Authors’ reply

We thank Professor Smith for his interesting comments. Professor Smith refers to an “obvious omission...any doctor or patient derived clinical parameters”. Clearly, we had measured the knee circumference of the target knee in this situation and we were using knee swelling as a clinical parameter; in table 1 of our paper it can be seen that there was no significant change in the knee circumference in any of the treatment or placebo groups during the study. Although we did not show the data in the results section, we stated that there was no statistically significant improvement in the doctor’s assessment of knee synovitis over the study period. Therefore, we do not suggest that there was a marked clinical response to treatment in these patients. We agree that there was a marked disparity in the baseline CRP levels within the three groups, but this was a result of randomisation and therefore something over which we had no control.

As regards the changes in MRI measurements, and the quantitative maps showing the reduction in gadolinium uptake, we believe that the trend towards the dose response across the three groups was clearly the most important interpretation of these results. We do not agree, however, with the reader’s interpretation that a possible range of change of 25% is small, especially as the patients had longstanding, resistant disease. The mean duration of disease for these patients was about 12 years and they had undergone multiple treatments with disease modifying antirheumatic drugs.

Professor Smith’s final point about anti-CD14 antibodies, which label macrophages as well as T cells, we clearly discussed in the third paragraph of the discussion—“There are a number of possible explanations for this apparent reduction in the number of CD14+ cells, which may represent a reduction...or macropages...”

In summary, we believe that this was an important study, firstly, as a proof of concept approach for therapeutic studies in rheumatoid arthritis, and secondly, as a unique comparison of the number of Reiter cells, which may represent a reduction in T cells or macrophages...

P EMMER
School of Medicine, Rheumatology and Rehabilitation Research Unit, University of Leeds, 36 Clarendon Road, Leeds LS2 9NZ, UK
Email: p.emery@leeds.ac.uk

LETTERS TO THE EDITOR

CD36 and CD14 immunoreactivity of Reiter cells in inflammatory synovial fluids

Reiter cells are macrophages containing ingested polymorph nuclei that are commonly found in most inflammatory synovial fluids. Available data indicate that CD36 and CD14 on human monocyte derived macrophages are adhesion molecules involved in several biological processes. Of interest, their role in the process of adhesion and phagocytosis of apoptotic cells has been recently demonstrated.

Jones and colleagues demonstrated reduced Reiter cells in the synovial fluids from patients with rheumatoid arthritis. This observation is consistent with the hypothesis that Reiter cells play a regulatory part in preventing autolysis of polymorphonuclear neutrophils (PMN) and thus local tissue damage.

The purpose of this study was to evaluate by histochemical technique whether Reiter cells express CD36 and CD14 in inflammatory synovial fluids.

We analysed the synovial fluids obtained from the knee joints of 10 patients suffering from inflammatory joint diseases of recent onset (<6 weeks). Three patients had sero-positive active rheumatoid arthritis, four patients had seronegative spondyloarthritis (two reactive arthritis, one psoriatic arthritis, one enterorheumatic), and two patients had crystal induced arthritis (two cases of acute gout and one case of acute pseudogout). Synovial fluids were processed within one hour of aspiration. Two slides were stained with May-Grunewald-Giensa (MGG) reagent. Reiter cells were counted on the basis of the first 500 cells encountered on MGG stained slides. In addition, two cytocentrifuge monolayer preparations were processed for immunohistochemistry using the monoclonal anti-human-CD36 antibody (Boehringer Mannheim-Germany) diluted to 3.5 mg/ml and the monoclonal antihuman monocyte CD14 antibody (DAKO-Denmark) diluted 1:10 in TRIS-HEPES buffer. In brief, specimens were air dried, fixed with acetone and then stored at −70°C until processing. The specimens were incubated for 60 minutes at room temperature with the primary antibody. For the conjugation of peroxidase an En Vision+TM Kit (Dako) was used. The monoclonal were then incubated for five minutes with a prediluted diamino-benzidine solution (DAKO) and counterlabelled with Mayer’s haematoxylin. All incubation steps were pre-ceded by washes in 0.1 M PBS (five minutes × three). The slides were examined at 400× magnification.

