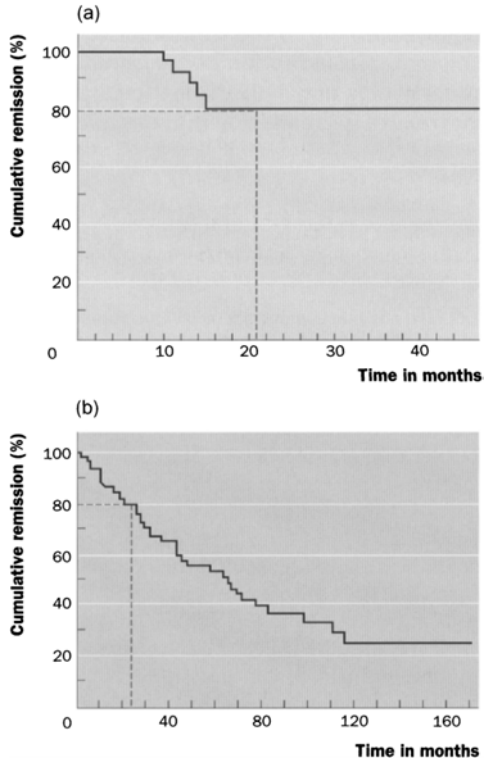

Fig. 11.2 (a) Kaplan-Meier plot of the time to disease relapse in 31 patients with WG treated with a regimen of cyclophosphamide and glucocorticoid for remission induction and methotrexate for remission maintenance. (b) Kaplan-Meier plot of the time to disease relapse in 60 historical WG patients treated with a regimen of cyclophosphamide and glucocorticoids. Source: Langford *et al.* (2000).

Comment

This study is an extension of a previously reported trial |2| of 42 patients with non-life-threatening WG treated with MTX and prednisolone. Twenty-one of 24 had active GN at entry, median follow up was 76 months (20–108). Only two patients had an increase in serum creatinine (>0.2 mg/dl), 12 had stable renal function and six improved. These patients all had normal/near normal serum creatinine at entry. MTX is renal excreted and should not be used in patients with significant renal impairment. However, the presence of GN should not preclude the use of MTX and corticosteroids as initial therapy in patients with near normal creatinine. Patients with life-threatening WG should continue to receive cyclophosphamide.

Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity.
D R W Jayne, H Chapel, D Adu, *et al. Q J Med* 2000; **93**:433–9.
BACKGROUND. Intravenous immunoglobulin (IVIg) is a possible alternative treatment for ANCA-associated systemic vasculitis (AASV), which is associated with less toxicity than conventional immunosuppressive drugs. This study investigated the efficacy of IVIg in patients with persistently active AASV in a randomized placebo-controlled trial.

INTERPRETATION. Treatment with a single course of IVIg (total dose 2 g/kg) reduced disease activity in persistently active AASV, but this effect was not maintained beyond 3 months. There were mild reversible side-effects following IVIg (headaches, rise in serum creatinine and fatigue).

Comment

Thirty four patients were randomized (17 in each group) to either placebo or IVIg. Significant responses were seen in 14 of 17 of the IVIg group and six of 17 of the placebo group (P=0.015, OR 8.56 95% CI 1.74–42.2). These patients had persistent disease activity despite conventional therapy with glucocorticoids, cyclophosphamide and azathioprine. Clinical response was not seen beyond 3 months, C-reactive protein (CRP) levels fell following IVIg. No changes were seen in ANCA levels, but only 10 of 34 had raised ANCA levels at the time of treatment. There was no difference in glucocorticoid or cytotoxic drug exposure in either group. The study did not address whether IVIg is suitable for induction regimens or an adjunct to conventional therapy in elderly patients to permit lower doses of corticosteroids or cytotoxic agents. IVIg is an alternative treatment for AASV with persistent disease activity after standard therapy.

Evaluation of antineutrophil cytoplasmic antibody seroconversion induced by minocycline, sulfasalazine, or penicillamine.

H K Choi, M C Slot, G Pan, *et al. Arthritis Rheum* 2000; **43**: 2488–92.

BACKGROUND. Case reports suggest that minocycline, sulphasalazine and penicillamine are associated with AASV. This study evaluated ANCA seroconversion in serum samples obtained during three randomized double-blind controlled trials.

INTERPRETATION. There was no evidence to support the notion that minocycline, sulphasalazine or penicillamine induced ANCA seroconversion.

Comment

Samples were obtained from three trials: (a) 48-week trial of minocycline versus placebo in 132 patients with early rheumatoid arthritis (RA); (b) 37-week trial of sulphasalazine versus placebo in 89 patients with RA; and (c) 104-week trial of high-dose versus low-dose penicillamine in 27 patients with early systemic sclerosis. ANCA were measured by IIF and by antigen-specific ELISA for PR3 and MPO antibodies. Twelve (5%) patients were seropositive at baseline for MPO-ANCA. No patients demonstrated seroconversion in active study drug groups. No subject was positive for PR3 in combination with cytoplasmic ANCA. There was no clinical evidence of vasculitis in any patient. The number of paired sera is relatively small, and a low seroconversion rate cannot be ruled out. The study did not rule out seroconversion rates of <1.6% (95% CI 0–5.8%) for minocycline, <1.7% (0–7.2%) for sulphasalazine and 3.7% (0–13.7%) for penicillamine. The study duration was similar to drug exposure duration for reported cases of minocycline-induced AASV, but less for both sulphasalazine and penicillamine. The study raises issues of causality in previous case reports, but does not exclude rare, sporadic cases of either ANCA seroconversion or true AASV.

Treatment of refractory, symptomatic hepatitis C virus related mixed cryoglobulinaemia with ribavirin and interferon-α.

E Zuckerman, D Keren, G Slobodin, *et al. J Rheumatol* 2000; **27**:2172–8.

BACKGROUND. HCV infection is closely associated with mixed cryoglobulinaemia. Antiviral therapy with IFN-α is effective in many cases. The aim of this study was to investigate the role of combination therapy with IFN-α and ribavirin.

INTERPRETATION. The combination was clinically effective in refractory mixed cryoglobulinaemia associated with HCV, producing a fall in cryoglobulin levels and improvement in mixed cryoglobulinaemia related symptoms

(arthralgias, proteinuria and skin vasculitis), even without a complete virological or biochemical response.

Comment

Nine patients with type II symptomatic mixed cryoglobulinaemia who had failed IFN-α monotherapy were treated with IFN-α (3 million units three times weekly) and ribavirin (15 mg/kg per day) for 6 months. Cryoglobulins became undetectable within 6 weeks of therapy in seven patients and deceased in two others. Arthritis, proteinuria and skin vasculitis were all improved with 10 weeks of therapy but neuropathy persisted. All patients were HCV-RNA positive and five had cirrhosis. Complete virological response was only seen in two patients. Normalization of alanine aminotransferase was achieved in four patients. Cryoglobulinaemic symptoms recurred in four patients on stopping combination therapy, resumptionresulted in further symptomatic improvement. Therapy may, therefore, need to be long term or even lifelong. The study suggests that IFN-α/ ribavirin is a useful combination in refractory patients but a larger, longer study is required to confirm the findings.

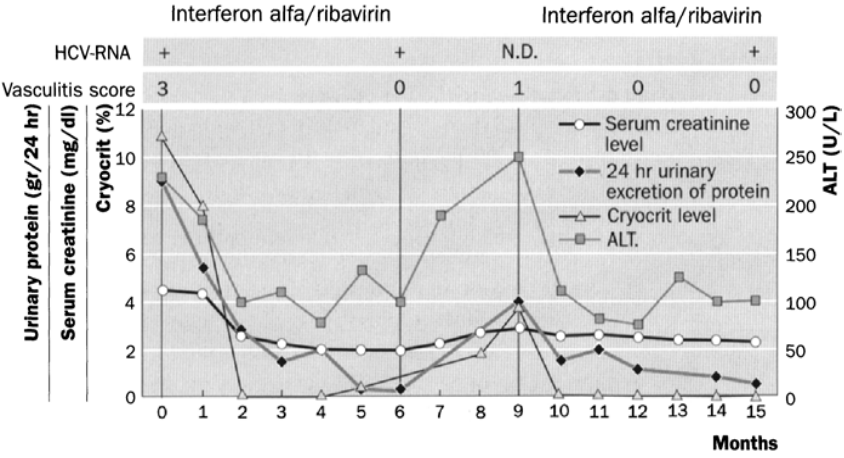

Fig. 11.3 Response of mixed cryoglobulinaemia associated syndrome to combination therapy with IFN-α and ribavirin in patient 2. Within 8 weeks of combination therapy, cryoglobulin became undetectable and there was substantial improvement in 24 h urinary excretion of protein and disappearance of vasculitic purpura. Cessation of treatment led to increase of cryocrit and relapse of symptoms. Re-treatment resulted in a second remission. N.D., not done. Vasculitis score (clinical score): 0, absence of active skin lesions; 1: mild vasculitis—rare (<10) purpuric spots on the lower limbs; 2, moderate vasculitis—≥10 purpuric spots on the lower limbs; 3, severe vasculitis— extension of acral purpura to the trunk and/or upper limbs; 4, presence of skin ulcers and/or gangrene. Source: Zuckerman *et al.* (2000).

Conclusion

Vasculitis is now treatable and the treatments are clearly life-saving. However, some conditions, particularly WG are prone to relapse. Consequently, the issues current in treatment of vasculitis revolve around minimizing the toxicity of therapy (in particular, that associated with cyclophosphamide) and appropriate maintenance therapy once remission has been achieved; therefore, relapses are dealt with promptly without exposing the patients to premature or unnecessary therapy. These papers emphasize the need for accurate clinical assessment, aided by the measurement of ANCA levels, in determining the likelihood of relapse. It will also be important in future studies to define subsets of patients for whom effective therapy can be provided without recourse to cyclophosphamide.

B.
Giant cell arteritis

Introduction

Giant cell arteritis (GCA) is the most common of the systemic vasculitides occurring chiefly in those aged 60 or over. The aetiology is unknown, but results from an interplay of genetic and environmental factors. Recent studies have looked at the possible roles of *Chlamydia pneumoniae* infection, tumour necrosis factor (TNF) microsatellite polymorphisms and angiogenesis in the aetiopathogenesis.

GCA is associated with significant morbidity associated with corticosteroid therapy. Various regimens have been proposed to reduce the total exposure to corticosteroids, including the use of intravenous pulse methylprednisolone in patients with uncomplicated GCA.

Whether GCA and polymyalgia rheumatica (PMR) are closely linked and represent opposite ends of a spectrum is controversial; some immunogenetic evidence suggests that there are differences between the two conditions (e.g. TNF micro-satellite polymorphisms). A recent clinical study of peripheral arthritis in GCA and PMR suggests that arthritis only occurs in patients with PMR.

Cell adhesion molecules in the development of inflammatory infiltrates in giant cell arteritis. Inflammation induced angiogenesis as the preferential site of leukocyte-endothelial cell interactions.

M C Cid, M Cebrián, C Font, *et al. Arthritis Rheum* 2000; **43**:1749–55.

BACKGROUND. The aim of this study was to investigate the expression pattern of endothelial cell adhesion molecules and their leucocyte receptors in temporal arteries from patients with GCA.

INTERPRETATION. Inflammation-induced angiogenesis is the main site of leucocyte-endothelial cell interactions leading to the development of inflammatory infiltrates in GCA.

Comment

Immunohistochemistry was performed on 32 temporal artery specimens from GCA patients and 12 control samples from patients with other diseases. Constitutive (PECAM-1, ICAM-1, ICAM-2 and P-selectin) and inducible (E-selectin and VCAM-1) adhesion molecules were mainly expressed by adventitial microvessels and new vessels in inflammatory infiltrates. There was preferential use of VLA-4/ VCAM-1 and LFA-1/ICAM-1 at the adventitia and Mac-1/ ICAM-1 at the intimamedia junction. There was a correlation between the intensity of staining for inducible endothelial adhesion molecule expression (E-selectin and VCAM-1) and the severity of the previous inflammatory response. Specific interactions occur at different sites in the artery allowing transmigration of different functional cell subsets. Corticosteroid therapy did not completely suppress expression of inducible adhesion molecules in the patients examined in this study. New therapeutic targets directed against adhesion molecules or angiogenesis might be beneficial in GCA and complement conventional therapy with corticosteroids.

Detection of *Chlamydia pneumoniae* in giant cell vasculitis and correlation with the topographic arrangement of tissue-infiltrating dendritic cells.

A D Wagner, H C Gérard, T Freseman, *et al. Arthritis Rheum* 2000; **43**: 1453–51.

BACKGROUND. GCA may be an antigen-driven disease, however, the antigen is unknown. Clustering of cases suggests an infectious aetiology. The aim of this study was to determine whether *Chlamydia pneumoniae* could be detected in temporal artery biopsy specimens.

INTERPRETATION. *C. pneumoniae could* be detected in eight of nine temporal artery biopsy specimens from patients with GCA and showed a strong topographic relationship with dendritic cells.

Comment

C. pneumoniae was detected either by immunohistochemistry or by polymerase chain reaction (PCR). Six of nine GCA samples were positive using both methods, and one each by immunohistochemistry or by PCR. One of four specimens from PMR patients was positive only by immunohistochemistry. All nine control samples were negative by both methods. Immunohistochemistry demonstrated that *C. pneumoniae* were located in the adventitial layer of the temporal artery close to granulomatous infiltrates, and there was a topographic

relationship with dendritic cells. The role of both *C. pneumoniae* and dendritic cells in the pathogenesis of GCA needs to be further clarified. Chlamydiae are capable of causing persistent infection under certain conditions, with the host response to the infection being the major contributor to the development of disease. The authors speculate that *C. pneumoniae* might be persistent in the arterial wall after the primary infection leading to an inflammatory response. Dendritic cells may represent the antigen-presenting cells in this situation.

Association of giant cell arteritis and polymyalgia rheumatica with different tumour necrosis factor microsatellite polymorphisms.

D L Mattey, A H Hajeer, A Dababneh, *et al. Arthritis Rheum* 2000; **43**: 1749–55.

BACKGROUND. GCA and PMR are closely related conditions. The aim of this study was determine whether GCA and PMR are associated with different TNF microsatellite polymorphisms.

INTERPRETATION. GCA and PMR are associated with different TNF microsatellite polymorphisms. The primary associations (TNF-α2 and TNF-β3) seem to influence susceptibility independently of HLA-DRB1 associations.

Comment

A case-control methodology was used to compare 136 patients with GCA/PMR with 147 ethnically matched controls. Samples were obtained from residents of Lugo, northwest Spain. In patients with isolated GCA the primary association was with TNF-α2, which was independent of the association with HLA-DRB1*0401 and *0101. A negative association was found with TNF-α10. Patients with isolated PMR had a positive association with TNF-β3, which was independent of the HLA-DRB*13/*14 association in isolated PMR; TNF-δ4 was negatively associated with PMR. The study provides evidence for the genetic heterogeneity of GCA and PMR. Further studies in different ethnic groups are required to determine whether these TNF microsatellite polymorphisms are common in other populations.

A randomised, multicentre, controlled trial using intravenous pulses of methylprednisolone in the initial treatment of simple forms of giant cell arteritis: a one year follow up study of 164 patients.

P Chevalet, J-H Barrier, P Pottier, *et al. J Rheumatol* 2000; **27**:1484–91.

BACKGROUND. Corticosteroids are effective in preventing blindness in GCA, but the optimum dose regimen is controversial. The aim of this study was to investigate the corticosteroid sparing effect of an initial intravenous pulse of methylprednisolone in simple GCA.

INTERPRETATION. Methylprednisolone pulses have no significant long-term, corticosteroid sparing effects in the treatment of simple GCA and should be limited to complicated patients.

Comment

One hundred and sixty-four patients with biopsy proven GCA, or fulfilling American College of Rheumatology criteria for GCA with a negative biopsy, were studied. Patients with recent (<1 month) ocular or vascular involvement, isolated PMR, neoplasia, other inflammatory disease and aged >85 years were not included. Three treatment groups were compared: (i) intravenous pulse of 240 mg methylprednisolone, followed by 0.7 mg/kg per day prednisolone; (ii) 0. 7 mg/kg per day prednisolone no intravenous pulse; (iii) 240 mg methylprednisolone intravenous pulse, but followed by a lower dose of prednisolone at 0.5 mg/kg per day. Corticosteroid dosage was reduced after normalization of two biological inflammatory variables to obtain half-dosage after 4 weeks in groups i and ii and 20 mg/day after 2 weeks in group iii. Tapering was systematically attempted from the sixth month of treatment. After 1 year cumulative doses of prednisolone were the same for all three groups (P=0. 39). No significant differences were seen in the time required for the normalization of CRP, corticosteroid resistance (failure to normalize CRP in 3 weeks or abolish pain/fever in 1 week) (13.5% of patients), or corticosteroid side-effects (39% of patients, P=0.37). Corticosteroid-resistant patients received large doses of prednisolone and developed more GCA complications (P=0.02). The main corticosteroid side-effects seen were infections. The overall mortality was 3% after 1 year. Intravenous methylprednisolone should be reserved for patients with complicated forms of GCA.

The incidence and clinical characteristics of peripheral arthritis in polymyalgia rheumatica and temporal arteritis: a prospective study of 231 cases.

J T Gran, G Myklbust. *Rheumatology* 2000; **39**:283–7.

BACKGROUND. The occurrence of peripheral arthritis in PMR is controversial. The aim of this study was to evaluate the incidence and clinical features of peripheral arthritis in PMR and temporal arteritis.

INTERPRETATION. Peripheral arthritis and the development of RA only occurred in patients with PMR and did not occur in temporal arteritis patients, suggesting aetiopathological differences between the two conditions.

