Search for Infective Agents in Undifferentiated Oligoarthritis in Papua New Guinea

Sir—Arthritis is well described in the indigenous population of Papua New Guinea (PNG) [1] and is a significant health problem. Most patients have oligoarticular peripheral arthritis associated with HLA-B27 [2]; rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis are apparently rare [3]. It is likely, therefore, that many patients have a form of spondyloarthropathy. Infectious diseases, including sexually transmitted, respiratory and parasitic infections, are common in PNG so that the possibility that arthritis may, in some cases, be associated with infections by Chlamydia trachomatis, Chlamydia pneumoniae, enteric pathogens and others exists.

Studies of reactive arthritis in Europe and the USA have demonstrated likely roles for a variety of bacteria, with bacterial membrane antigens, DNA and RNA being detectable within the joint [4]. Similar findings are now emerging in undifferentiated forms of spondyloarthropathy in which no other evidence of a precipitating infection can be found. We have therefore investigated joint material obtained from patients in PNG to search for antigens and DNA of bacteria associated with reactive arthritis and Lyme disease, along with antigens of selected commensal bacteria.

Synovial fluid (SF) and synovial biopsies were obtained from 15 adult (two female) Papua New Guinean highlanders presenting with knee swelling of 10 days–8 weeks duration. One had typical Reiter’s syndrome, three had recently had diarrhoea and other specific diagnoses were excluded.

SF was stored at −20°C and was treated with bovine testicular hyaluronidase prior to testing. Bacterial antigen detection was undertaken using a standard indirect immunofluorescence technique, with the exception of the two commercial antibodies, Imagen (Dako) and microtrak (Syva), which were supplied by one of us (DW). Monoclonal antibodies were established and all slides were counterstained with Evans Blue, mounted in buffered glycerol with antifade and covered with a glass coverslip. All slides were read by two observers (JH and AK). Positive control slides, consisting of smears of pure cultures of individual bacteria, were included in each batch of slides.

Detection of Chlamydia and Borrelia DNA was undertaken using the polymerase chain reaction (PCR). Two Borrelia burgdorferi gene targets were amplified: conserved regions from the gene encoding the Osp A protein [5] and the chromosomal region of the 16S rRNA gene [6] as described elsewhere [7]. PCR products were confirmed by hybridization using oligonucleotide probes as described elsewhere [7]. Chlamydia gene sequences were identified using nested PCR. Chlamydia pneumoniae major outer membrane protein (MOMP) sequence was amplified and detected as previously described [8]. The MOMP gene of C. trachomatis was amplified as described elsewhere [9]. The specificity of the C. trachomatis PCR product was confirmed by restriction digestion with EcoRI.

Synovial biopsies were fixed in buffered formaldehyde in sections stained with haematoxylin and eosin, Congo red and Perl’s prussian blue. Where appropriate, Gram’s stain, Ziehl–Neelsen and periodic acid–Schiff reactions were also used. Slides were examined by light microscopy by one of us (DW).

On light microscopy, most of the biopsies showed a prominent chronic synovitis characterized by an increase in the number of lining layer cells with overlying fibrin deposition, together with underlying inflammatory cell infiltrates of lymphocytes, plasma cells and macrophages. In four of the biopsies, there was haemosiderin accumulation within the

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Designation/origin</th>
<th>Monoclonal/polyclonal</th>
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</thead>
<tbody>
<tr>
<td>Bacteroides fragilis</td>
<td>QUBF5 Dr S. Patrick, Belfast, UK</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>Camp 1 CPHL</td>
<td>Polyclonal (rabbit)</td>
</tr>
<tr>
<td>Esherichia coli</td>
<td>B357 Dakopatts, Denmark</td>
<td>Polyclonal (rabbit)</td>
</tr>
<tr>
<td>Klebsiella aerogenes</td>
<td>CPHLK1 CPHL*</td>
<td>Polyclonal (rabbit)</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>Salm E1 CPHL*</td>
<td>Polyclonal (rabbit)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>SM014 CPHL*</td>
<td>Polyclonal (rabbit)</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>TOMA6 Prof P.</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Streptococcus pyogenes group A</td>
<td>Toivanan, Turku, Finland</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>M6600 Dako UK</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>Dr L. Cropper, CPHL*</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Chlamydia spp.</td>
<td>Imagen, Dako</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>Mirotrak, Syva</td>
<td>Monoclonal</td>
</tr>
</tbody>
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*Central Public Health Laboratory, Colindale, UK.
macrophages. Two further biopsies had an active chronic synovitis with neutrophils associated with the chronic inflammatory cells. No bacteria, crystals or amyloid were identified in any of these biopsies.

Aliquots of all 15 synovial fluid samples were stained with all 12 antibodies. All positive controls yielded bright specific apple green fluorescence with characteristic bacterial morphology. No specific staining was observed in any of the patients' samples.

Specific DNA amplification by PCR for genomic sequences of *C. pneumoniae*, *C. psittaci*, *C. trachomatis* and *Borrelia burgdorferi* also yielded negative results, and no sample contained significant titres of *B. burgdorferi* antibody.

These findings do not provide support for the hypothesis that arthritis in these patients results from the deposition of bacterial antigens in synovium or SF.

Inevitably, clinical data are incomplete, the patient group is probably heterogeneous and the sample size small. However, these negative findings may usefully inform future studies into this important rheumatic disorder in PNG.

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**Combination Therapy with Sulphasalazine and Azathioprine**

Sir—Some combination second-line therapies in rheumatoid arthritis (RA) are advocated as an improvement compared to using either drug alone [1]. Waterworth [2] has reported a favourable impression of the combination of sulphasalazine (SAS) and azathioprine (AZA) in RA. There is no theoretical reason why a combination of these drugs would be more effective than either drug alone. SAS may, in part, act by competitive inhibition of folate uptake. AZA, an oral purine analogue, prevents the synthesis of adenosine and guanine, and is incorporated into mitotic cellular genetic material, thus eventually causing cell death. This report concerns the use of this drug combination in routine clinical rheumatology practice.

The case notes of 38 patients known to have received the drug combination (SAS/AZA) were reviewed. Thirty-seven patients had RA and one psoriatic arthritis (mean age 57.1 yr, range 34-81 yr; 19 females, 19 males; mean disease duration 36 yr, range 1-50 yr). Most patients had been taking SAS (mean duration 18 months) prior to the combination therapy. The mean drug dose for the combination was 2.1 g SAS, 92.8 mg AZA. Improvement was based on clinical impression at 6 months: improvement occurred in 17 (45%), no change in eight and 13 withdrew because of side-effects (three rash, seven gastrointestinal upset, and one each of leucopenia, generally unwell and nephrotic syndrome). Changes in plasma viscosity, haemoglobin, white cell count, AST and ALT are given in Table I.

Some patients have done remarkably well on this drug combination. In general, however, the combination is poorly tolerated with only 45% remaining on the drugs at 6 months. Most withdrawals were because of gastrointestinal side-effects or rash. The data do not allow a critical appraisal of the drug combination—the improvement seen may be mainly due to AZA; a

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**TABLE I**

<table>
<thead>
<tr>
<th>PV</th>
<th>Hb</th>
<th>WBC</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved</td>
<td>-0.11</td>
<td>1.03</td>
<td>-0.97</td>
<td>8.3</td>
</tr>
<tr>
<td>No effect</td>
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<td>-0.07</td>
<td>0.01</td>
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<tr>
<td>Side-effects</td>
<td>0</td>
<td>-0.83</td>
<td>-0.21</td>
<td>13.9</td>
</tr>
</tbody>
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