Omission of primary antisera, use of normal rabbit serum, or one of subsequent steps in the staining method were included as controls for specificity.

Macrophages as well as Reiter cells could be observed on MGG stained slides. Reiter cells were more abundant in synovial fluids from patients with seronegative spondyloarthritis and crystal induced arthritis compared with synovial fluids from RA (table 1).

On immunohistochemistry preparations, numerous mononuclear cells showed a CD36 positive reaction, while all the Reiter cells observed displayed a positivity for the thrombospondin receptor. CD14+ mononuclear cells outnumbered CD36+ cells; similarly, all the Reiter cells observed were immunoreactive for the anti-CD14 antibody (fig 1A, 1B).

Our findings show that Reiter cells do express both CD36 and CD14 adhesion molecules.

CD36 expression on Reiter cells seems to support the notion of the involvement of this receptor in the clearance of apoptotic PMN during synovial inflammation. In vitro data have shown that thrombospondin receptor and CD14 are some of the most important adhesion molecules involved in cell clearance.

The expression of the thrombospondin receptor turns an amateur phagocyte into a professional one. It has been hypothesised that dysregulation of this receptor and the ensuing impairment of inflammatory cell elimination could play a part in inducing chronicity as well as tissue damage and scarring. Recently, CD14 has been demonstrated to mediate recognition and phagocytosis of apoptotic cells. This interaction depends on a region of CD14 that is supposed to be identical to a region that binds bacterial lipopolysaccharide, triggering the release of proinflammatory cytokines from macrophages. On the other hand, the interaction with self components acts as an initial step leading to apoptotic cell elimination. A major role for CD36 in the uptake of apoptotic neutrophils has been recently hypothesised, but it seems likely that microenvironmental modifications could promote the switch from a CD36 dependent pathway to pathways using other adhesion molecules such as CD14. The removal of inflammatory PMN is mediated by several surface molecules and modulated by microenvironmental modifications; it seems to be a crucial, although only partially understood event for the control and resolution of inflammation. Our results suggest that CD14 and CD36 could be involved in the adhesion of the macrophage to the apoptotic cell, the first step of

<table>
<thead>
<tr>
<th>Sample</th>
<th>RA (n=3)</th>
<th>SsA (n=4)</th>
<th>CIA (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reiter cells (n)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

RA: rheumatoid arthritis, SsA: seronegative spondyloarthritis, CIA: crystal induced arthritis.
Non-periodic leg pain in patients with familial Mediterranean fever

Familial Mediterranean fever (FMF) is characterised by recurrent bouts of fever, peritonitis, pleuritis, arthritis or cryopyelas-like skin disease. Between the episodes, FMF patients are free of symptoms and appear healthy.1 However, interestingly we observe leg complaints after prolonged standing or sitting, or both, in FMF patients, who usually experience these painful manifestations during or after long distance bus trips. Thus we conducted a questionnaire study on 40 FMF patients (age, mean (SD); 21.6 (2.7) years; F: M; 2: 38) and 180 healthy male subjects (age, 21.3 (0.2) years) to ascertain the frequency of these complaints, and some of them were also included in a test to provoke these symptoms. Table 1 shows the questionnaire. Positive cases were also questioned for the presence of swelling or redness during these painful periods, and whether these complaints followed by an episode. Although 14 of the 180 healthy subjects responded positively to the first question (question A), none of them were considered to be positive for further questions (questions B). All FMF patients reported foot or leg pain after prolonged standing periods (first part of question A). They described that, at the onset, the pain was merely confined to the foot. However, other sites (the ankles, the calves, the knees or even the thighs) were involved in an additive manner as the intensity of pain increased unless resting ensued. The severity of FMF patients has experienced foot pain (with or without subcutaneous swelling) during or after long distance bus travelling and they also described an area of redness, which typically located on the ankles and the instep of those occurrences. Thirty five patients defined a period of fatigue accompanied a low grade fever subsequent to the episodes with severe lower extremity symptoms.