Comment

Two hundred and thirty-one patients (187 pure PMR, 29 cases temporal arteritis, and 15 PMR and temporal arteritis coexistent) were prospectively identified. Only 16 patients (6.5%) were lost to follow-up. Pre-existing RA was excluded.

During follow-up, 11 cases developed RA; 10 of 11 had pure PMR and one of 11 PMR/temporal arteritis overlap; the mean interval was 62.3 months after the development of PMR. A peripheral arthritis was observed at some time in 38.5% of cases. This occurred at diagnosis alone in 14.7% of cases, in 7.8% of patients, peripheral arthritis was present both at diagnosis and subsequently, while in 16% peripheral arthritis developed subsequent to diagnosis. At diagnosis, peripheral arthritis was not seen in temporal arteritis, but one patient developed a transient monoarticular arthritis during follow-up. The most commonly involved joints were the knees (46.7%), metacarpophalangeal joints (42.0%) and wrists (42.1%). The sternoclavicular joints were only involved in 2.8% of patients. At diagnosis, few patients required additional therapy to corticosteroids; however, during the course of treatment other therapies, including non-steroidal anti-inflammatory drugs and intra-articular steroids were required. Only seven patients required disease-modifying antirheumatic drugs. The study suggests that synovial tissues are important in PMR and that there may an aetiological difference between PMR and temporal arteritis.

The importance of skip lesions in temporal arteritis.
D N Poller, Q van-Wyk, M J Jeffrey. *J Clin Pathol* 2000; **53**(2): 137–9.

BACKGROUND. To determine the frequency of skip lesions in an unselected series of temporal artery biopsies and compare the results with other series. This was a retrospective review of 102 consecutive temporal artery biopsies taken in a 5-year period (1992–97) in one large hospital.

INTERPRETATION. In this series skip lesions were relatively rare, accounting for 8.5% of cases of active vasculitis. The degree of inflammation in temporal arteritis is discontinuous. Immunostaining for inflammatory cells, for example, using antibodies to leucocyte common antigen (CD45) and CD15, may be helpful in identifying the presence of an inflammatory cell infiltrate in skip lesion segments of the temporal artery.

Comment

Temporal arteritis is a syndrome that can be diagnosed by specific clinical and laboratory criteria, and by a temporal artery biopsy showing mononuclear cell infiltrates or a granulomatous process with multinucleated giant cells. While the temporal artery is commonly examined, the occurrence of skip lesions (that is, one or more areas of typical arteritis, while other sections of the same artery have no features of arteritis) presents potential difficulties. The incidence of skip lesions is important in relation to the predictive value of a negative temporal artery biopsy, but the incidence is not clear, with reports varying from 0 to 28%.

In this study, although 102 cases were evaluated, only 35 cases (34.3%) showed evidence of active cranial vasculitis. All biopsies were of reasonable size

(at least 6 mm). Three of these cases (8.5%) showed apparent skip lesions. Unfortunately, no clinical information was available for these cases, although by the very fact of the biopsy having been taken, it can be assumed they had features of GCA.

Although ultrasound and colour/power Doppler techniques are being reported in the diagnosis of temporal arteritis, temporal artery biopsy is likely to remain an important investigation. In this context skip lesions must be considered when reviewing the biopsy findings.

Lack of association between infection and onset of polymyalgia rheumatica.
J Narváez, M T Clavaguera, J M Nolla-Sole, *et al. J Rheumatol* 2000; **27**(4): 953–7.

BACKGROUND. The aetiology of GCA is unknown, but its sudden onset and the wide variation in incidence reported from various parts of the world suggest a genetic predisposition and/or the influence of environmental factors, such as infectious agents or a seasonal effect. This study is a retrospective analysis of the influence of season on GCA in the authors' area over the period 1985–97, as well as the possible association between infection and onset.

INTERPRETATION. No seasonal pattern or association between infection and the onset of GCA in 143 cases was observed.

Comment

This is a retrospective study that addressed an important question. The authors evaluated those patients with GCA diagnosed in their area from 1985 to 1997. To evaluate seasonal variation in disease onset, the month of onset of the first symptoms related to GCA was used to calculate season-specific incidence rates. To test for an association between infection and GCA onset, only infections that occurred within 2 months before the onset of disease were considered. This may have resulted in some false negatives in the sample. In addition, many infections are subclinical and detection would be difficult.

Because of the difficulty in determining whether an infection was present using only the clinical and laboratory data recorded in patients' medical charts, the authors categorized the likelihood of patients having infection into three groups: no infection, probable infection and definite infection, with only the latter group having objective evidence of infection. The authors noted no seasonal variation in disease onset during the 13-year period and only one (0.7%) of 143 patients was categorized as a probable infection, whereas definite infection was not observed in any case. From these results the authors concluded that the hypothesis of an infectious cause for GCA seems highly improbable.

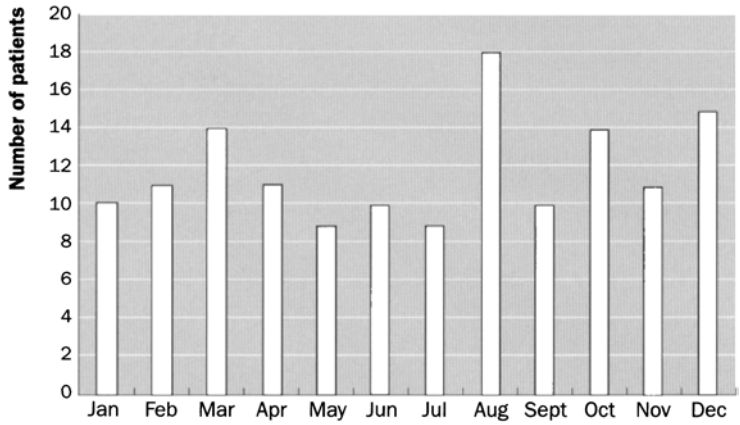

Fig. 11.4 Monthly incidence of 143 cases of GCA during the period 1985–97. Source: Narváez *et al.* (2000).

This is an extremely difficult area to study, as many infections may be subclinical and both the specific infections that may be involved, and the time period prior to diagnosis where infection may be relevant, remain undefined.

Conclusion

Of the vasculitides, GCA/PMR is the most common and generally the most satisfying to treat in view of the gratifying and rapid response to corticosteroids, although we still need to optimize treatment by minimizing exposure to steroids. However, as demonstrated in recent papers, we still know little about its pathogenesis. The association with age might suggest an autoimmune aetiology—as mechanisms controlling autoimmunity decline with age, hence the increasing prevalence of auto-antibodies with age. However, equally likely is a change in the relationship with a persistent and relatively benign pathogen—the obvious parallel is *Herpes zoster* reactivation many years after chicken pox. In this context it is natural that *C. pneumoniae* should attract attention as it persists in arteries in relation to atherosclerotic plaques, but evidence for this idea remains limited. The idea that GCA follows contact with a new pathogen is less attractive—by 55 we are likely to have encountered most of the common pathogens in our environment—so the lack of evidence for seasonal attacks is not too surprising.

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Part V

Degenerative disorders and soft tissue rheumatism

12
Osteoarthritis

Osteoarthritis (OA) remains one of the most common causes of pain and reduced function in the community. The year 2000 has seen increased understanding of the underlying pathology together with clarification of the most appropriate strategies to patient care (see review articles such as Dieppe |**1**|. Original articles selected here reflect the breadth of research interest in this area.

A randomised controlled trial comparing topical piroxicam gel with a homeopathic gel in osteoarthritis of the knee.
R A van Haselan, P A G Fisher. *Rheumatology* 2000; **39**:714–19.

BACKGROUND. Complementary medicine is increasingly popular, despite the lack of a formal evidence base to support its use. The pharmacological basis for topical treatments for gonarthrosis is also debated, although a meta-analysis of smaller trials has demonstrated efficacy of these agents compared with placebo.

INTERPRETATION. Patients with symptomatic, radiologically established OA of the knee (*n*=184) were randomized in a quasi-blinded fashion to a topical homeopathic remedy or to topical piroxicam. At 4-week review both treatments appeared efficacious and equivalent, with no difference in the incidence of adverse effects. *Post-hoc* analysis suggested that the topical non-steroidal anti-inflammatory drug (NSAID) was most efficacious in those who continued to take an oral NSAID.

Comment

The study was designed and powered to test for equivalence of the two agents, which was subsequently established. The outcome measures selected at the time the study was designed did not include some which would now be considered useful, such as walking tests, SF-36, or Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). In particular, the one-joint Richie test is, as acknowledged by the authors, not validated in OA. However, physician and patient assessments are validated and equivalence was shown in these

parameters. The authors adjusted the reduction in pain (on a Visual Analogue Scale [VAS]) for a significant difference in pain at baseline between the two treatment groups; however, other effects of this difference might not be adjusted for, and the study must be interpreted with some caution in view of this. The incidence of adverse effects was reassuringly low; however, a significant difference cannot be excluded with these data.

Manipulating the data, a significant ($P<0.05$) difference could be found on patient overall assessment, favouring the homeopathic remedy. This may also be suggested by the fact that, on weighing the tubes of gel as a measure of compliance, the homeopathic users had applied an average 3.3 g/day of gel, compared with 2.7 g/day by the piroxicam users; Are patients likely to use more of a treatment they perceive to be effective? However, in contrast, the use of other analgesia was not significantly reduced in the homeopathic users.

The authors state that a separate study comparing the homeopathic treatment against placebo is to be reported soon. This, and the comparison against other treatments (conventional and complementary, oral and injected), will be important to fully establish the place of topical remedies in general, and of complementary medicine in particular, in OA.

Home-based exercise therapy for older patients with knee osteoarthritis: a randomised controlled trial.
R J Petrella, C Bartha. *J Rheumatol* 2000; **27**:2215–21.

BACKGROUND. Quadriceps and range of motion (RoM) exercises are frequently advocated for knee OA. This study evaluates the effectiveness (i.e. including compliance) of such a regime in older patients for whom any reduction in requirement for analgesia and NSAIDs is particularly welcome.

INTERPRETATION. Contrary to previous studies, all patients were given a single NSAID at a fixed dose. The 'sham' group were given simple exercises, which would not increase muscle strength or knee RoM. The intervention group actively increased knee RoM (e.g. holding the knee flexed using a towel to grip the foot) and increased quadriceps strength (lowering to a 'seated' position leaning against a wall, sustained for increasing periods, before rising again). Over 8 weeks, pain was reduced to a greater extent in the intervention group (18% versus 11% reduction in VAS). Self-paced walking and stepping tests were faster in both groups, more so in the exercising group (26 s versus 9 s faster, $P<0.002$), as was the improvement in the WOMAC score.

Comment

These patients were 68–80 years of age with osteoarthrosis of the knee. While not specifically stated, hip osteoarthrosis was unlikely to be significant given the exercises involved. While it was an important step to standardize NSAID use in this study, we are not told how many were not previously taking NSAID

regularly nor the compliance with NSAID use during the study; therefore, the relative contribution of this intervention to the overall improvement cannot be assessed. None the less, unless there was a significant difference in compliance between groups, the difference between groups may reasonably be attributed to the exercise programme. The step and walk time tests are important functional assessments, and it is perhaps significant that the improvements seen in these parameters were greater than the reduction in pain. Considering the substantial resource implications of putting a programme such as this in place, it would be invaluable if the authors reviewed this cohort in, say, 6 months' time to see how many have voluntarily kept up the exercises, and whether the benefits are sustained over a longer period than this 2-month study.

[1] A meta-analysis of chondroitin sulfate in the treatment of osteoarthritis.
B F Leeb, H Schweitzer, K Montag, J S Smolen. *J Rheumatol* 2000; **27**: 205–11.

[2] Glucosamine and chondroitin for treatment of osteoarthritis; a systematic quality assessment and meta-analysis
T E McAlindon, M P LaValley, J P Gulin, D T Felson. *JAMA* 2000; **283**: 1469–75.

BACKGROUND. Increasingly patients, 'informed' by lay publications and the internet, are turning to non-prescription medication for OA (and many other complaints). To date, studies of chondroitin and of glucosamine, two leading preparations in the OA field, have been equivocal. These papers (and others reported during the year investigating possible methods of action) have clarified the advice we can offer our patients regarding these treatments.

INTERPRETATION. [1] Selecting randomized, placebo-controlled studies that included some, at least, of the recommended European League Against Rheumatism criteria for OA trials, seven of the 16 trials identified were further evaluated. All concerned OA knee or hip only. Compared with a reduction in patients on placebo to 65–80% of the level of symptoms recorded at baseline, chondroitin reduced symptoms to 57% of baseline; the difference first reaching statistical significance after 4 months of treatment (suggesting chondroitin is a 'symptom-modifying slow-acting OA drug'). The effect was sustained in studies up to 3 years.

Comment [1]

While meta-analysis is often regarded as the optimal evidence base, the technique shares equivalent disadvantages to retrospective studies—selection bias and publication bias being the most important in this setting. Of concern with this study, the authors state that their selection criteria for inclusion were determined after reviewing the retrieved papers, and it is not clear whether this

assessment of study quality was carried out blinded to the study results. Further caveats with the conclusions of the study were the variation in dose (from 800 to 2000 mg/day) and the use of NSAIDs. The authors of this paper found no correlation between dose and change in VAS for pain. Authors of the original papers in general reported a reduced use of NSAID during their studies suggesting that the benefits could reasonably be attributed to the chondroitin alone. Only two criteria (Lequesne index and VAS for pain) were sufficiently consistent in all studies to be suitable for metaanalysis, and other conclusions such as 'a treatment advantage for chondroitin' in patient and physician global assessments are not supported by the data in the meta-analysis. Intention-to-treat analysis was not used in any of the studies, although drop-out rates were found to be comparable for treatment and placebo groups, and the drug is not considered to have significant adverse effects. On balance, it appears chondroitin does offer a small treatment advantage over placebo in patients already taking NSAIDs—its place as an alternative to NSAIDs remains unknown.

INTERPRETATION. [2] All 15 studies examined concerned knee OA. Study quality ranged from 12 to 55% (of a maximum possible score), with intention-to-treat analysis in only one chondroitin and one glucosamine study. Using the primary outcome originally reported, the effect size (where 0 represents no effect and 0.8 a large effect), was 0.44 for glucosamine (95% Cl 0. 24–0.64) and 0.96 (Cl 0.63–1.3) for chondroitin. Recalculating the effect size using the meta-analysis authors' hierarchy of outcomes, similar effect sizes of 0. 49 and 0.88, respectively, were found.

Comment [2]

This meta-analysis appears to be more rigorously carried out, with a number of the standard devices to identify bias (heterogeneity tests, funnel plots, sensitivity analysis, etc.) reported. The authors identified one study (of chondroitin), which had a con siderably greater treatment effect; when this was excluded, the heterogeneity resolved, and the effect size was reduced from 0.96 to 0.78, which obviously remains considerable and convincing.

The paper is also commendable in that the primary data were reviewed to attempt a more valid meta-analysis using a hierarchy of desirable outcome measures |**3**|, analysing effect size in all studies using this uniform set of outcomes. As most papers had in fact, used at least one of these parameters the effect size did not alter significantly with this re-analysis. Four studies of each drug reported outcomes at 4 weeks, where the effect size appeared to be smaller. This is likely to be a true observation, as effect sizes tend to be overestimated when there are fewer data points. It is also consistent with Leeb's meta-analysis discussed above suggesting that chondroitin is slow acting. It remains to be seen from ongoing research whether this slow onset of effect is related to a disease-modifying effect, although it has been suggested that glucosamine alters

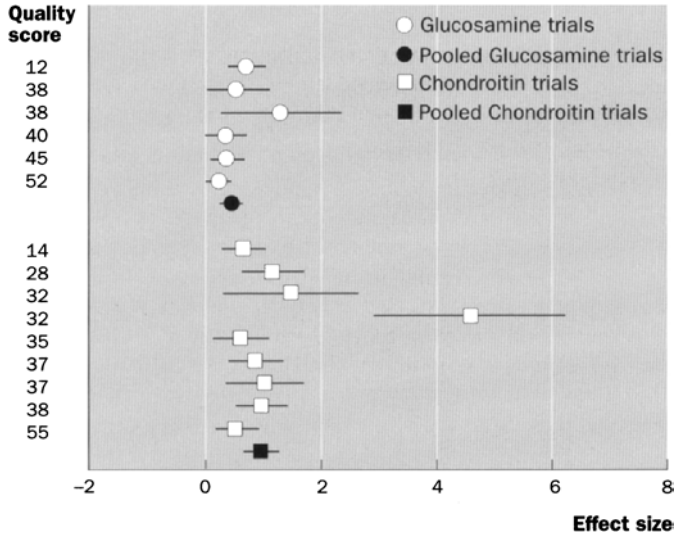

Fig. 12.1 Forest plot of effect sizes for trials and pooled effects. 95% confidence intervals are shown using lines extending from the symbols. Effect size is based on the scale proposed by Cohen |2| in which 0.8 reflects a large effect, 0.5 a moderate effect and 0.2 a small effect. Source: McAlindon *et al.* (2000).

dysregulated matrix metalloproteinase activity. It is important to bear in mind in this regard, that while these studies report only those with OA of the knee, it does not rule out the possibility that other symptomatic joints might also benefit, even if the treatment effect is smaller.

Adhesion of transplanted chondrocytes onto cartilage *in vitro* and *in vivo*.
P J Doherty, H Zhang, V Manolopolous, *et al. J Rheumatol* 2000; **27**: 1725–31.

BACKGROUND. Chondrocyte transplantation has been reported as a novel approach to isolated cartilage defects and has the added benefit of enhanced synthesis of matrix molecules. However, the application of this technology to more diffuse disease has not previously been reported.

