LETTERS TO THE EDITOR

Normal production of neutrophil activating peptide 1/interleukin 8 in monocytes from subjects with previous yersinia triggered reactive arthritis

Sir: Ankylosing spondylitis and reactive arthritis, a form of sterile joint inflammation triggered by an infection elsewhere in the body, belong to a family of seronegative spondyloarthropathies. Reactive arthritis can follow enteric infections caused by salmonella, shigella, campylobacter, or Yersinia enterocolitica, urogenital infection caused by chlamydia, and, possibly also, infections caused by Borrelia burgdorferi, among others.1 About 80% of patients with reactive arthritis carry the HLA-B27 antigen. The mechanism of this disease is not known but non-specific immunological reactions of the host with increased generation of inflammatory mediators or increased responsiveness to them may play a part.1 For instance, subjects with previous yersinia triggered reactive arthritis show enhanced neutrophil chemotaxis and increased oxygen radical production.2 Neutrophil activating peptide 1/interleukin 8 (NAP-1/IL-8) is a newly characterised chemotactic cytokine, produced by stimulated mononuclear phagocytes and a variety of tissue cells.3 Production of NAP-1/IL-8 may be induced by microbial antigens, which were recently found in the inflamed joints of patients with reactive arthritis triggered by chlamydia,4 yersinia,5 or salmonella.6 To find out whether subjects susceptible to the development of reactive arthritis show aberrant production of cytokines we cultured supernatants from 100 ml of whole blood of subjects with previous yersinia arthritis, uncomplicated yersinia enteritis, and without a history of yersinia infection.

Subjects were allocated to five groups (I-V, table 1) on the basis of HLA-B27, previous yersinia triggered reactive arthritis (yersinia arthritis), and previous uncomplicated yersinia enteritis. Twenty one subjects with previous yersinia arthritis were selected from a follow-up patient group,6 nine subjects with previous non-complicated yersinia enteritis had been treated at the Helsinki University Central Hospital, and 24 subjects without a history of yersinia infection were healthy laboratory and hospital personnel. Diagnosis of yersinia infection was based on a stool culture positive for the organism or a raised agglutination antibody titre (>1/160) for Yersinia enterocolitica (O:3 or O:9). At the time of this study the subjects had recovered completely from yersinia infection/arthritis, did not use anti-inflammatory drugs, and were free from signs and symptoms of infection.

Blood samples from two subjects with previous yersinia arthritis (one HLA-B27 positive, the other HLA-B27 negative), one subject with previous yersinia enteritis, and two controls (one HLA-B27 positive, the other HLA-B27 negative) without a history of yersinia infection were taken and studied concurrently on a given day. In three series of experiments from group I and group III subjects (see table 1) were not available for study.

Buffy coat cells were prepared from peripheral blood anticoagulated with heparin (10 U/ml). Mononuclear cells were separated by dextran sedimentation and Ficol-Isoaque density gradient centrifugation.4 The purified cells were washed three times with Hank's balanced salt solution and then suspended in RPMI 1640 medium supplemented with 1-25% (vol/vol) pyrogen-free albumin solution (40 mg/ml; Finnish Red Cross Blood Transfusion Service, Helsinki), 1 mM HEPES buffer, 2 mM l-glutamine, and antibiotics. Samples (1 ml) containing 1×10⁵ monocytes were cultured in 24-well tissue culture plates (Costar, Cambridge MA, USA; No 3424) for 24 hours at 37°C in 5% CO₂ and 95% air in the presence of 0 to 10 μg/ml Escherichia coli O3:K6 lipopolysaccharide (Difco, Detroit, Michigan Company, St Louis, MO, USA). The culture supernatants were then collected and stored at −80°C before analysis for NAP-1/IL-8 content.