In provocation test, 30 volunteer male FMF patients (age, 21.2 (1.8)) without proteinuria and 30 volunteer male healthy subjects (age, 21.1 (0.8)) were kept in an upright position (standing, walking or dependent sitting) for six hours. At the beginning, all participants were symptom free and none of them had any other disorder that may cause foot pain. Thirteen FMF patients were receiving colchicine treatment. Bilateral ankle and the knee, circumference were measured from the marked points at the onset and the termination of the test. The mean change in circumference per measurement site (mean (SD)) was 3.1 (0.7) mm and 1.3 (1.5) mm in the patient and the control group, respectively. Although the comparison was statistically significant (p<0.014; Mann-Whitney U), we think that our method was not reliable to detect those small changes precisely.

At the end of the provocation test, none of the healthy controls had lower extremity pain or tenderness. Apart for one patient, all FMF patients had intense foot or calf pain, which interfered with walking. Tenderness was so profound that it could be elicited even by a gentle touch. Widespread tenderness was detected in 12, whereas localised tenderness was detected in 17 of the patients. Although swelling was not noticed in anyone, focal erythematous areas (not crypyelas) were seen in five patients. After five hours of resting, palpation showed that tenderness was sustained (14 widespread and 16 localised). A localised pain and tenderness was also developed in the symptom free patient. Colchicine use did not change the results of provocation test (p=0.240; Fisher’s test).

Although leg pain induced by exercise or prolonged standing has already been discussed in FMF patients,2 we are unaware of any report about leg pain and swelling episodes after prolonged sitting in these patients. Increased hydrostatic pressure in the lower extremities may be the main factor responsible for those symptoms experienced during bus trips. It was suggested that FMF is related to catecholamine metabolism as metaraminol infusion may provoke an acute episode,1 and episodes may be prevented by prazosin hydrochloride, as reported recently.3 Leuco-ocytes may need adequate perfusion (driving) pressure to pass through capillaries in microcirculation. These findings raise the possibility that catecholamines may increase the hydrostatic pressure of capillary bed, which may be an inciting factor for episodes.

Our findings show that an inflammatory reaction involving lower extremities occurs after prolonged standing and sitting periods in FMF patients. We think that genetically low level of inhibitory activity (that is, mutated pyrin) may not be able to compensate the inflammatory reaction that is probably initiated in a stressful microenvironment caused by not only microtrauma,4 but also increased hydrostatic pressure.

I am greatly indebted to Professor Hasan Yazer for critical discussion and help in preparation. A DINÇ

Department of Rheumatology, Internal Medicine, Gülhane School of Medicine, Ankara, Turkey

Correspondence to: Dr Dinç, GATA Romatoloji Blim Dalı, Etilik, 06010 Ankara, Turkey


CORRECTION

Table 1 Questionnaire on lower extremity complaints

<table>
<thead>
<tr>
<th>A</th>
<th>Have you ever had foot or leg pain events after prolonged standing and/or bus travel lasted more than six hours?</th>
<th>If the answer is yes,</th>
<th>B</th>
<th>Has it been existed since childhood or adolescence?</th>
<th>Does it occur mostly bilateral?</th>
<th>Does it persist at least 30 minutes after rest?</th>
<th>If all of the answers are yes, then the case was considered to be positive.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>No</strong></td>
<td><strong>Yes</strong></td>
<td><strong>No</strong></td>
<td><strong>Yes</strong></td>
<td><strong>No</strong></td>
<td><strong>Yes</strong></td>
<td><strong>Yes</strong></td>
</tr>
</tbody>
</table>


We regret that the references in this article are incorrectly numbered. Owing to the splitting of reference 7, references numbered from 9 onwards in the text are listed as 10 onwards in the reference list.