INTERPRETATION. Using an animal model of OA (transection of the anterior cruciate ligament), the authors demonstrate that degraded cartilage binds transplanted chondrocytes more avidly, thus targeting treatment. Adenoviral transfection to increase matrix production was also achieved, and the implanted chondrocytes could, *in vitro,* be re-infected more readily than native cells. On histology, the implanted cells were buried deep within matrix, indicating their ability to integrate with existing matrix, and/or to produce significant amounts of new matrix.

Comment

The adhesion of chondrocytes to articular cartilage is essential if the implanted cells are to have a sustained effect, and this study has provided good evidence that this can be achieved. The degradation of cartilage is relatively uniform in this model, however, and it is unclear to what extent the results will be reproducible in human OA, which is more patchy and at different stages at different locations within the joint. Viral transfection of cells is an increasingly useful technique, and the apparent ability of these cells to produce new matrix is potentially valuable. However, others have found that the matrix produced in this way lacks the biomechanical properties of native collagen matrix. Leaving this aside, a further difficulty has been to sustain the transfected population of cells in the joint. The approach in these *in vitro* studies, of re-infecting the cells as virally expressed activity wanes, is interesting— however, avoiding acquired immunity to the transfecting vector is a significant problem that will have to be overcome before this approach can be applied in practice. No doubt we will see further developments in this technology in coming years.

Risk factors for the incidence and progression of radiographic knee osteoarthritis.
C Cooper, S Snow, T E McAlindon, *et al. Arthritis Rheum* 2000; **43**: 995–1000.

BACKGROUND. According to the WHO, knee OA is set to become the fourth greatest cause of disability among women, and the eighth most common in men. Previous studies (including one from the same leading author) were cross-sectional and unable to distinguish between risk factors for incidence and for progression of gonarthrosis. As this might have a significant impact on health promotion strategies, a prospective study was carried out.

INTERPRETATION. In this report, a cohort first recruited in 1990–91 was re-examined. In summary, obesity was the only factor strongly associated with incidence of OA [odds ratio (OR) 18.3] over a 5-year period, with marginally increased OR for previous knee injury (2.9, 95% Cl 1.2–6.7) and for regular sports participation (3.2, Cl 1.1–9.1). In terms of progression, obesity continued to be significant, while pain at baseline evaluation and presence of Heberden's nodes also carried increased ORs. The authors also compared these risk factors using Kellgren/Lawrence grade 1+ or 2+ for the diagnosis of OA. They propose that some risk factors are more associated with osteophyte formation (e.g. sports participation), while others are associated with joint-space narrowing (e.g. obesity).

Comment

Of note in this study, follow-up was by invitation, introducing bias towards worse and/or progressive disease—in all, 76% of the original cohort were reviewed.

It is indeed plausible that 'known' factors influence incidence and progression differently. This is suggested by the relatively small proportions who rapidly deteriorate compared with the large proportion of the population with some degree of OA and by data from genetic studies.

The authors extrapolate from the differences in radiological grades to propose that these risk factors influence different aspects of disease. This is an interesting observation, and one that could be supported by theoretical understanding of OA. Sheer stresses in sports or injured knees could lead to osteophyte formation while loading of the joint in obesity might accelerate joint-space loss. However, the K/L grades were not established to distinguish different types of OA (osteophytic versus joint-space narrowing). Grading implies linear progression, rather than branching into categories; i.e. these risk factors can only be interpreted as influencing incidence or progression, but cannot, on the basis of this study, be implicated in creating different 'subtypes' of knee OA. None the less, a number of studies have been published this year indicating increases in the progression of OA in those with valgus compared with varus deformity, again implying that mechanical factors do play a more complex part than simply loading the joint.

Whether these observations can be translated into risk reduction remains to be seen. That risk factors are more strongly associated with incidence rather than progression would favour primary prevention strategies. Obesity has a sufficiently increased OR in both incidence and progression to be particularly important. However, prevention of knee injury or of sports participation is considerably more contentious.

Linkage analysis of candidate genes as susceptibility loci for osteoarthritis—suggestive linkage of COL9A1 to female hip osteoarthritis.

Z Mustafa, K Chapman, C Irven, *et al. Rheumatology* 2000; **39**: 299–306.

BACKGROUND. OA has not escaped the enthusiasm for identifying genetic associations with disease. Twin studies have estimated the heritability of radiographic OA at certain sites to range from 36 to 68%, with first-degree relatives having over twofold increase in risk, but they also point to a multifactorial aetiology.

INTERPRETATION. Families with at least two members who had had hip or knee replacement surgery were studied in a linkage analysis strategy considering 11 candidate genes. Subgroups (males only affected, females only, hips only, knees only and combinations of these) were analysed in a two-stage approach. Two markers are identified as possible susceptibility loci for hip OA in

women: COL9A1, and a considerably weaker association with a site on chromosome 6p21.3 for HLA/COL11A2.

Comment

Because OA is a multifactorial disease with, at best, a polygeneic association, standard linkage approaches are not sufficiently discriminatory, and the authors offer sound reasoning for their approach. The selection of candidate genes is reasonable, taking into consideration previous studies, what is known of some uncommon monogeneic or Mendelian traits, and genes that could reasonably be expected to be implicated in OA. The usual 5% level of significance was chosen; however, in view of the relatively small group (in terms of genetic linkage studies), it is possible that a type II error may have occurred. That said, the only associations that were excluded by this threshold were at the same loci as those found to be significant in females with hip arthritis, but in different subgroups. This association with a relatively sparse collagen type offers interesting insights into the pathophysiology of joint degeneration, which the authors briefly discuss.

Conclusion

This section has reviewed several treatments for OA, ranging from alternative medicines to experimental transplantation of chondrocytes. The cyclooxygenase (COX) -2-specific anti-inflammatory drugs are also important in treatment of OA and are reviewed elsewhere in this volume. The prevalence (near ubiquity) of OA means that it is important to research thoroughly all proposed therapies, and it is particularly important that alternative or novel treatments are fully assessed. As a large proportion of OA patients is elderly with co-morbid conditions, the possible side-effects of therapies are particularly important. The use of non-selective anti-inflammatory drugs in OA has been criticized in the past because of the unfavourable risk/benefit ratio, but the previous recommendation that analgesics should be used instead has not always been welcomed by patients, many of whom derive more benefit from NSAIDs than from analgesics. The advent of COX-2-selective drugs is important, therefore, as life-threatening gastrointestinal side-effects can be avoided, although their effect on renal function remains of concern. The position of glucosamine and chondroitin in the therapy of OA requires further clarification as a large number of patients are already taking these drugs. In view of their relative safety, even small benefits would be worthwhile, especially if they were more marked in particular subgroups of patients.

The genetic basis of OA is now starting to be understood better; abnormalities in some genes clearly result in premature OA (e.g. type II collagen in Stickler's syndrome); however, even if the same genes are not implicated in 'normal' OA, such findings may give insight into the pathogenic pathways that result in OA.

This in turn may lead to the development of novel therapies or a reassessment of how current therapies may be used to best advantage. Finally, the finding that obesity is a major risk factor for OA suggests that its incidence will increase substantially and strengthens the case for public health measures to decrease levels of obesity. Unfortunately, the other public health message aimed at combating cardiovascular risk factors—take more exercise/play sports—may also increase OA incidence!

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13
Osteoporosis

Introduction

Osteoporosis continues to be a leading public health concern. The selection of papers here aims to reflect the breadth of ongoing osteoporosis research, highlighting both positive and negative studies that one can expect to influence clinical practice in the coming years and those areas where the reader may wish to follow emerging trends.

Alendronate for the treatment of osteoporosis in men.
E Orwoll, M Ettinger, S Weiss, *et al. N Engl J Med* 2000; **343**: 604–10.

BACKGROUND. The Fosamax International Trial (FOSIT) and Fracture Intervention Trial (FIT) trials of alendronate involved postmenopausal women. As there may be significant differences in the pathophysiology of osteoporosis in men, this study is an important addition to the evidence base.

INTERPRETATION. Treatment: alendronate 10 mg or placebo (+500 mg calcium, 400–450 iu vitamin D for all). On intention-to-treat analysis, there were significant increases over baseline bone mineral density (BMD) at all three sites for the treatment group (7.1%, 2.5% and 2% for the neck of the femur, lumbar spine and total body, respectively), which were also significant when compared with placebo. Vertebral fracture rates, assessed by published quantitative and semiquantitative methods, were reduced only by the quantitative method (7.1% versus 0.8% incidence of new fractures, P=0.02). Alendronate was as well-tolerated as placebo.

Comment

Study entry required T-scores of <−2 at the neck of the femur and <−1 at lumbar spine, or <−1 at the neck of the femur together with radiological vertebral deformity or history of osteoporotic fracture. As only half of both groups had one or more vertebral fractures at baseline, the remainder did not have osteoporosis

by WHO criteria. Of note, testosterone levels were assessed at baseline to stratify participants, but men with other secondary causes of osteoporosis were excluded.

This study demonstrates the efficacy of alendronate in increasing BMD in a wide age range of men, irrespective of testosterone level, although the effect was, as expected, greater in eugonadal men. However, fracture prevention is the only rational basis for treatment of osteoporosis. The study is, therefore, deficient in this respect, although the reduction in vertebral fracture rate is certainly encouraging. It is important to bear in mind that the proportion with prevalent vertebral fractures (who are, therefore, at higher risk of further fracture irrespective of their BMD) was high, such that the treatment effect is likely to be smaller in an unselected population. Further studies, with fracture as the end-point, will be necessary before alendronate can be routinely recommended for males with reduced BMD.

A randomised trial of the effects of risedronate on vertebral fractures in women with established osteoporosis.
J Y Reginster, H W Minne, O H Sorensen, *et al.,* on behalf of the Vertebral Efficacy with Risedronate Therapy (VERT) Study Group. *Osteoporosis Int* 1999; **11**(1):83–91.

BACKGROUND. In view of the increased risk of further fracture in the months following a first (usually vertebral) fracture, new bisphosphonates continue to be developed with the aim of a more rapid onset of action. Improved oesophageal tolerability is also desirable.

INTERPRETATION. Treatment: risedronate 2.5 mg or 5 mg/day or placebo (+1g calcium +500 iu vitamin D if pre-treatment level was <40 nmol/l). A statistically significant reduction in relative risk of vertebral fracture rate was evident at the end of the first year in the 5-mg group (61%, number-needed-to-treat=14). The difference at the end of 3 years, although lower remained significant (49%, reflecting a lower rate in both groups in the second and third years). Relative risk of fractures at other sites (a secondary end-point) was reduced by a significant 33% overall in the 5-mg group, but the difference was not significant at the hip, the most important site. A remarkable 91% and 92% in the treatment and placebo groups, respectively, reported an adverse effect. The rates of oesophageal symptoms in non-steroidal anti-inflammatory drug (NSAID) or aspirin users were comparable with placebo at 26%.

Comment

This 3-year trial involved post-menopausal women with established osteoporosis, having, in this case, two or more vertebral fractures. Intention-to-treat analysis of the 5-mg group is reported (the lower dose patients were converted to 5 mg after 2 years following interim analysis). For unexplained reasons, more complex statistical calculations are used rather than simply comparing the proportions with fractures in each group.

Vertebral fractures were identified by annual lateral spine radiographs, and other fractures by history and supporting radiographs. This trial demonstrates a high anti-fracture efficacy in the spine, and shows that this can be achieved in the first year where risk is greatest. However, a treatment option for osteoporosis should protect against all fractures, and hip fracture reduction was not demonstrated in this study. It must be emphasized that these were post-menopausal women with vertebral fractures, and the results should not be applied to women whose only risk is low bone mass. This new bisphosphonate would be particularly welcome if it offered an advantage over existing therapy. The most appropriate comparison is with the first part of the FIT trial with alendronate—the number-needed-to-treat for vertebral fractures here was also 14 (at 3 years). The reported rate of upper gastrointestinal adverse effects was higher in the FIT study (40% in both placebo and treated groups). Certainly it is reassuring to prescribers that upper gastrointestinal adverse effects from risedronate were not increased in users of aspirin or NSAIDs. However, the ideal would be a direct comparison of the two drugs to determine best the place of risedronate in the hierarchy of treatment options.

Prevalence of bone loss with risedronate in glucocorticoid treated rheumatoid arthritis patients.
R Eastell, J P Devogelaer, N F A Peel. *Osteoporosis Int* 2000; **11**:331–7.

BACKGROUND. The place of alendronate and etidronate in the prevention and treatment of corticosteroid-induced osteoporosis is established. Now risedronate is tested in a corticosteroid-induced osteoporosis risk group in whom rheumatoid arthritis poses additional risks, through immobility and systemic disease activity.

INTERPRETATION. Treatment: 2 years cyclical risedronate (15 mg/day for two out of every 12 weeks) or continuous risedronate (2.5 mg/day). During the 2 years, active treatment with risedronate maintained BMD while those on placebo lost bone. In a third follow-up year without treatment, bone loss occurred but was not accelerated in the treatment groups. The daily regimen appeared to be more efficacious. Risedronate was as well tolerated as placebo, even among NSAID users.

Comment

An unusual feature compared with most intervention trials with bisphosphonates is that, rather than universally supplement with calcium (with or without vitamin D), this study adjusted for daily calcium intake (by questionnaire) in their analysis. The effect of this is not clear, but is likely to be trivial as the mean intake was in excess of 800 mg in all groups.

While analysis of covariance for per cent changes in BMD was used during treatment, slopes of regression lines to determine differences in rate of bone loss

were used for the third, off-treatment, year. The reasons for not consistently using the same method are not made clear to a non-statistician reader. In any event, the differences throughout the study are small bearing in mind the coefficient of variation in repeated BMD measurements, and the fairest conclusion is that BMD is probably maintained while on 2.5 mg daily treatment. The difference between the daily and cyclical regimens is surprising, given the findings of equivalence in preliminary studies comparing daily and pulse or cyclical regimens of alendronate or ibandronate. While the dual-energy X-ray absorptiometry (DXA) scan at week 97 (study end) came 3 months after the last treatment for the cyclical group, such a rapid loss of effect is unlikely to be the explanation for the difference between cyclical and continuous therapy, given that a single remodelling cycle takes about 100 days.

A final point to emerge in the study is the significant rate of BMD loss in the placebo group, even though only 23% were taking doses of 7.5 mg or more of prednisolone, the threshold for intervention in the most recent guidelines.

Reduction of vertebral fracture risk in post-menopausal women with osteoporosis treated with raloxifene.
B Ettinger, D M Black, B H Mitlak, *et al. JAMA* 2000; **282**:637–45.

BACKGROUND. The efficacy of oestrogen in fracture prevention has been demonstrated in only one small study. The anti-fracture efficacy of raloxifene, a selective oestrogen receptor modulator (SERM), which inhibits bone resorption without stimulating the endometrium or breast, is now investigated.

INTERPRETATION. Treatment: 3 years of 60 mg or 120 mg raloxifene daily or placebo, +500 mg calcium, 400–600 iu vitamin D. Women with T scores <−2.5 only were randomized separately from those who additionally had fractures (those with two or more fractures or >25% loss of vertebral height were included irrespective of their BMD). There was a significant reduction in vertebral fracture risk on treatment (reduction in relative risk 30–50%). The two doses gave comparable results in the non-fracture group, with a small advantage from the 120-mg dose in the prevalent fracture group. Hip and other non-vertebral fracture risk was not reduced. There was a threefold higher risk of venous thromboembolic events (despite excluding those with a past history of these conditions), and a higher rate of withdrawal compared with placebo, mainly because of hot flushes. Breast cancer risk was reduced by a third.

Comment

Most women abandon hormone replacement therapy (HRT) within 1 year of treatment due to concerns about breast cancer risk, together with a high rate of adverse effects. Tamoxifen, the prototype SERM, with its beneficial effect on breast cancer, appeared also to have beneficial effects on BMD. Now, fracture prevention has also been demonstrated in a SERM, with a number needed to

treat of 10–16, comparable with risedronate or alendronate in those with prevalent fracture. The authors state that there was no significant difference in the 60 and 120 mg doses in the non-fracture group, although numbers needed to treat are given as 46 and 59, respectively. All vertebral radiographs were assessed with robust quality control in a single centre, an important point in a study carried out in 125 centres. Other small points relating to statistical methods do not upset the validity of their conclusions (e.g. intention-to-treat analysis is described, although the statistics are provided on a table where the data are said to be from completers only). As already discussed with respect to risedronate (see above), treatment should protect against all fractures; however, the authors point out that this study only had 12% power to detect a significant difference in hip fracture rates, and they are following the cohort for a further year to evaluate the cumulative fracture incidence. This study confirms and clarifies the place of raloxifene in post-menopausal osteoporosis.

Osteoporosis in elderly men and women: effects of dietary calcium, physical activity and body mass index.
T V Nguyen, J R Center, J A Eisman. *J Bone Miner Res* 2000; **15**:322–31.

BACKGROUND. Conflicting evidence from observational studies has suggested that physical activity, calcium intake and body mass may influence BMD and/or fracture risk. This study aimed to investigate the impact of these factors in an older population, where the fracture risk is greatest.

INTERPRETATION. Calcium intake, body mass index (BMI) and quadriceps strength correlated with BMD. In a mathematical model combining these, using the lowest tertile as a cut-off, the prevalence of osteoporosis increased fivefold (in women) to 30-fold (in men) in the small proportion (4.4 and 5.5%, respectively) with all three 'risk factors'.