Concentrations of NAP-1/IL-8 in the culture supernatants were determined by a microtitre plate assay for elastase release from human peripheral blood buffy coat neutrophils.7 In brief, 100 μl portions of a neutrophil suspension (10⁶ cells/ml) treated for 10 minutes with 5 μg/ml cytochalasin B were dispensed into the wells containing 125 μmol of elastase substrate (N-methyl-D-aspartic-acid-alanine-pro-val-7-amido-4-methylcoumarin, Bachem, Bubendorf, Switzerland) and the test sample in 150 μl phosphate buffered saline containing 2-5 mg/ml of bovine serum albumin (Fluka AG, Buchs, Switzerland). For each plate, background and NAP-1/IL-8 controls (0.3, 1, 3, 10, 30, 100 mmol/l) were included. The assay was performed at room temperature using a Titrtek Fluoroscan (Eliasb, Helsinki, Finland), and the titres in samples were determined from an NAP-1/IL-8 standard curve.

The frequency distributions of NAP-1/IL-8 values were significantly skewed, but could be normalised by logarithmic transformation, and therefore the data are expressed as geometric means (SD). To evaluate the responsiveness of the cells, differenials were determined by subtracting the control value in the absence of lipopolysaccharide from the values in the presence of lipopolysaccharide. The significance of the difference between the groups was evaluated by Student's t test.

The results showed that monocyte and lymphocyte counts were similar in the five subject groups (table 1). In all cases low levels of NAP-1/IL-8 production were seen in the absence of lipopolysaccharide and the yields increased markedly in parallel with the lipopolysaccharide concentration, reaching a maximum on exposure to 10 μg/ml lipopolysaccharide (table 2). No significant difference was obtained in the yield of NAP-1/IL-8, in the presence and absence of lipopolysaccharide, among groups I to V (table 2; figure). The differentials at each lipopolysaccharide concentration among the five groups were also similar (data not shown).

In patients with reactive arthritis consequent upon chlamydia, yersinia, or salmonella, antigens of the triggering microbe are detected in the inflamed joint.8,9 In two cases yersinia

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Table 1 Characteristics of the subject groups and differential counts of mononuclear cells after Ficol-Isoaque centrifugation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>12</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Men/women</td>
<td>7/5</td>
<td>2/7</td>
<td>1/8</td>
<td>2/10</td>
<td>3/9</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td>40(26-59)</td>
<td>56(48-70)</td>
<td>45(26-29)</td>
<td>44(29-63)</td>
<td>45(35-47)</td>
</tr>
<tr>
<td>Mean period in years (range)</td>
<td>15(10-20)</td>
<td>16(14-19)</td>
<td>6(2-14)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Mean (SD) differential count (%)</td>
<td>Monocytes</td>
<td>26(6)</td>
<td>23(5)</td>
<td>25(5)</td>
<td>27(7)</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>71(8)</td>
<td>73(7)</td>
<td>72(7)</td>
<td>73(6)</td>
</tr>
</tbody>
</table>

1: HLA-B27 positive subjects with previous yersinia arthritis; II: HLA-B27 negative subjects with previous yersinia arthritis; III: subjects with previous yersinia infection without extraintestinal manifestations (two of these were HLA-B27 positive); IV: HLA-B27 positive subjects without a history of yersinia infection; V: HLA-B27 negative subjects without a history of yersinia infection.

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Table 2 Neutrophil activating peptide 1/interleukin 8 (NAP-1/IL-8) concentrations in the media of mononuclear cells cultured for 24 hours in the presence of different concentrations of lipopolysaccharide (LPS).* Results are given as the geometric mean (−SD; +SD)

<table>
<thead>
<tr>
<th>LPS (μg/ml)</th>
<th>NAP-1/IL-8 (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>174 (6:2;48)</td>
</tr>
<tr>
<td>1</td>
<td>110 (3:8;31)</td>
</tr>
<tr>
<td>0.1</td>
<td>10 (4:5;22)</td>
</tr>
<tr>
<td>0.01</td>
<td>10 (3:5;10)</td>
</tr>
<tr>
<td>0</td>
<td>3 (2:0;8)</td>
</tr>
</tbody>
</table>

*For explanation of groups see table 1.
antigen was detected as long as three and 17 years after yersinia infection, suggesting that the microbial structures can persist in the tissues. The antigens are found in mesenchymal cells and cervical lymph nodes and in the skin, and, during the acute phase of infection, in peripheral blood cells of almost every patient, including patients who will not develop reactive arthritis. Thus the presence of antigens in the blood provides an explanation for extensive antigen dissemination, but not for the development of reactive arthritis and other clinically significant extrarticular manifestations.