Comment

This study highlights the apparent consequences of deficiency in calcium intake in an elderly population, but cannot be interpreted as evidence that calcium supplementation will improve BMD, although studies have found that calcium with vitamin D reduced hip fracture risk in the elderly. Quadriceps strength continued to have a positive correlation with BMD, although a physical activity index did not, when corrected for age, BMI and the other parameters in the multiple regression model. This, and the correlation with BMI itself, may relate to constitutional factors and body habitus, which may indirectly influence BMD (e.g. vitamin D receptor gene polymorphisms correlate with body mass and with BMD). Overall, the effect of each was small, accounting for only 1–4% of the variance in BMD measurements. By comparison genetic factors account for 60–80% of the variance in BMD in reported studies. However, a recently reported

study in an elderly population of twins, found concordance rates for fracture even among monozygotic twins to be only 10%. Therefore, while BMD is an important risk factor for fracture, numerous other factors are important, particularly in the elderly (falls, cardiovascular disease, etc.). It is disappointing that the authors reported correlations with BMD instead of with fracture risk, despite having reported the fracture incidence in this same cohort in a study in 1994. Cross-sectional studies are of limited value as causal relations between variables cannot be identified with certainty. However, it would appear that the generally accepted benefits of exercise and calcium intake may also be applied to older age groups.

No permanent reduction in bone mineral density during treatment of polymyalgia rheumatica and temporal arteritis using low dose corticosteroids.

G Haugeberg, G Myklebust, H Dovland, *et al. Scand J Rheumatol* 2000; **29**(3):163–9.

BACKGROUND. The study examined BMD in patients with polymyalgia rheumatica (PMR) or temporal arteritis (TA), currently or previously treated with prednisolone. BMD (using single X-ray absorptiometry or DXA) was measured in radius, spine, and hip in 26 currently and 28 previously prednisolone-treated patients with PMR (*n*=38) or TA (*n*=16). The prednisolone-treated patients were compared with 30 newly diagnosed patients with PMR (*n*=26) or TA patients (*n*=4) examined prior to starting prednisolone, and 70 healthy controls.

INTERPRETATION. BMD was not shown to be substantially reduced in PMR and TA patients currently or previously treated with mean low-dose prednisolone. However, a tendency to a lower BMD was found in PMR/TA patients currently treated with prednisolone and in the prednisolone-treated TA patients. A small tendency to an increase in vertebral fracture rate was also seen in the treated groups, although it was statistically insignificant in this small sample size.

Comment

This is an important clinical question but difficult to study due to sample sizes, variation in patient characteristics, dose and duration of steroid, and coexisting disease. Several confounding factors existed. There was a large variation in individual doses of steroid, in particular in the PMR group. A number of different antiresorptive drugs were used in the treated groups and three patients had coexisting thyroid disease. Disease duration is not clearly stated. The currently treated and previously treated groups are combined for some analyses, which may be inappropriate.

In spite of the difficulties with this study, it does address an important clinical question and larger studies are warranted.

[1] Six months daily administration of parathyroid hormone and parathyroid hormone-related protein peptides (PTHrP) to adult ovariectomized rats enhances bone mass and biomechanical properties: a comparison of human parathyroid hormone 1–34, parathyroid hormone-related peptide 1–36, and SDZ-parathyroid hormone 893.

A F Stewart, R L Cain, D B Burr, *et al. J Bone Miner Res* 2000; **15**:1517–25.

[2] Enhancement of bone mass in osteoporotic women with parathyroid hormone followed by alendronate.

R S Rittmaster, M Bolognese, M P Ettinger, *et al. J Clin Endocrinol Metab* 2000; **85**:2129–34.

BACKGROUND. The value of parathyroid hormone (PTH) in osteoporosis as a bone-forming agent has been recognized for some time, although there have been concerns about rapid loss of the bone so gained. Further clinical trials are adding to our understanding of how to use this therapeutic option.

INTERPRETATION. [1] Studies with biochemical markers suggest PTHrP may be a purely anabolic agent (the other forms increasing bone turnover with a net gain), and the Sandoz (SDZ-893) variant was developed for greater receptor affinity. Using a variety of biochemical, densitometric, histomorphometric and biomechanical tests, SDZ-893 was the most potent agent, but at the expense of hypercalcaemia in most animals (fatal in 13%), and was associated with a trend towards increased serum creatinine. Human PTH 1–34 was intermediate in potency with hypercalcaemia in one animal, while PTHrP was significantly less efficacious on histomorphometry parameters, and correspondingly less impressive in the other areas. However, there were no adverse effects in these animals either.

Comment [1]

In terms of methodology, these authors have carried out a comprehensive investigation of PTH effects on bone, itself a useful publication, although replicating work carried out by various authors in the past. It further reassures that increases in cancellous bone density and biomechanical properties are not necessarily at the expense of cortical bone. Note that total bone mineral (ash weight) of the radius increased (comprising both cortical and trabecular bone in differing proportions along its length), although this does not prove that cortical bone was not lost.

The most significant result, considering how PTH might be used in the future, is the fatal hypercalcaemia induced by the most potent agent. As dose-ranging was not a part of this study, it is unclear whether this is a specific toxicity/benefit ratio that applies to the SDZ-893 agent, or a dose-response effect that will be seen in all PTH therapy. In particular, the authors state that the dose of PTHrP was merely chosen to be the same as the other agents. However, studies in humans have shown that PTHrP is absorbed and cleared more rapidly, thereby

requiring a higher daily dose to achieve the same steady-state levels. This study, therefore, cannot be taken as conclusive evidence favouring human PTH over analogues.

INTERPRETATION. [2] Following a 1-year placebo-controlled study of recombinant PTH (1–84) daily by subcutaneous injection at varying doses, 75 women took alendronate 10 mg daily, with no placebo arm. Results are for 66 women who completed the study. There was no significant change in BMD at lumbar spine or femoral neck during the second year, with the substantial gains over placebo from PTH therapy maintained.

Comment [2]

PTH-induced increases in BMD are considerably more than seen in bisphosphonate studies. However, a small decrease, reaching statistical significance, in whole body BMD, reflects concerns that PTH may cause bone loss at peripheral, cortical bone sites. Oestrogen taken concurrently can prevent this, and has also been shown to maintain PTH gains when taken after PTH. Taking alendronate, the gains at hip and spine were maintained, and indeed there was a further increase in all groups (as might be expected from alendronate, irrespective of previous treatment). Reassuringly, the whole body BMD also recovered. It is not known whether a bisphosphonate could be given concurrently with PTH, although there are physiological reasons to suspect this would be counterproductive (to increase bone formation requires increased turnover, which would be inhibited by the bisphosphonate). The fact that PTH is a bone forming agent and that alendronate is not is reflected in the changes in bone turnover markers, with significant rises in the formation markers osteocalcin and bone alkaline phosphatase while on PTH, which then return to baseline. Unfortunately, there was no placebo arm in this follow-on study, so we cannot judge the rate at which bone loss might have occurred in this cohort without follow-on therapy. This study increases understanding of how PTH might best be used, although whether the gains in BMD will translate into equivalent gains in fracture prevention will require larger studies.

Stimulation of bone formation in vitro and in rodents by statins.
G Mundy, R Garrett, S Harris, *et al. Science* 1999; **286**:1946–9.

BACKGROUND. The search for bone-forming agents continues, in an effort to recover bone already lost in osteoporotic individuals. While such activity has been reported for a small number of compounds (PTH being the most promising), identifying other potent options would be welcome.

INTERPRETATION. A luciferase reporter gene was linked to the BMP-2 gene promoter in an osteoblast cell line; the statin lovastatin had a positive

effect, i.e. it produced increased levels of luciferase, which is easily measured due to its ability to emit light. This implies that the statin acts on the BMP-2 promoter and would increase BMP-2 levels. Simvastatin was then assessed by a different BMP-2 assay, again proving to have a stimulatory effect on BMP-2 production. The bone-forming effect of a number of related statins was then demonstrated *ex vivo* and *in vivo,* simvastatin and lovastatin being further tested as oral agents in ovariectomized and control rats by histomorphometric analysis of trabecular bone. A mechanism of action is proposed that parallels that suggested for the aminobisphosphonates, interfering with prenylation of intracellular signalling proteins such as Rho, Ras, but acting primarily in osteoblasts instead of osteoclasts.

Comment

This beautifully simple assay allowed over 30 000 natural compounds to be screened for their effects on BMP-2 production. The methods are scientifically robust, with appropriate controls, although there are no details of the actual numbers treated in some of the biological assays (graphs are simply illustrated with significant *P* values); there were 10 animals in each of the oral treatment groups. While osteoporosis is broadly a combination of reduced bone formation and increased resorption, the balance and coupling of these processes varies according to the precise aetiology. To date, oestrogen withdrawal has primarily been associated with release from inhibition of osteoclastogenic factors. Therefore, it is remarkable that the statins had such a marked effect on ovariectomized animals, and it will be interesting to confirm and compare these findings in osteoporosis where reduced formation dominates. Currently available statins, having a high first-pass metabolism, have targeted cholesterol synthesis in the liver. If agents are to be used as bone-forming therapy in osteoporosis, more systemically available forms will be required, which may adversely affect their tolerability. However, a reduced risk of fractures has been found in case-control studies of patients taking statins |**1**|.

Monitoring osteoporosis therapy with bone densitometry.
S R Cummings, L Palermo, W Browner, *et al. JAMA* 2000; **283**: 1318–21.

BACKGROUND. It is desirable with any intervention to be able to assess individually benefit from that treatment. With osteoporosis this is somewhat more difficult, as efficacy would mean a fracture not occurring, and bone density is used as a surrogate marker. However, the reliability of such monitoring has not been assessed to date.

INTERPRETATION. Using the data from the low-dose arm of FIT (alendronate) and Multiple Outcomes of Raloxifene Evaluation (MORE) (raloxifene) trials, the authors compared the change in BMD at the end of the first year, with the overall change at the end of the 2-year treatment or placebo. This study illustrates the phenomenon of regression towards the mean, where BMD

changes in the first year ranged from over 10% gained to more than 6% loss (in the case of alendronate), yet the majority of women had a net gain after 2 years of 3–6% (hip and spine, respectively). The same was observed in the placebo group of both studies, indicating that this phenomenon relates to random error in measurement and is independent of the biological effect of the treatment.

Table 13.1 Change in femoral neck bone mineral density (BMD) during year one compared with the probability of gaining BMD and the mean percentage gain in BMD during the second year of treatment with raloxifene hydrochloride*

Percentage change in BMD during Year 1	No. of participants	No. (%) who gained during Year 2	Mean percentage change during Year 1†	Mean percentage gain during Year 2†
<−4 (loss)	317	251 (79)	−6.5 (−6.7 to −6.2)	4.0 (3.4 to 4.5)
−4 to <−2	412	280 (68)	−2.8 (−2.9 to −2.8)	1.8 (1.4 to 2.2)
−2 to 0	663	454 (68)	−0.9 (−1.0 to −0.9)	1.6 (1.3 to 1.8)
0 to < 2	845	469 (56)	−1.0 (1.0 to 1.0)	0.4 (0.2 to 0.6)
2 to < 4	733	318 (43)	3.0 (2.9 to 3.0)	−0.4 (−0.7 to −0.2)
4 to < 6	503	190 (38)	4.9 (4.8 to 4.9)	−1.2 (−1.5 to −0.8)
6 to < 8	242	88 (36)	6.9 (6.8 to 7.0)	−1.2 (−1.7 to −0.7)
> 8 (gain)	239	53 (22)	10.7 (10.4 to 11.1)	−2.8 (−3.4 to −2.2)

*Data from the Multiple Outcomes of Raloxifene Evaluation.
†95% confidence intervals are in parentheses.
Source: Cummings *et al.* (2000).

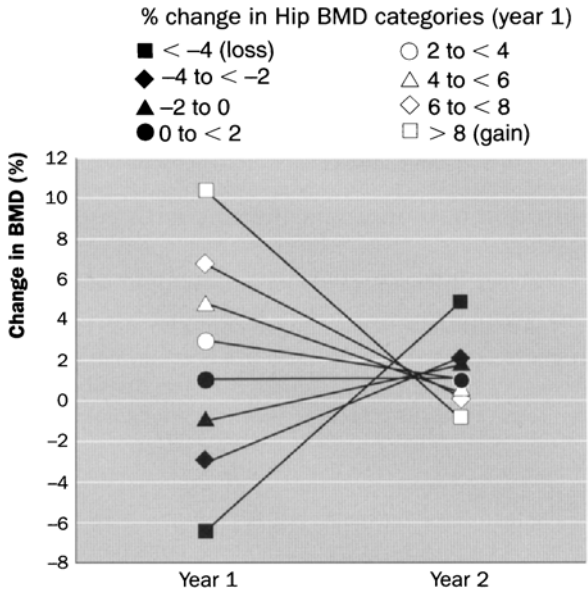

Fig. 13.1 Change in total hip BMD during the first year of treatment with 5 mg/day of alendronate sodium compared with the probability of gaining hip BMD and the average change in BMD observed the next year of treatment. Source: Cummings *et al.* (2000).

Comment

This study demonstrates the weakness inherent in repeated measurements when the test has a high coefficient of variation (1–2%) in relation to expected change on treatment (3–6% in most 2–3-year studies). The authors raise a number of concerns about the value of monitoring, and indeed the significance of repeated measurements in those not taking treatment in deciding if they are losing bone at an accelerated rate. The only drawback in the study was that it analysed data from the low-dose alendronate arm, rather than the higher dose currently used in practice. However, while the magnitude of the treatment effect was greater with the higher dose, regression towards the mean is independent of treatment (as demonstrated by the placebo group) and it is unlikely that the results, or the conclusion, would differ significantly.

Quantitative ultrasound or clinical risk factors—which best identifies women at risk of osteoporosis?
A Stewart, D M Reid. *Br J Radiol* 2000; **73**:165–71.

BACKGROUND. While guidelines for referral for bone densitometry based on clinical risk factors have been published, they are not specific (68% of patients referred were found to be normal on DXA scanning in a recent study), and a more selective screening tool would help reduce demands on DXA services. Ultrasound is being investigated in this role.

INTERPRETATION. In a cohort of 250 women already 'selected' by being referred for DXA by their general practitioner, calcaneal quantitative ultrasound (QUS) was more sensitive and specific for, and had a higher agreement with, the T-score category defined by DXA, as compared with a questionnaire identifying clinical risk factors for osteoporosis.

Comment

We are not told the questions asked in the questionnaire, and cannot therefore judge whether a weighting system would have been appropriate. While most published questionnaires have been designed to identify risk of having osteoporosis, the DXA results reflect the risk of fracture. The comparisons of these risk profiles, then, may not be valid—as acknowledged by the authors. The agreement as a κ statistic between spine and hip DXA T-scores is used as the benchmark in the discussion. As identified in a number of published reports, the agreement was relatively low (0.33). However, both measures are taken simultaneously, and the patient diagnosed as osteoporotic if the T-score at either site is <-2.5. Therefore, a fairer comparison would be between the QUS or risk factors and the combined DXA result at either site. Similarly, a stiffness index combining the broadband ultrasonic attenuation and velocity of sound data from QUS have been shown to improve sensitivity for fracture risk, and this comparison would have been helpful. Of some concern, supporting the worries

of many clinicians in the field, is the poor agreement (κ=0.23) between broadband ultrasonic attenuation measured by two different machines. The study suggests that QUS is superior to a simple questionnaire in selecting patients for DXA, but also highlights the fact that, unless and until T-score or alternative categories are defined for QUS, BMD remains the definitive test for osteoporosis.

[1] Monitoring individual response to hormone replacement therapy with bone markers.
P Delmas, P Hardy, P Garnero, M P Dain. *Bone* 2000; **26**:553–60.

[2] Early response in biochemical markers predicts long-term response in bone mass during hormone replacement therapy in early postmenopausal women.
N H Bjarnason, C Christiansen. *Bone* 2000; **26**:561–9.

BACKGROUND. If monitoring treatment with BMD is unhelpful for individual treatment decisions, monitoring with biochemical markers of bone turnover might be more useful. These have previously been shown to correlate with changes in BMD in osteoporosis, and with response to treatment in cohorts of patients.

INTERPRETATION. Osteocalcin and bone-specific alkaline phosphatase (formation markers), and the resorption markers, serum and urine c-telopeptide of type I collagen (sCTX, uCTX or Crosslaps®), were analysed in two 2-year studies of HRT. Receiver-operator curves and logistic regression analysis were used to determine best combinations and optimal cut-offs for optimal sensitivity and specificity. Resorption markers were more sensitive than formation markers and responded earlier (within 6 months compared with 12 months). Per cent change over baseline was more sensitive than absolute values. uCTX performed best overall in both studies.

Comment

Delmas *et al.* categorized patients as responders or non-responders if BMD increased or decreased by at least 2.26% (the least significant change given the coefficient of variation for BMD). Emphasis was on identifying responders. However, in clinical practice, the concern is to identify non-responders so that treatment can be changed without delay. With specificity set at 90%, best sensitivity (combined 3 and 6 months' per cent change in uCTX) was 74%, positive predictive value 91%. Therefore, if decisions were based on the bone turnover markers, only 64% would have had appropriate treatment advice (10% told to continue treatment but would be non-responders, 26% told to stop treatment but would be responders). However, the authors' definition of 'responders' may be disputed, as patients who were rapidly losing bone would be biological responders if their BMD merely remained stable, and this would be reflected in reduced turnover markers. The apparent utility of turnover markers

in their analysis is, therefore, diminished—by increasing 'false positives', i.e. patients with reduced turnover markers but defined by BMD as 'non-responders'.

The second study chose to define response as any gain in BMD, and non-response as any fall, a threshold that is probably more appropriate. However, as the potential numbers of responders and non-responders is greater, there is an apparent difference in sensitivity and specificity compared with the results of Delmas *et al.* Receiver-operator curves were again used, these authors identifying two cut-off values to offer different levels of specificity, reporting the corresponding sensitivity, positive predictive value and negative predictive value. However, analysis was again directed at identifying responders.

Appraisal of these studies would suggest that the enthusiasm for monitoring treatment efficacy is not yet justified. Until greater sensitivity to change can be achieved, considerable numbers will have inappropriate changes to their treatment, compared with the outcome without knowledge of turnover markers.

Conclusion

This chapter has highlighted several developments in osteoporosis research; further support for the use of bisphosphonates is provided, including the newer compound risedronate, and raloxifene represents an additional useful drug in post-menopausal osteoporosis. However, in each of the trials reported it is important to note precisely what a drug has been demonstrated to do. Does it increase bone density, and if so at what site? Does it decrease fracture rate, and if so at what site? In what group of patients was efficacy shown? Because large trials are required to demonstrate statistically significant effects on fracture rate, such data may not be generated in the first trials of a new drug, and BMD measurements reported instead. However, it is important to continue to conduct trials with the power to determine that clinically relevant events such as fracture or loss of height are being affected by a particular treatment. Meanwhile experimental studies point to the possible use of statins in osteoporosis and alternative therapies such as PTH.

In a condition that has such a high prevalence as osteoporosis it is particularly important to devise cost-effective measures to identify the population that requires treatments and can tell the physician whether treatment has been successful. An alternative to DXA would, therefore, be very desirable, given the problems of time and expense not to mention exposure to X-ray radiation. QUS continues to be developed, but better still would be biochemical analyses, which lend themselves to rapid screening of large numbers of patients. This area needs more development. Perhaps the identification of genes involved in the pathogenesis of osteoporosis will also aid in defining the at-risk population or the subjects most likely to respond to particular treatment modalities.

References

1. Cummings SR, Bauer DC. Do statins prevent both cardiovascular disease and fractures? [Editorial]. *JAMA* 2000; **283**:3255–7.

14
Soft tissue rheumatism

The group of disorders sometimes grouped together as 'soft tissue rheumatism' have a very high prevalence, account for the largest numbers of consultations in primary and even secondary care, and have significant socio-economic effects. Therefore, research into their diagnosis, aetiology and treatment is of considerable importance.

The papers included in this chapter deal with several major disorders: Carpal tunnel syndrome (CTS); tendinitis, particularly of the tendo Achilles; disorders of the shoulder; and fibromyalgia. In carpal tunnel the emphasis is on accurate clinical diagnosis, which should lead to optimal management. For tendon disorders, new imaging modalities (magnetic resonance imaging [MRI] and ultrasound) are also providing useful information on the precise structures involved, while results of treatment and the pathological processes underlying disease have received attention in the last year. Shoulder pain is responsible for significant morbidity and loss of employment in the community, but with a history of relatively little investigation into causation and effective treatment (as emphasized in the Cochrane review—see Green *et al.*). Better definition of the components of shoulder movement implicated in different clinical disorders may lead to better management. Lastly, fibromyalgia continues to provide challenges diagnostically and the search for a possible underlying 'physiological' abnormality continues, although many would regard the disorder as being primarily one of pain perception, which could 3 to secondary abnormalities.

A diagnostic algorithm for carpal tunnel syndrome based on Bayes' theorem.
D O'Gradaigh, P Merry. *Rheumatology* 2000; **39**:1040–1.

BACKGROUND. The aim of this study was to construct a diagnostic algorithm based on Bayes' theorem and simple clinical tests to allow accurate diagnosis without resort to nerve conduction studies. A cohort of patients with possible CTS had clinical and electrophysiological testing, from which sensitivity, specificity and prevalence were made, and then used in the formula of Bayes' theorem. The algorithm was then tested in a prospective study.

INTERPRETATION. An algorithm was developed that proved to be reliable and was similar to nerve conduction studies when tested prospectively.

Comment

The diagnosis of CTS is dependent upon clinical symptoms, nerve conduction studies and response to treatments. Clinicians vary greatly in their views on the relative importance of these approaches, and in particular the role of nerve conduction studies is a topic of much debate.

The patients in the initial phase of the study were defined as either classical of probable CTS on the basis of the distribution of their pain. Each then had a Tinel's and a Phalen's test, and then nerve conduction studies. The sensitivity and specificity of the clinical tests were measured against the nerve conduction study, taken as the definitive test. It could be argued that a more appropriate standard of reference would have been a response to decompression. Some parameters assessed in nerve conduction studies have been shown to be sensitive and specific, but the variation in those parameters measured, as discussed elsewhere, is underestimated and may be dependent upon the observer.

The results from this study do indicate that when the clinical suspicion is strong, nerve conduction studies are not necessary, but when the diagnosis is less clear on clinical grounds, studies are warranted.

A neurophysiological grading scale for carpal tunnel syndrome.

J D Bland. *Muscle Nerve* 2000; **23**(8):1280–3.

BACKGROUND. The severity of CTS can be defined in terms of clinical and neurophysiological criteria and numerous scores exist for this purpose. This paper aimed to describe a new neurophysiological grading score and to examine the relationship between the neurophysiological grading and a numerical score derived from the clinical history.

INTERPRETATION. A neurophysiological grading score was described. A highly significant linear relationship between the neurophysiological and clinical grading scores was noted. Patients with more characteristic stories of CTS generally had higher neurophysiological grades.

Comment

Laboratories vary in the specific neurophysiological parameters used in the diagnosis of CTS and the specific cut-off levels set as being indicative of CTS. In this study a numerical scale was developed, which included six grades of severity based on electrophysiological characteristics. Clinical symptoms were also recorded using a single page questionnaire and a logistic regression model was generated using the presence of absence of neurophysiological abnormality as a

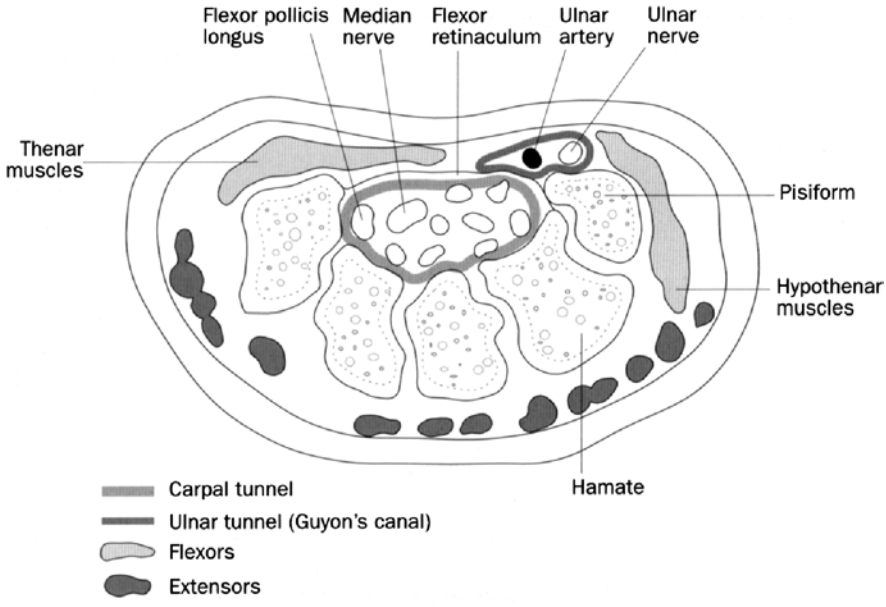

Fig. 14.1 The ulnar tunnel (Guyon's canal). Because of the close relationship of the ulnar tunnel to the carpal tunnel, pathology affecting one may simultaneously affect the other. Source: Bland (2000).

dependent variable. This model was found to have a predictive value in finding an electrophysiological abnormality at the wrist in 80%, although an 'appreciable' false positive rate was also noted.

The symptom scores were then compared with the neurophysiological gradings. In bilateral cases the side with the worst neurophysiological grading was taken for comparison. The sample size was large (8501), with subjects falling in particular into the normal (grade 0) and severe (grades 5 and 6) ends of the neurophysiological grading spectrum. Linear regression (Pearson) coefficient for clinical symptoms versus neurophysiological gradings was 0.4728 (P<0.0001). Comparison of each pair of adjacent groups (on the basis of nerve conduction studies) showed each group to be significantly distinct from the rest.

Test-retest reliability of combined sensory index: implications for diagnosing carpal tunnel syndrome.
H L Lew, L Wang, L R Robinson. *Muscle Nerve* 2000; **23**(8): 1261–4.

BACKGROUND. The combined sensory index (CSI), the sum of individual nerve conduction tests, has previously been reported to have superior sensitivity and specificity compared with single nerve conduction tests for the diagnosis of CTS. In this paper, the test-retest reliability of

single nerve conduction tests (ring-diff, thumb-diff and palm-diff; see below for definition of these terms) versus a CSI was examined.

INTERPRETATION. The CSI had the highest test-retest reliability (Spearman rho=0.95), when compared with ring-diff, thumb-diff and palm-diff (Spearman rho=0.67, 0.75 and 0.74, respectively). The authors proposed the use of the CSI as not only an accurate but also a reliable method for diagnosing CTS.

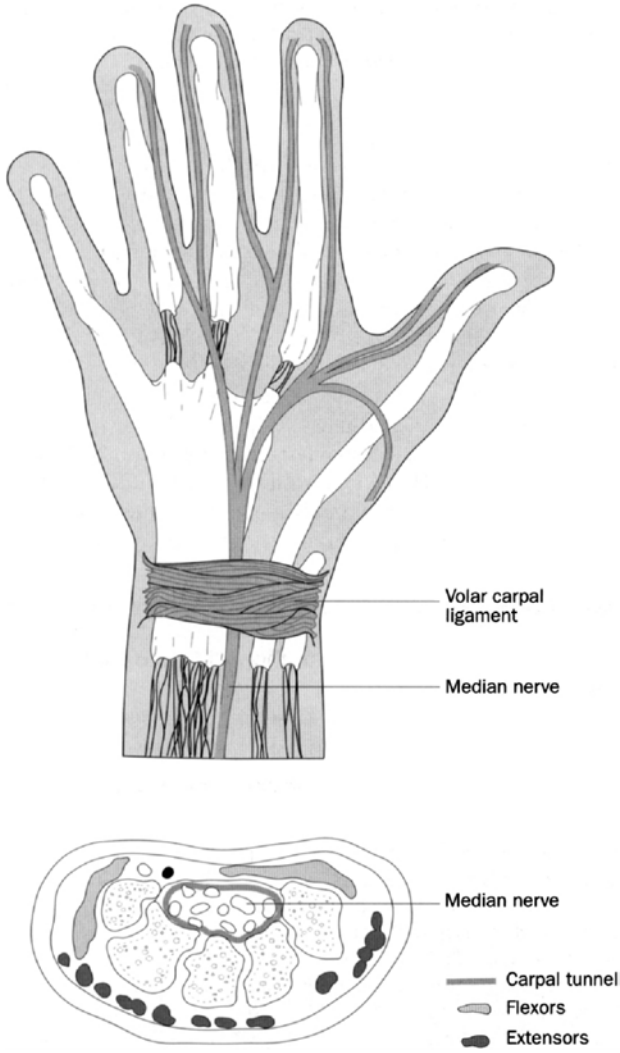

Fig. 14.2 The carpal tunnel and the course of the median nerve. The space for the median nerve and flexor tendons is very confined. The median nerve courses under the region occupied by the palmaris longus and flexor carpi radialis tendons. Source: Bland (2000).

Comment

There are many nerve conduction tests used for the diagnosis of CTS and there is no uniform opinion as to which is the best. The mean sensory nerve latency across the wrist is popular as sensory fibres are commonly affected early in the condition. Although findings in the symptomatic hand can be compared with the contralateral hand, it is often preferred to compare those in the affected median nerve to those in the radial and ulnar nerves in the same hand. The CSI combines multiple test results with the aim of improving sensitivity and specificity.

The CSI is the sum of: (a) median-ulnar ring finger antidromic latency difference at 14 cm (ring-diff); (b) median-radial thumb antidromic latency difference at 10 cm (thumb-diff); and (c) median-ulnar midpalmar orthodromic latency difference at 8 cm (palm-diff). The high test-retest reliability of the CSI demonstrated by one observer is encouraging. However, inter-tester reliability has not been assessed and it is important for a test to be clinically useful in practice and in research. The relationship between the CSI and the extent of clinical symptoms and prognosis is undetermined.

Autonomic dysfunction in idiopathic carpal tunnel syndrome.
J Verghese, A S Galanopoulou, S Herskovitz. *Muscle Nerve* 2000; **23**(8): 1209–13.

BACKGROUND. A prospective, systematic study of autonomic disturbances in 139 limbs (76 patients) with CTS.

INTERPRETATION. Autonomic symptoms were reported in 76 limbs (47 patients). Sympathetic skin response (SSR) had a sensitivity/specificity ratio of 34/89% in CTS with autonomic symptoms. The presence of autonomic disturbances was significantly associated with female gender (odds ratio 4.06, 95% Cl 1.5–11.4, P=0.007), SSR abnormalities (odds ratio 4.3, 95% Cl 1. 6–11.4, P=0.003), and severity of electromyographic findings (odds ratio 1.8, 95% Cl 1–3.3, P=0.04) but not age, duration of disease, or clinical severity in a binary logistic regression model. Autonomic disturbances are common (55%) in CTS, occurring with increasing severity of electrophysiological findings.

Comment

Although the sensorimotor manifestations of CTS are well known the autonomic disturbances, although recognized, are not well documented. This study aimed to investigate autonomic symptoms in patients with a diagnosis of CTS. The authors developed a questionnaire for this purpose and validated the questionnaire, obtaining a good inter-rater reliability (κ value of 0.85) and excellent internal consistency (Cronbach's a coefficient of 0.9). Subjects were asked about the presence or absence of swelling, skin dryness, Raynaud's phenomenon, fingertip ulceration and nail changes. Sympathetic skin response

(an electrophysiological test) was also evaluated. Patients were examined to confirm symptoms of swelling and skin dryness.

Unfortunately, there was no control group in this study and most subjects (not surprisingly) were women. Hormonal and occupational effects were not explored. The effect of treatment of CTS on the autonomic disturbances in this group is being evaluated.

Automatic carpal tunnel syndrome tester.
E Stalberg, S Stalberg, L Karlsson. *Clin Neurophysiol* 2000; **111** (5): 826–32.

BACKGROUND. The authors aimed to develop and evaluate a device for automatic testing of sensory latency across the carpal tunnel.

INTERPRETATION. A device for automatic testing of sensory latency across the carpal tunnel is described.

Comment

The authors aimed to develop an automatic technique for the assessment of sensory changes across the carpal tunnel. This included the construction of a mechanical device, the development of analysis algorithms and the evaluation of the sensitivity, accuracy and usefulness of this approach.

The device consisted of a fixed bar containing a stimulating electrode distally and a bipolar recording set up with the two poles at 7 and 14 cm distance proximally. The device is thus based on the so-called 14/7 method, using orthodromic testing. Automatic testing of electrode resistance and muscle relaxation precedes the self-triggered start of stimulation. The latencies are analysed according to a special algorithm. The proximal/distal latency index is increased in CTS. Healthy controls (32 hands in 32 subjects) and patients referred with suspicion of CTS (258 hands) were studied with the new device, by conventional technique and sometimes by inching (centimetring) across the carpal tunnel. A positive CTS result with the tester was in accordance with the routine test in 94% of the patients. Routine studies showed CTS in 118 hands; among those, the tester agreed in 44, showed no signals in 70, and indicated normal findings in four hands. In cases with a combination of polyneuropathy and CTS, the CTS was detected in five of six cases. A complete test takes about 1 min per hand.

The advantages of this approach are its speed and sensitivity for mild and moderate cases of CTS and it avoids inter-observer variability. Additional neurological disorders will not be detected by this technique. Larger studies are needed to assess the method further. Although, as the authors acknowledge, this study did not evaluate the relationship with clinical findings, this is an important area to examine in the future.

Tethered median nerve stress test in the diagnosis of carpal tunnel syndrome.
F Raudino. *Electromyogr Clin Neurophysiol* 2000; **40**:57–60.
BACKGROUND. The sensitivity of the median nerve stress test (stress test) described by LaBan *et al.* |1| was evaluated in 140 arms with CTS.
INTERPRETATION. The test was generally of low sensitivity.

Comment

A wide number of 'diagnostic' tests are described in the literature for the diagnosis of specific soft tissue complaints. However, most have not been validated and, unfortunately, many serve to add to the diagnostic challenge of making an accurate diagnosis. In this paper the median nerve stress test was evaluated in 140 subjects in whom abnormal nerve conduction studies suggestive of CTS, have been demonstrated. This test was described by LaBan *et al.* |1| and is performed by hyperextending the supinated wrist and the distal interphalangeal joint of the index finger for one minute and looking for pain in the proximal forearm. The stress test was positive in 60 hands (42.8%), the Phalen's sign in 79 (56.4%) and the Tinel's sign in 59 (42.1%). Hypoaesthesia to pinprick in the distribution of the median nerve was found in 45 hands (32.1%) and weakness or wasting of thenar eminence in 17 (12.1%). The study is limited by the use of a single observer only. This demonstrates the poor sensitivity of all clinical tests in the evaluation of patients with symptoms suggestive of CTS.

The incidence of recurrence after endoscopic carpal tunnel release.
M J Concannon, M L Brownfield, C L Puckett. *Plast Reconstr Surg* 2000; **105**(5):1662–5.
BACKGROUND. The goals of this study were to attempt to define the recurrence rate after endoscopic carpal tunnel release and to determine if it differs from that of the open technique. A retrospective review of the charts of 191 consecutive CTS patients treated operatively was performed.
INTERPRETATION. A statistically higher incidence of recurrence of CTS after endoscopic release compared with the traditional 'open' release was noted in this sample.