Possibly, extrarticular symptoms derive from inflammatory hyperreactivity of the patient to the antigenic stimulus. It has been shown that patients with yersinia reactive arthritis show enhanced neutrophil migration in response to a chemotactic stimuluum in vitro and in vivo, as do patients with ankylosing spondylitis, at least in vitro. Furthermore, neutrophils from patients who have a history of severe active yersinia reactive arthritis, or with sequelae, show increased generation of oxygen radicals in vitro. NAP-1/IL-8 stimulates neutrophil chemotaxis and oxygen radical production and might thereby contribute to the neutrophil hyperactivity.

In conclusion, the results show that both control and lipopolysaccharide induced NAP-1/IL-8 production by monocytes was similar in subjects with past yersinia arthritis or enteritis and unaffected subjects, and did not differ between HLA-B27 positive and negative subjects. This seems to rule out an aberrant function of monocytes, at least for the synthesis and release of NAP-1/IL-8, one of their major products, in the triggering of reactive arthritis.

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Hand radiography: an indicator of upper cervical disease in rheumatoid arthritis

Sir: One of the most dangerous complications of rheumatoid arthritis is upper cervical disease, which can cause sudden deaths and serious neurological problems. In previous surveys, reported prevalences of atlantoaxial subluxations range between 19 and 71% according to the method of radiological techniques used. Although direct radiographic investigations can show atlantoaxial subluxations, computed tomography (CT) and magnetic resonance imaging are the best procedures for detecting the myelopathy and bone soft tissue pathologies in this region. As these methods are expensive, however, the question then has to be asked: which patients with rheumatoid arthritis should be examined by CT or magnetic resonance imaging?

Conlon et al were the first group reporting a relation between atlantoaxial subluxations and severe peripheral joint destruction. Later, reports from Stevens et al and Rasker and Cos\footnote{The names were not visible in the image.} supported this view. We attempted to determine if the CT findings correlate with hand radiographic findings or not. We studied 40 patients chosen randomly from 138 out patients at Ankara Hospital, diagnosis as having rheumatoid arthritis according to the 1987 revised criteria of the American Rheumatism Association. Anteroposterior and lateral radiographs were taken and then we carried out CT of the cranio cervical junction in the axial and coronal planes with the patient's neck in maximum flexion and neutral position by using a 320 workation scanner. We noticed not only atlantoaxial subluxations but also erosions of the odontoid process or atlas arcus, thickness of the ligaments, dis- tension of the synovial cavities, and ligament ruptures.

Of the 40 patients studied, 12 had completely normal or slightly abnormal (periarticular soft tissue swelling, periarticular calcifications, slight joint space narrowing) hand radiographs. In this group there was no evidence of upper cervical disease or atlantoaxial subluxations in CT scans, except in one patient who had a slightly thickened transverse ligament. One remaining group of 28 patients, who had erosions and marked joint space narrowing in hand radiographs, we found CT abnormalities of all kinds in 25 and atlantoaxial subluxations in 19 patients. As expected, atlantoaxial subluxations were associated with severe and mutilating abnormalities in the hand radiographs. We suggest that if the hand radiographs are normal, upper cervical spine CT should not be expected in rheumatoid arthritis and there is no need for sophisticated examination of this region.

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Positive antinuclear antibodies in malignancies

Sir: I read with interest the recent case report of a patient with Raynaud’s phenomenon and positive antinuclear antibodies in a malignancy. I would like to report two additional cases of malignancies, which were associated with Raynaud’s phenomenon and a high titre of antinuclear antibodies, and make some comments on the subject.

CASE 1
A 61 year old man presented for rheumatology evaluation with a six month history of arthralgias in both hands and a six week history of severe Raynaud’s phenomenon. Eight months earlier he was admitted to the hospital because of hemiparesis of his left arm and leg, which lasted a couple of days with fever of 39-35°C. He was checked thoroughly in a neurological and a medical clinic, but with no neurological deficit and the fever was found. Finally, he was suspected to have an unknown vasculitis and given high dose steroids, which relieved his symptoms. Antinuclear antibodies were negative at that time. When he was referred to

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