Comment

Surgical treatment for CTS is the most frequent surgical procedure of the wrist and accounts for $1 billion annually in the USA in direct costs alone. There is continued debate over the advantages and disadvantages of endoscopic versus open carpal tunnel decompression and this paper addresses this. The main stated

advantage of endoscopic release is that patients have less incision pain post-operatively.

Recurrence in this study was defined as documented CTS in patients in whom the symptoms had resolved post-operatively but then recurred. The limitations of the study are that it is retrospective and the follow-up period relatively short (mean of 29 months in the open surgery group and 22 months in those treated endoscopically). Patients were allowed to choose which technique they wanted. It is not clear who performed the surgery, with the author stating that is was performed by the Plastic Surgery Division, suggesting a large number of operators, which makes interpretation of data more difficult. It is possible that the success of either procedure is dependent upon the skill of the operators. However, there are several reports suggesting that endoscopic release does not result in better long-term results compared with the open technique.

MR imaging of overuse injuries of the Achilles tendon.
P T Karjalainen, K Soila, H J Aronen, *et al. Am J Roentgenol* 2000; **175**: 251–60.

BACKGROUND. This study aimed to illustrate and classify the abnormalities found on high-resolution MRI of symptomatic Achilles tendons in 100 athletic adult patients with 118 painful Achilles tendons.

INTERPRETATION. Lesions in the Achilles tendon and in the peritendinous structures can have similar clinical presentation. MRI detects and characterizes these changes. A more specific diagnosis and prognosis can be made with the use of MRI than with clinical examination alone.

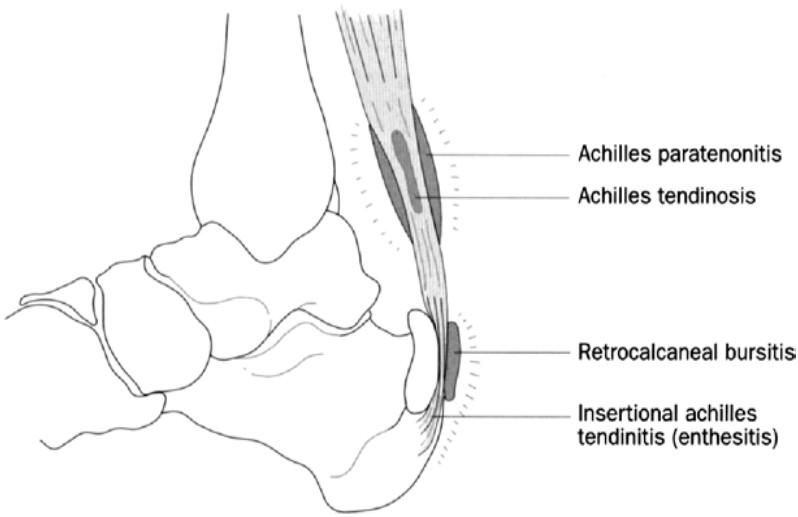

Fig. 14.3 The Achilles tendon and related disorders. Source: Karjalainem *et al.* (2000).

Comment

This paper illustrates the part that imaging (in this case MRI) has to play in the diagnosis and understanding of specific soft tissue lesions. In this study the Achilles tendon, peritendinous tissues, tendon insertion and musculotendinous junction tendons of all subjects were imaged using MR with a 1.5-T magnet. Twenty-eight patients underwent surgery, and histopathological samples were taken in 13. Long-term follow-up was performed, on average, 3.4 years after MRI. Of the 118 painful Achilles tendons, abnormalities in the tendon (n=90), surrounding structures or both were detected in 111. The relationship between MRI findings and macroscopic appearance was further examined in 28 subjects who went to surgery and samples in the 13 in whom histopathological samples were taken. Of the 21 macroscopic foci of tendinosis at surgery, 20 were revealed on MRI. Bursitis was noted on MRI in 11 patients and 10 of these at surgery. A thickened paratenon was demonstrated by MRI in 12 of the 19 patients in whom it was noted macroscopically at surgery. Histopathological changes consistent with tendinosis were noted in the 13 specimens taken from subjects with findings suggestive of intratendinous lesions at surgery and on MRI.

MRI is highly sensitive in the demonstration of specific Achilles tendon and paratenon pathologies. Further such studies are required of other soft tissue complaints.

Achilles tendon ruptures in south-east Finland between 1986–1996, with special reference to epidemiology, complications of surgery and hospital costs.

T Nyyssonen, P Luthje. *Ann Chir Gynaecol* 2000; **89**:53–7.

BACKGROUND. The incidence of Achilles tendon ruptures appears to be increasing. The study evaluated the annual incidence, aetiology, operative complications and direct hospital costs of Achilles tendon ruptures.

INTERPRETATION. The incidence of Achilles tendon ruptures is increasing in south-east Finland. The rate of major surgical complication was low (4.5%) and comparable with earlier studies.

Comment

This study involved a retrospective study of 93 consecutive patients operated on for Achilles tendon rupture over a 10-year period, from 1986 to 1996 at a district hospital in Finland with a population of 92 500. During the observation period no patient with an Achilles tendon rupture was treated conservatively. Ninety-five patients with Achilles tendon ruptures were treated including one re-rupture (1%) and one patient with two ruptures. There were seven patients with an open Achilles tendon rupture. The total annual incidence in the hospital area was 8.6 ($\pm$4.3)/10^5 and for closed Achilles tendon ruptures 8.0 ($\pm$3.8)/10^5 inhabitants per

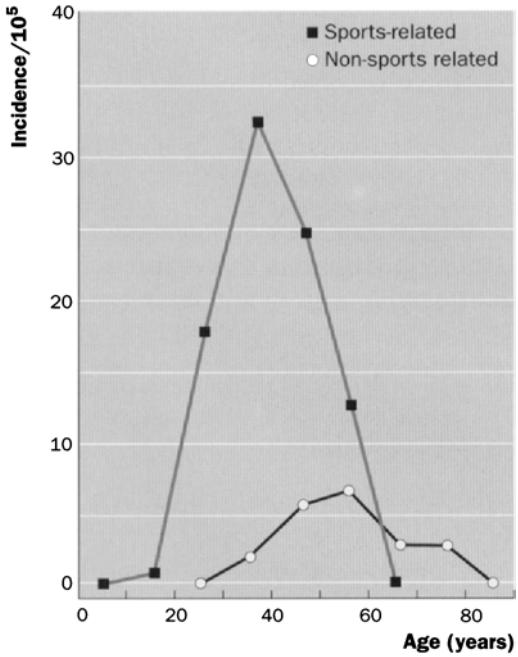

Fig. 14.4 The incidence of Achilles tendinopathies appears to be increasing |2|

year. The total incidence was 9.3 (±4.6)/10^5 and for closed Achilles tendon ruptures 8.6 (±4.1)/10^5. Most of the injuries were sport related, the most frequent sport being volleyball. Major surgical complications were seen in 4.5% of patients who underwent surgery for closed Achilles tendon rupture and the total complication rate was 11%. The average direct hospital costs per patient was US $1375.

The sample in this study was small, and much larger injury surveillance studies are needed to define the aetiology, incidence and outcomes in specific soft tissue injuries. Estimated costs should include not only the direct cost to the hospital, but costs of lost time at work, sick pay, lost revenue in industry and cost of treatments before and after surgery. Injury surveillance studies are notoriously difficult to perform and it is likely that the more severe injuries will be surveyed, due to a greater ease of detection and follow-up.

The mechanism of formation of bony spurs (enthesophytes) in the Achilles tendon.
M Benjamin, A Rufai, J R Ralphs. *Arthritis Rheum* 2000; **43**(3): 576–83.

BACKGROUND. Bony spurs are observed in some patients with Achilles enthesitis. The early stages in the formation of bony spurs in relation to normal enthesis development were evaluated in the rat.

INTERPRETATION. As a part of normal development, bone grows into the Achilles tendon as the calcaneus enlarges. Ossification is preceded by vascular invasion, which occurs along rows of enthesis fibrocartilage cells. Small bony spurs develop when ossification at one point on the enthesis outstrips that on either side.

Comment

Bony spurs are well recognized at the attachments of many tendons and ligaments, in particular at the iliac crest, patella, spine and calcaneus. Although they are common in degenerative, inflammatory and metabolic enthesopathies and in diffuse idiopathic skeletal hyperostosis, they are most commonly seen in healthy individuals, particularly with increasing age. They may be asymptomatic or painful. Their mechanism of formation is not known, but it has been suggested that they form in response to an inflammatory reaction and/or due to repetitive trauma or tensile forces. In this study the early stages of formation of spurs in the Achilles tendon of the young rat were studied. It was noted that spurs developed as a result of endochondral ossification of fibrocartilage at the enthesis of the Achilles tendon, implying that damage does not have to be present for such spurs to develop. The authors suggested that the increased surface area created at the tendon-bone junction may be an adaptive mechanism to ensure the integrity of the interface in response to increased mechanical loads.

The rats were kept in animal house cages and were not exposed to any exercise regimen and it seems unlikely that the tissues were exposed to significant tensile forces or overuse. It is not known how such information can be extrapolated to humans, but the aetiology and effects of bony spurs are important to the prevention and treatment of specific enthesopathies.

Relationship between calf muscle size and strength after Achilles rupture repair.
J Leppilahti, S Lahde, K Forsman, *et al. Foot Ankle* Int 2000; **21** (4):330–5.

BACKGROUND. A study of the relationships between calf muscle size and strength in 85 patients an average of 3.1 years after repair of Achilles tendon rupture.

INTERPRETATION. The isokinetic calf muscle strength results were excellent or good for 73% of the patients, whereas calf muscle size was normal in only 30%.

Comment

The long-term effects of soft tissue injury and subsequent management is an area where little research has been performed. This study demonstrated a significant reduction in calf muscle cross-sectional area (measured by computed tomography) in comparison with the opposite side, some 3 years after repair of Achilles tendon rupture. However, no significant decline in isokinetic plantar flexion strength compared with the other side was noted in three-quarters of patients. Such findings emphasize the importance of strength gain through neural adaptation rather than muscle hypertrophy.

Chronic Achilles tendon overuse injury: complications after surgical treatment. An analysis of 432 consecutive patients. M Paavola, S Orava, J Leppilahti, *et al. Am J Sports Med* 2000; **28**:77–82.

BACKGROUND. Achilles tendon injuries are common complaints and often difficult to treat. The spectrum of injury includes paratenonitis, tendinosis, retrocalcaneal bursitis, enthesitis and partial or complete tear. Surgery is frequently performed on chronic cases. This is a retrospective study to evaluate complications of surgery in 432 patients who underwent surgery as considered appropriate for their specific condition.

INTERPRETATION. Complications occurred in 46 cases (11%) during the 5-month follow-up period. These complications necessitated re-operation in 14.

Comment

This retrospective study included subjects with a wide spectrum of Achilles tendinopathies, who accordingly underwent different surgical procedures. Distribution of complications by diagnosis is given in this paper. Complications appeared higher for interventions for peritendinitis/paratenonitis and partial rupture and all cases of seroma formation and four of five cases of sural nerve irritation occurred in those with peritendinitis. All had surgery as day cases and 76 cases were performed under local anaesthetic, with the remainder being performed under spinal or general anaesthesia. The complications included 14 cases of skin edge necrosis (resulting in further surgery), 11 superficial wound infections (two had further surgery), five seroma formations (two had surgery), five haematomas (one had more surgery), five fibrotic scar formation (one had surgery), four sural nerve irritations, one new partial rupture and one deep vein thrombosis.

Surgery for Achilles tendon injuries is widely advocated. However, the post-operative complication rates quoted here are consistent with other reports and are higher than those quoted for many other types of lower limb surgery. This may be related to difficulties in wound healing in this area, previous corticosteroid injections, inadequate haemostasis and technical aspects of the surgery. The

proximity of the sural nerve to the tendon in the lower leg cannot be overemphasized.

Tenocytes from ruptured and tendinopathic Achilles tendons produce greater quantities of type III collagen than tenocytes from normal Achilles tendons.
N Maffulli, S W B Ewen, S W Waterston, *et al. Am J Sports Med* 2000; **28**(4):499–505.

BACKGROUND. In degenerative tendinopathy, an increase in the proportion of type III collagen relative to type I collagen is thought to weaken the tendon and predispose it to rupture. While investigating the hypothesis that the cellular activity is altered in tendinopathy, the authors studied the collagen types produced by normal and pathological tenocytes in an *in vitro* model of tendon healing.

INTERPRETATION. Immunological staining of type III collagen was found to be increased in pathological tenocytes compared with normal tenocytes after damage to the cell monolayer in culture.

Comment

The processes underlying the broad spectrum of tendinopathies encountered in clinical practice are still not fully understood. Alterations in cellular and extracellular matrix constituents have been described and the description of many of the changes that are noted as 'degenerative' is an oversimplification.

Unfortunately, methodological shortcomings cast doubt on the validity of the data of this paper. Immunohistochemistry with antibodies of unspecified provenance and unknown (not shown) specificity is not sufficient to show altered collagen expression. In addition, this is not a quantitative method, and cannot be used to compare levels of expression between cell cultures. The presence of type III collagen needs to be confirmed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting, or possibly by molecular methods (gene expression).

Other studies have shown no difference in the collagen types produced by different tenocyte populations grown in culture (Dr Graham Riley, Cambridge, personal communication). Cultured cells grown on plastic in monolayers have a very different phenotype compared with cells *in vivo*.

Pes anserinus tendino-bursitis: what are we talking about?
J Uson, P Aguado, M Bernad, *et al. Scand J Rheumatol* 2000; **29** (3): 184–6.

BACKGROUND. To evaluate the ultrasonographic features of the pes anserinus insertion (PA) and subcutaneous medial knee fat in patients clinically diagnosed of pes anserinus tendino-bursitis (PATB) syndrome.

INTERPRETATION. Patients diagnosed of PATB syndrome in rheumatology rarely have ultrasonographic evidence of tendinitis or bursitis of the pes anserinus.

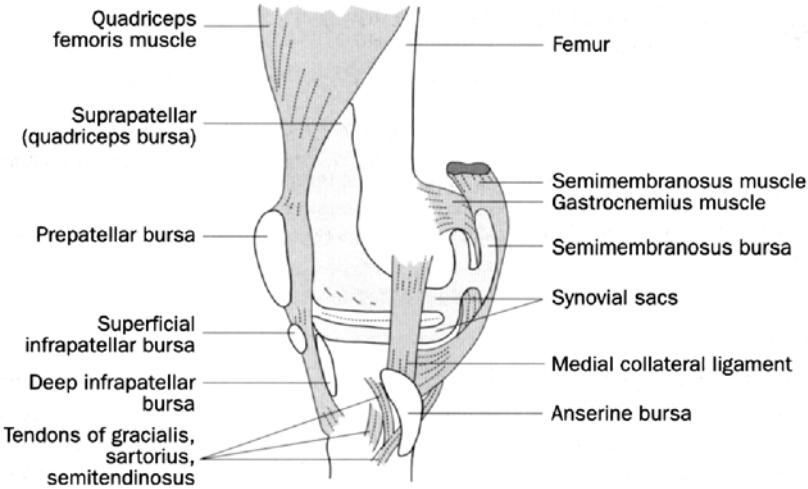

Fig. 14.5 Some of the bursa in the region of the knee. The pes anserinus bursa lies between the medial collateral ligament and the overlying pes anserinus (the tendons of the sartorius, gracilis and semitendinosis). Source: Uson *et al.* (2000).

Comment

Ultrasonographic features of the pes anserinus insertion and subcutaneous medial knee fat pad were examined in 37 female patients with a clinical diagnosis of PATB. In 23 patients with a clinical diagnosis of unilateral PATB, the painful knee was compared with the painless opposite knee, and six patients with bilateral PATB were compared with six healthy controls. All patients had radiographic OA of the knee and 75% had a body mass index greater than 27. Pes anserinus tendinitis was found in one symptomatic knee and pes anserinus bursitis in two symptomatic and one asymptomatic knee. Although PATB was excluded in most cases the cause of the symptoms was not clarified.

This paper shows the value of ultrasonography in the evaluation of musculoskeletal disorders, where clinical tests may have poor sensitivity and specificity. Ultrasound is highly dependent upon the experience and expertise of the operator and this is likely to be an important factor in such studies. The operator in this case is stated to be an experienced ultrasonographer. More studies of this nature are needed in order to describe the accuracy of clinical evaluation and the relationship between imaging and examination.

Novel use of laser Doppler imaging for investigating epicondylitis.
W R Ferrell, P V Balint, R D Sturrock. *Rheumatology* 2000; **39**: 1214–17.

BACKGROUND. Objective measures of soft tissue inflammation are limited. This paper evaluates the use of laser Doppler imaging in conjunction with ultrasound and power Doppler to evaluate soft tissue perfusion.

INTERPRETATION. Laser Doppler appears to be a sensitive measure of soft tissue perfusion.

Comment

Assessment of the presence or absence of inflammation in soft tissue lesions is extremely important. It has been clearly demonstrated that many of the changes seen in chronic soft tissue lesions are degenerative, with no cellular evidence of inflammation. The degree of inflammation in earlier lesions is undefined. Nevertheless, many of the therapeutic approaches used in the management of these lesions are anti-inflammatory. There is a great need for a reliable and sensitive measure of inflammation in these complaints and this is the issue addressed in this study.

The authors used laser Doppler flowmetry, which can be used to generate a spatial map of skin perfusion at a chosen site. They combined this with standard ultrasonography in grey-scale and power Doppler modes in a study of a single case of lateral epicondylitis before and after a steroid injection. Grey-scale imaging identified the lesion and power Doppler localized increased blood flow to deeper structures—possibly periosteum rather than the common extensor tendon. Laser Doppler imaging identified increased perfusion over the epicondylar area. After a local steroid injection perfusion on laser Doppler imaging returned to normal at the same time as the resolution of symptoms, while perfusion on power Doppler took a week longer.

This single case study does indicate potential for laser Doppler imaging in the assessment of soft tissue lesions. However, it is not clear whether it is simply measuring skin perfusion rather than that of deeper structures, and the reliability of both Doppler imaging and power Doppler in this situation are yet to be established. Resolution of symptoms correlated with a return to normal perfusion on laser Doppler imaging; it could be argued that the more delayed return to normal perfusion levels of deeper structures on power Doppler is a more helpful indication of a full tissue response to treatment.

Occupational risk factors for shoulder pain: a systematic review.
D A van der Windt, E Thomas, D P Pope, *et al. Occup Environ Med* 2000; **57**(7):433–42.

BACKGROUND. This review systematically evaluated the available evidence on occupational risk factors of shoulder pain.

INTERPRETATION. It seems likely that shoulder pain is the result of many factors, including physical load and the psychosocial work environment. The available evidence was not consistent across studies, however, and the associations were generally not strong.

Comment

Relevant reports were identified by a systematic search of the literature and the methodological quality of the publications was assessed by two independent reviewers using a standardized checklist. Details were extracted on the study population, exposures (physical load and psychosocial work environment), and results for the association between exposure variables and shoulder pain. The 29 studies (three case-control and 26 cross-sectional studies) included in the review had a median method score of 60% of the maximum attainable score. Potential risk factors related to physical load and included heavy work load, awkward postures, repetitive movements, vibration, and duration of employment. In studies with high method scores (≥75%), positive associations were reported for repetitive movements, vibration and duration of employment. However, the odds ratio varied widely (1.4–46). Nearly all studies that assessed psychosocial risk factors reported at least one positive association with shoulder pain, but the results were not consistent across studies for either high psychological demands, poor control at work, poor social support or job dissatisfaction. Substantial heterogeneity across studies for methods used for exposure assessment and data analysis impeded statistical pooling of results. Future longitudinal research should evaluate the relative importance of each individual risk factor and the role of potential confounding variables—such as exposure during leisure time—in order to set priorities for the prevention of shoulder pain in occupational settings.

A review of current literature on physiological tests and soft tissue biomarkers applicable to work-related upper limb disorders.

J M Saxton. *Occup Med London* 2000; **50**(2):121–30.

BACKGROUND. A review of key literature on physiological tests and biochemical markers of musculoskeletal stress/injury, which are applicable to studies of work-related upper limb disorders.

INTERPRETATION. Specific tests are described and the relative lack of biochemical markers of soft tissue injury is emphasized.

Comment

Work-related upper limb disorders are among the most commonly reported occupational illnesses and epidemiological evidence of work-relatedness has

been reported for some specific conditions, including CTS, hand/wrist tendinitis, shoulder tendinitis and hand-arm vibration syndrome. Occupational health surveillance requires objective tests for diagnostic and monitoring purposes. In this paper the author reviews physiological and biochemical tests that may be used in this context. Nerve conduction studies and psychophysical tests used in the assessment of neurological dysfunction associated with work-related upper limb disorders are discussed with respect to their strengths and limitations. However, no conclusions are drawn nor guidelines given for the use of such investigations.

With respect to biochemical markers of soft tissue injury, the author emphasizes the relative lack of such markers. The literature relating to studies of markers is described and the conclusion is that there is no reliable biochemical indicator of soft tissue injury relating to work-related complaints. The author moves on to suggest new ways that testing might be implemented during occupational health surveillance to enable early warning of impending problems and to provide more insight into the underlying nature of soft tissue disorders. This includes well-controlled studies that monitor workers in high-risk occupations, performing studies that examine the effects of experimental interventions during physiological testing, and laboratory studies to develop specific reliable and sensitive markers of soft tissue damage.

The value of this paper is not in the presentation of new research, but in the illustration of the relative lack of reliable tests for the diagnosis and monitoring of many soft tissue complaints.

Interventions for shoulder pain.

S Green, R Buchbinder, R Glazier, A Forbes. Interventions for shoulder pain. *Cochrane Database Syst Rev* 2000 (2):CD001156.

BACKGROUND. A Cochrane review of the efficacy of common interventions for shoulder pain.

INTERPRETATION. There is little evidence to support or refute the efficacy of common interventions for shoulder pain. As well as the need for further well designed clinical trials, more research is needed to establish a uniform method of defining shoulder disorders and developing outcome measures that are valid, reliable and responsive in these study populations.

Comment

This is a thorough and much needed review that is carried out to the high standard of the Cochrane system. The authors emphasize that in spite of the frequency of shoulder complaints and their significant socio-economic impact, there is a lack of well designed clinical trials to support most of the treatment approaches. Many studies have been limited by their inclusion of heterogeneous

study populations, inappropriate outcome measures, short-term follow-up and other methodological flaws.

The relevance of the moment arm of shoulder muscles with respect to axial rotation of the glenohumeral joint in four positions.
D K Kuechle, S R Newman, E Itoi, et *al. Clin Biomech* 2000; **15** (5):322–9.

BACKGROUND. This study was undertaken to determine the efficiency of the shoulder girdle muscles during axial humeral rotation based on measurements of the moment arms.

INTERPRETATION. The findings in this paper indicate that, due to its larger size, infraspinatus is the most effective external rotator, regardless of position, followed by teres minor, posterior deltoid (neutral position) and supraspinatus (coronal plane). Subscapularis and pectoralis major are the most effective internal rotators (independent of position), followed by latissimus dorsi, teres major and the three components of deltoid (sagittal plane). This information may be useful in the development of exercise programmes in physical therapy.

Comment

Muscle force production is a function of moment arm, muscle size, length/tension relationships and electromyographic activity. Measurement of the moment arms of specific shoulder muscles is, therefore, important in the understanding of shoulder movements and pathologies. However, it is a difficult area to study, mainly due to complexities of the articulation and measurement techniques. The technique described in this paper used tendon excursion and joint displacement to calculate the moment arms of shoulder muscles during four specific movements of axial humeral rotation, an important movement in everyday tasks.

There were some limitations to the study, including neglecting some of the portions of muscles during moment arm calculations, and the estimation of the relationship between the humerus and the scapula may not be truly representative of that *in vivo*.

The intra-articular component of the subscapularis tendon: anatomic and histological correlation in reference to surgical release in patients with frozen-shoulder syndrome.
A W Pearsall 4th, T F Holovacs, K P Speer. *Arthroscopy* 2000; **16**(3):236–42.

BACKGROUND. The intra-articular subscapularis tendon (IASS) has been implicated in the limitation of abduction and/or external rotation of the shoulder in some studies of patients with frozen shoulder. For this reason some surgeons release this structure arthroscopically in selected patients. The purpose of the current study was to assess the anatomy and histology of

the subscapularis muscle, including its intra-articular component. Patients with frozen shoulder who underwent arthroscopic capsular release, including release of the IASS were also assessed.

INTERPRETATION. The anatomy of the IASS was described. The authors concluded that the IASS can be released during arthroscopic capsular release for frozen shoulder with minimal risk of secondary anterior instability to the patient.

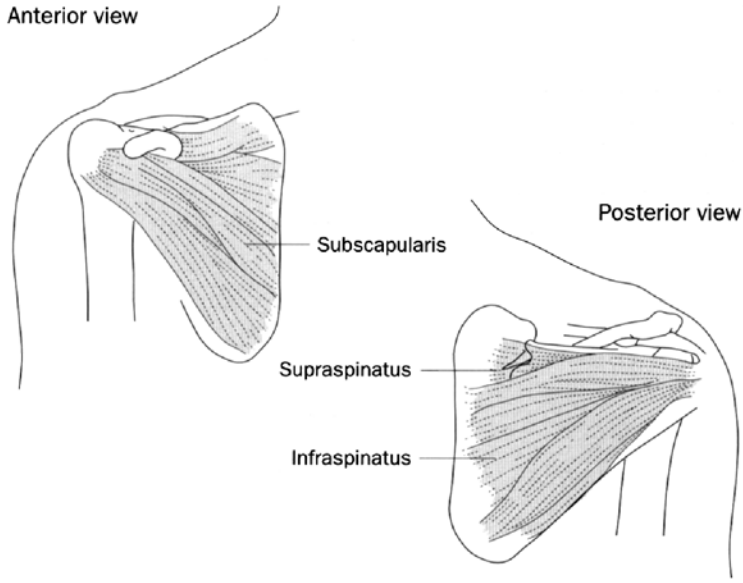

Fig. 14.6 The Rotator Cuff. Source: Pearsall *et al.* (2000).

Comment

The involvement of the IASS in glenohumeral restriction is an important question, but has not been answered by this study. Although the morphology has been described in a small number of patients, its involvement in frozen shoulder was not specifically evaluated. The 35 patients who underwent arthroscopic capsular release completed an unvalidated questionnaire relating to pain and function and from this the authors concluded that all patients had reduction of pain and improved function at an average of 22 months after surgery. Only one patient had mild subjective symptoms of instability. No control group, which had not had release of the IASS, was included.

Ultrasonography of the rotator cuff. A comparison of ultrasonographic and arthroscopic findings in one hundred consecutive cases.

S A Teefey, S A Hasan, W D Middleton, *et al. J Bone Joint*

Surg Am 2000; **82**(4):498–504.

BACKGROUND. A retrospective study performed to determine the diagnostic performance of high-resolution ultrasonography compared with arthroscopic examination for the detection and characterization of rotator cuff tears.

INTERPRETATION. Ultrasonography was highly accurate for detecting full-thickness rotator cuff tears, characterizing their extent, and visualizing dislocations of the biceps tendon. It was less sensitive for detecting partial-thickness rotator cuff tears and ruptures of the biceps tendon.

Comment

The value of ultrasound in the diagnosis of soft tissue lesions is now well recognized and there have been several publications to date that demonstrate that in many cases it can be as sensitive as MRI. Ultrasound also has the advantage that procedures, such as injection, can be performed under direct imaging. In this paper the authors performed a retrospective evaluation of 100 consecutive shoulders in 98 patients with shoulder pain who had undergone pre-operative ultrasonography and subsequent arthroscopy. The high sensitivity of ultrasound for the diagnosis of full-thickness tears, but limitations with respect to other pathologies in particular partial thickness tears, has also been well documented. However, other workers have suggested that ultrasound is sensitive in the diagnosis of biceps tendon lesions, and more work is needed in this area.

Limitations of this study are that it is retrospective and it appears that the arthroscopist was not blinded to the ultrasound findings. Nevertheless it is a study that may help to reduce some diagnostic arthroscopies being performed, being replaced in part by imaging in the form of ultrasound and/or MRI.

Ankylosing spondylitis and the shoulder: commonly involved but infrequently disabling.

R Will, G Kennedy, J Elswood, *et al. J Rheumatol* 2000; **27**: 177–82.

BACKGROUND. Shoulder involvement in patients with ankylosing spondylitis (AS) and the frequency of shoulder pain, stiffness and loss of movement and function in this group was assessed. The inter-relationship between shoulder symptoms, function, range of movement and radiology was also studied.

INTERPRETATION. Shoulder symptoms and loss of shoulder mobility are common in patients with AS, and correlate with higher pain scores and influence of AS on their lives as assessed by the Arthritis Impact Measurement Scale (AIMS), but are rarely disabling. Involvement of the shoulder joint in AS correlates with involvement of other peripheral joints as well as the extent of radiographic change on shoulder radiographs.

Comment

A cross-sectional design was used in both studies. In study A, a self-administered questionnaire was sent to 2500 patients with AS and data were available in 1515 individuals. The questionnaire consisted of questions on pain and stiffness in hips, knees and shoulders, an assessment relating to spinal mobility, and the multi-dimensional AIMS questionnaire, which addresses physical, emotional and social aspects. Unfortunately, there was no questionnaire specifically relating to shoulder function. Fifteen per cent and 13.8% of subjects had severe/very severe shoulder pain or stiffness, respectively. Patients with severe/very severe shoulder pain were more likely to have significant hip and knee involvement. Significant shoulder involvement appears to be as common as involvement of the hip joint.

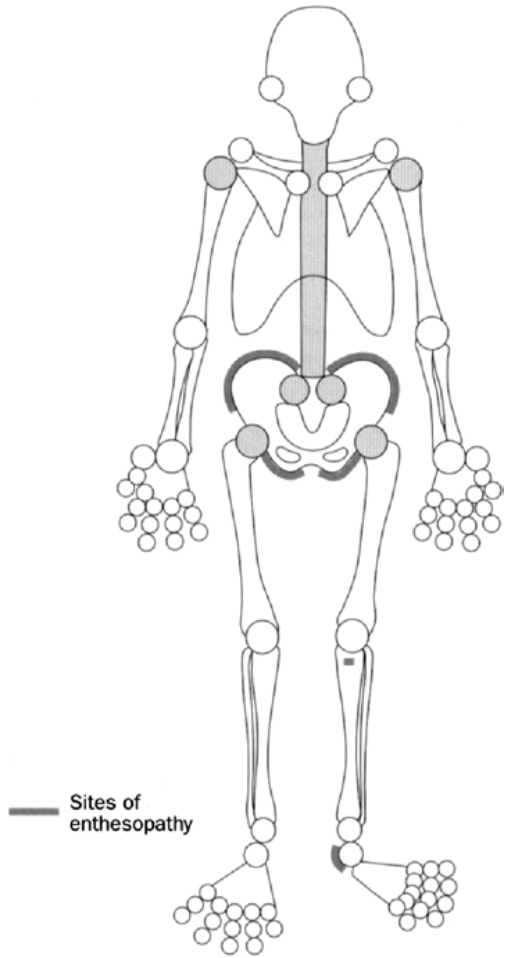

Fig. 14.7 Spinal pain, stiffness and reduced mobility are not the only features of AS. Peripheral joint involvement and enthesopathy are common. The shaded joints represent those commonly affected. Source: Will *et al.* (2000).

In study B, a clinical assessment of 88 patients with AS was undertaken that included plain radiographs in 26 consecutive patients. The nature of the examiner was not made clear. Shoulder pain, stiffness and function were assessed by questions, although this was not in the form of a validated questionnaire. Shoulder range of motion was measured using a goniometer. The shoulder was not formally examined otherwise. Severe/very severe pain or stiffness was reported in 9.6% and 17.6%, respectively. Patient-reported disability associated with shoulder involvement was uncommon. Radiological changes were common, being present in 31% of patients, but were often minor. There was a significant correlation between the sum of the stiffness, abduction and flexion scores for both shoulders, and the total radiological score.

It is clear that shoulder involvement in AS is common, although this study suggests that it is not a source of significant disability. Further studies relating to the natural history, specific lesions and impact of shoulder involvement in rheumatic diseases is warranted.

Magnetic resonance-based motion analysis of the shoulder during elevation.
H Graichen, T Stammberger, H Bonel, *et al. Clin Orthop* 2000; **370**: 154–63.

BACKGROUND. No *in vivo* information exists about the relative positions of the shoulder girdle bones and the supraspinatus muscle in three-dimensional space. This study involved a motion analysis of these structures during passive arm elevation using open MRI and three-dimensional image processing.

INTERPRETATION. The study shows specific three-dimensional motion patterns for each bone of the shoulder girdle and the supraspinatus muscle during passive elevation.

Comment

Changes in shoulder motion patterns are relevant in various shoulder diseases, but understanding is poor of the relative positions of the structures of the shoulder girdle during movement. This has been a difficult area of biomechanical research, but the availability of MRI may improve the quality of work in this area. Three-dimensional processing of MR images was performed in 14 volunteers who were examined in five positions of abduction (30–150 degrees) with an open MR system. The axis of the supraspinatus, humerus, clavicle, and the plane of the glenoid were determined and the relative movements were calculated.

The technique and results have great potential for future studies in patients with pathological changes of shoulder girdle motion. Studies of a larger sample of normals must first be performed.

Frozen shoulder: a sympathetic dystrophy?
L P Muller, L A Muller, J Happ, *et al. Arch Orthop Trauma Surg* 2000; **120**(1–2):84–7.

BACKGROUND. Diagnostic and clinical features of the frozen shoulder syndrome and reflex sympathetic dystrophy are similar in many aspects. This study aimed to examine the bone mineral density (BMD) in patients with suspected frozen shoulder to examine whether a reduction in BMD (a recognized feature of algodystrophy) occurs in this disorder.

INTERPRETATION. Ten of 12 patients with primary frozen shoulder had BMD decreases greater 21% in the humeral head of the affected shoulder compared with the non-affected side.

Comment

Diagnostic and clinical features of the frozen shoulder syndrome and reflex sympathetic dystrophy are similar in that radioisotope bone scan shows an increased uptake in affected areas in both diseases, while native radiographs show a progressive demineralization. The finding of significantly reduced bone density in patients with frozen shoulder may indicate an algoneurodystrophy. The authors included an appropriate control group to examine the possibility of disuse as an aetiological mechanism. Further studies relating to the possibility of autonomic dysfunction in frozen shoulder are warranted.

Unilateral lower-limb musculoskeletal injury: its long term effect on balance.
H M Holder-Powell, O M Rutherford. *Arch Phys Med Rehabil* 2000; **81**: 265–8.

BACKGROUND. This study aimed to assess whether any long-term decrements in balance occur after unilateral musculoskeletal injury. The relationship between the size of the decrement and the dominance, injury type and the time since injury were also considered. Ten-second postural sway in both lower limbs of 48 patients with previous unilateral musculoskeletal lower limb injury 6 months to 42 years earlier, and 108 controls, were studied using a force platform system.

INTERPRETATION. Postural sway was significantly greater in the injured versus uninjured limb. No differences in balance between the dominant and non-dominant limbs of control subjects were demonstrated. Subjects with non-dominant injuries performed significantly better than those with dominant injuries. No relation was demonstrated between decrements in balance and type of injury or time since injury. The study implies that full recovery of balance is not achieved after injury.

Comment

Proprioception is an important factor in the performance of many weight-bearing activities, in particular in relation to sport and is often reduced after injury. For example, disability after ankle sprain is often related to this deficit and rehabilitation often focuses upon this. Long-term deficits have not previously been investigated. There are some limitations to this study in relation to variation on testing, a learning effect and the use of a range of different injuries and durations in a small sample. Nevertheless, this an interesting study that has significant relevance with respect to the rehabilitation of lower limb injuries. Further research relating to the effects of interventions upon long-term balance is warranted.

Do patients with ankylosing spondylitis have poorer balance than normal subjects?
H C Murray, C Elliot, S E Barton, *et al. Rheumatology* 2000; **39** (5):497–500.

BACKGROUND. This study investigated whether patients with AS had poorer balance than normal subjects.

INTERPRETATION. A significant number of patients with AS had poorer balance than normal subjects.

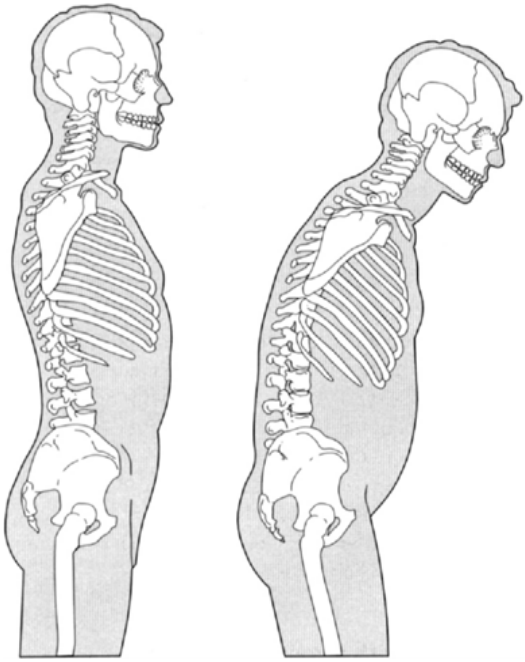

Fig. 14.8 The fusion of joints, stiffness, postural alterations, changes in the centre of gravity and joint and soft tissue changes may contribute to poorer balance in AS. Source: Murray *et al.* (2000).

Comment

The fusion of joints, stiffness, postural alterations and joint and soft tissue changes that can occur in AS may all contribute to a reduction in balance. Unlike the study by Holder-Powell and Rutherford (see above), the authors assessed balance by sway magnetometry, which measures sway at the hips in the horizontal plane using electromagnetic transmitter and receiver coils. This has previously been reported as being more sensitive than force platforms.

Balance was normal for the majority of subjects with AS. Abnormal balance was noted in 17% of subjects with AS with eyes open (all had moderate or severe AS) and 23% with eyes closed. A clear relationship with disease severity was not demonstrated. No significant relationships between balance and any quantitative descriptions of posture were noted.

This study has clinical relevance and the authors suggest that balance assessment using a sensitive system such as this is indicated as a routine assessment in individuals with AS.

Interexaminer reliability of the palpation of trigger points in the trunk and lower limb muscles.
C Y Hsieh, C Z Hong, A H Adams, *et al. Arch Phys Med Rehab* 2000; **81**(3): 258–64.

BACKGROUND. This study aimed to determine the inter-examiner reliability of palpation of three characteristics of myofascial trigger points (taut band, local twitch response and referred pain) in 26 patients with subacute low back pain and 26 controls. The effect of training on reliability was evaluated and differences in reliability between physiatrists and chiropractitioners were examined.

INTERPRETATION. Kappa scores for palpation of taut bands, referred pain and twitch responses were low between an expert and examiners both before and after training.

Comment

Myofascial pain syndrome is a condition characterized by musculoskeletal pain and other non-specific symptoms, and the presence of myofascial trigger points, defined as a highly localized painful or sensitive spot in a palpable taut band of skeletal muscle fibres. Several criteria have been stated for these trigger points and poor inter-examiner reliability has previously been reported in experienced examiners with no specific training sessions before the studies, but satisfactory values if prior training had been given. A range of levels of reliability from poor to good have been reported for inexperienced examiners given prior training. The only new element to this study is the inclusion of chiropractitioners and physiatrists, groups that had not been assessed before in relation to this issue.

The non-expert physicians included four chiropractors and four physiatrists, randomized into two groups (two of each per group). Only one group underwent training before the study. Three to seven patients were seen by both groups in each assessment session and all were examined by an 'expert' before the session commenced (this was taken as being the 'gold standard'). Findings were recorded as 'yes' or 'no' (present/absent). Examiners were blinded to each other's findings and to whether the subject was a patient or a control. The sequential examination of subjects introduces possible error due to changes in trigger point sensitivity after initial palpation. In addition, it may have been more appropriate to use the same examiners first untrained then trained, as any differences that may have existed between two separate small groups may not have been due to a training effect.

Kappa scores for the three characteristics noted on palpation of the myofascial trigger points ranged from 0.123 to 0.342 and 0.050 to 0.326, between expert and trained and untrained groups, respectively. Kappa scores for agreement between examiners ranged from −0.001 to 0.435 and −0.19 to 0.320 in the trained and untrained groups, respectively.

Although this study indicates the difficulty in obtaining good inter-examiner reliability in relation to myofascial trigger points in such patients, there are limitations in the study design that makes clear interpretation difficult.

An observer-blinded comparison of supervised and unsupervised aerobic exercise regimens in fibromyalgia.
C Ramsey, J Morreland, M Ho, *et al. Rheumatology* 2000; **39** (5):501–5.

BACKGROUND. This 12-week study compared a weekly supervised aerobic exercise programme with unsupervised home aerobic exercises in 74 subjects with fibromyalgia.

INTERPRETATION. Neither group showed an improvement in pain compared with baseline. There was some significant improvement in psychological well-being in the supervised group.

Comment

Fibromyalgia is considered to be related in part to deconditioning; supervised graded aerobic exercise programmes have been shown to have benefit, although having little impact upon pain. The supervision of such programmes is demanding upon resources and a home exercise programme would be considerably easier for patient and carers alike. However, a positive effect of contact with a carer may be important and difficulties with compliance have also previously been highlighted. This well-conducted study showed disappointingly little benefit from exercise in either group and raises the question of the value of dedicating resources to exercise programmes in fibromyalgia.

^{31}P magnetic resonance spectroscopy (MRS) in fibromyalgic syndrome (FMS).
H Sprott, R Rzanny, J R Reichenbach, *et al. Rheumatology* 2000; **39**: 1121–5.

BACKGROUND. The aim of this study was to measure levels of inorganic phosphate, phosphocreatine, adenosine triphosphate, and phosphodiesters in a common site of tenderness, the erector muscle, in 15 patients with fibromyalgia using ^{31}P-MRS.

INTERPRETATION. There were increases in the levels of phosphodiesters and inorganic phosphate in the spectra of fibromyalgic syndrome patients, but no difference in pH. Such findings may be related to the processes involved in the development of the fibromyalgic syndrome, but do not fully explain the symptoms.

Comment

The causation and pathophysiology of fibromyalgia are poorly understood. Earlier studies have indicated a correlation between symptoms of fatigue and metabolic changes in the muscle of patients with fibromyalgia. This was a well-designed study that avoided some of the possible methodological flaws seen in earlier studies. Potential flaws such as the effect of age on measured values and the problem of interpreting data in the form of ratios are discussed.

The authors emphasize the need to view such findings in terms of potential pathophysiological processes involved in fibromyalgia, but correctly point out that the notion that the metabolic abnormalities seen are directly responsible for symptoms is rather speculative. The cause of the metabolic abnormalities is not clear, although the authors suggest that ischaemia may play a part.

Conclusion

Although the topics included in the umbrella term ‘soft tissue rheumatism’ have attracted less research attention than traditional subjects such as rheumatoid arthritisand autoimmune disorders, the selection of papers reviewed shows that there is substantial activity in this field. This is certainly justified in view of the prevalence of the disorders; it is important that management of these conditions meets the same evidence-based criteria as those used in inflammatory arthritis. This is one justification for selecting a series of papers on CTS, a condition which costs the USA about 1 billion dollars annually to treat. Similar calculations could be made on the costs of shoulder disorders and tendinopathies, although in these conditions the effects of the conditions on fitness to work probably have more economic consequences than the costs of treatment.

One overall message that emerges in this chapter is that it is now both necessary and possible to define more precisely than has been customary, the

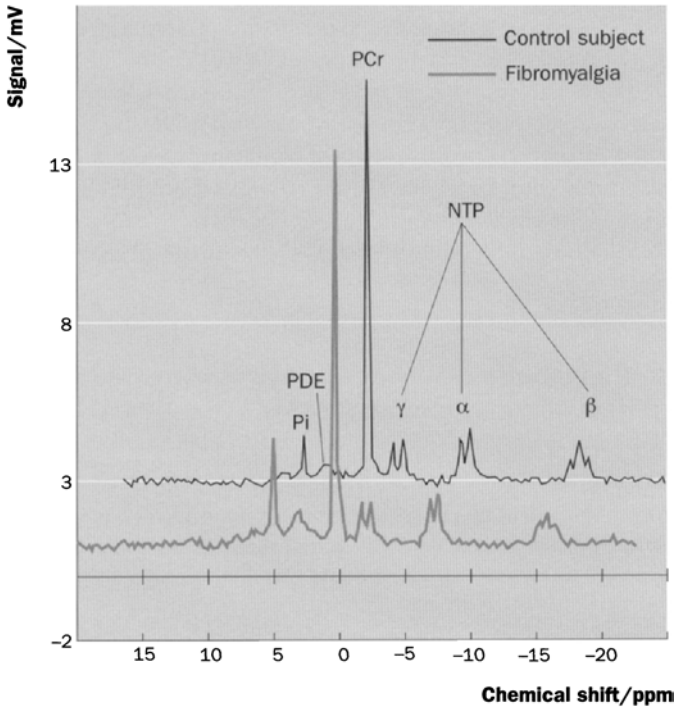

Fig. 14.9 ^{31}P-MRS spectra of the erector muscle in a control subject and a fibromyalgia patient. Source: Sprott *et al.* (2000).

tissues involved in the disorder and the nature of the pathological process. Advances in imaging are crucial here, but so is the design of well-validated instruments to measure pain and function (e.g. in shoulder pain), so that intervention trials can use these as outcome measures. Increasing research activity in this field, much of it carried out by physiotherapists and rheumatology specialist nurses, looks set to continue and expand in coming years.

References

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List of Abbreviations

AASV	ANCA-associated systemic vasculitis
AAV	Adeno-associated virus
ACA	anticardiolipin antibodies
ACE	angiotensin converting enzyme
ACR	American College of Rheumatology
AIMS	arthritis impact measurement scale
AM	antimalarial
ANA	antinuclear antibodies
ANCA	antineutrophil cytoplasmic antibody
APC	adenomatous polyposis coli
Apl	antiphospholipid antibodies
APS	antiphospholipid syndrome
ARA	American Rheumatism Association
AS	ankylosing spondylitis
ASA	acetylsalicylic acid
ATTRACT	anti-tumour necrosis factor alpha in rheumatoid arthritis patients receiving concomitant methotrexate
AVN	avascular necrosis
bFGF	basic fibroblast growth factor
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CA	chronological age
CAM	cellular adhesion molecule
CHAQ	Childhood Health Assessment Questionnaire
CI	confidence interval
CK	creatine kinase

CLASS	celecoxib long-term arthritis safety study
CMAS	Childhood Myositis Assessment Scale
CNS	central nervous system
cox	cyclooxygenase
CRH	corticotrophin-releasing hormone
CRP	C-reactive protein
CSI	combined sensory index
CT	computed tomography
CTS	carpal tunnel syndrome
DM	dermatomyositis
DMARD	disease-modifying antirheumatic drug
DTPA	diethylenetriaminepentaacetic acid
DXA	dual-energy X-ray absorptiometry
EASIA	enzyme amplified sensitivity assay
EBV	Epstein-Barr virus
EDU	endoscopic duodenal ulceration
EGU	endoscopic gastric ulceration
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
EMMPRIN	extracellular MMP inducer
ERA	enthesitis-related arthritis
ESR	erythrocyte sedimentation rate
ESSG	European Spondyloarthropathy Study Group
EULAR	European League Against Rheumatism
FAP	familial adenomatous polyposis
FIT	fracture intervention trial
FLIP	c-FLICE inhibitory protein
FMS	fibromyalgic syndrome
FOSIT	Fosamax international trial
FVC	forced vital capacity
GC	glucocorticoids
GCA	giant cell arteritis
GFR	glomerular filtration rate
GH	growth hormone
GI	gastrointestinal
GN	glomerulonephritis
GV	growth velocity

HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HRT	hormone replacement therapy
HSP	heat shock protein
IBD	inflammatory bowel disease
IASS	intra-articular subscapularis
ICAM	intercellular adhesion molecule
IFN	interferon
IGF	insulin-like growth factor
IIF	indirect immunofluorescence
IIM	idiopathic inflammatory myopathies
IL	interleukin
ILAR	International League Against Rheumatism
IVIg	intravenous immunoglobulin
IVMP	intravenous methylprednisolone
JAFAR	Juvenile Arthritis Functional Assessment
JCA	juvenile chronic arthritis
JIA	juvenile idiopathic arthritis
JPsA	juvenile psoriatic arthritis
JRA	juvenile rheumatoid arthritis
KS	keratan sulphate
LA	lupus anticoagulant
LAC	lupus anticoagulant
LCA	latent class analysis
LDL-C	low-density lipid-cholesterol
LFA	leucocyte function antigen
LLD	leg length discrepancy
LMP	latent membrane protein
LOD	logarithm of odds
LPS	lipopolysaccharide
MBL	mannose binding lectin
MBP	myelin basic protein
MCP	monocyte chemotactic protein
MHAQ	modified health assessment questionnaire
MHC	major histocompatibility complex
MI	myocardial infarction

MIP	macrophage inhibitory protein
MMP	matrix metalloproteinases
MMT	manual muscle strength testing
moAb	monoclonal antibody
MORE	multiple outcomes of raloxifene evaluation
MPO	myeloperoxidase
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRP	myeloid-related protein
MRS	magnetic resonance spectroscopy
MS	multiple sclerosis
MT	membrane type
MTX	methotrexate
NC	North Carolina
NK	natural killer
NOAR	Norfolk Arthritis Register
NOS	nitric oxide synthase
NP	nucleoprotein
NPSLE	neuropsychiatric systemic lupus erythematosus
NRAMP	natural resistance-associated macrophage protein
NSAID	non-sterodal anti-inflammatory drugs
NZB	New Zealand black
NZW	New Zealand white
OA	osteoarthritis
OPGL	osteoprotegerin ligand
OR	odds ratio
OSM	oncostatin M
PA	pes anserinus
PARC	psoriatic arthritis response criteria
PASI	psoriatic area and severity index
PATB	pes anserinus tendino-bursitis
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PECAM	platelet/endothelial cell adhesion molecule 1
PG	protective prostaglandin
PGN	proliferative glomerulonephritis
PICP	propeptide of type 1 procollagen

PMR	polymyalgia rheumatica
PPV	positive predictive value
PR3	proteinase 3
PTH	parathyroid hormone
PTHrp	parathyroid hormone-related protein peptides
Pyr	pyridinoline
QUS	quantitative ultrasound
RA	rheumatoid arthritis
RANKL	receptor activator of NF-kB ligand
RANTES	regulated upon activation, normal T-cell expressed, presumed secreted
RCT	randomized controlled trial
RE	radiographic outcome event
RF	rheumatoid factor
rhGH	recombinant human growth homone
RNA	ribonucleic acid
RoM	range of motion
rRNA	ribosomal RNA
RT-PCR	reverse transcription-polymerase chain reaction
SAPHO	synovitis, acne, pustulosis, hyperostosis, osteitis
SCF	stem cell factor
SCID	subacute combined immunodeficiency
sCTX	serum C-terminal crosslinked telopeptides of type I collagen
SD	standard deviation
SDS	standard deviation scores
SDZ	Sandoz
SE	shared epitope
SEM	standard error of the mean
SERM	selective oestrogen receptor modulator
SF	synovial fluids
SFC	spot-forming cell
SLE	systemic lupus erythematosus
SLEDAI	SLE disease activity index
SOJRA	systemic onset JRA
SPA	spondyloarthropathy
SPECT	single photon emission computed tomography
SR	slow release

SSR	sympathetic skin response
Stat	Signal Transduction and Transcription
sTfR	serum transferrin receptor
STIR	short tau inversion recovery
TA	temporal arteritis
TCD	thigh circumference discrepancy
Th	T-helper
TIMP	tissue inhibitors of metalloproteinase
TNF	tumour necrosis factor
TNFR	TNF receptor
TRAIL	TNF-related, apoptosis inducing, ligand
TRANCE	tumour necrosis factor-related activation-induced cytokine
TX	thromboxane
uCTX	urine C-terminal crosslinked telopeptides of type I collagen
UK	United Kingdom
UV	ultraviolet
VAS	visual analogue scale
VCAM	vascular cell adhesion molecule
VERT	vertebral efficacy with risedronate therapy
VIP	vasoactive intestinal peptide
VLA	very late activation antigen
VLDL-C	very low density lipid-cholesterol
WA	Washington
WG	Wegener’s granulomatosis
WHO	World Health Organization
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

Index of Papers Reviewed

General Index

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