Bacterial DNA in synovial fluid cells of patients with juvenile onset spondyloarthropathies

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Abstract

Objective. To identify bacterial DNA in synovial fluid cells of patients with active juvenile onset spondyloarthropathy (SpA).

Methods. The main group of study constituted 22 patients with juvenile onset SpA. In addition, five patients with adult onset SpA and nine with rheumatoid arthritis (RA) were studied. Polymerase chain reaction (PCR) with either genus- or species-specific primers was performed on synovial fluid cells to detect DNA sequences of Chlamydia trachomatis, Yersinia enterocolitica, Salmonella sp., Shigella sp., Campylobacter sp. and Mycobacterium tuberculosis. The presence of antibacterial antibodies in sera and synovial fluid was also determined by enzyme-linked immunosassay.

Results. The synovial fluid of nine patients with juvenile onset SpA, three with adult onset SpA and one with RA contained bacterial DNA. Five juvenile onset SpA samples had DNA of one single bacterium; two juvenile onset SpA and three adult onset SpA had DNA of two bacteria and two juvenile onset SpA had DNA of three bacteria. Overall, Salmonella sp. DNA was detected in seven synovial fluid samples, Shigella sp., Campylobacter sp. and M. tuberculosis were found in four samples each, and C. trachomatis was found in two. The bacterial DNA findings correlated with neither diagnosis nor disease duration. One RA synovial fluid had DNA of Campylobacter sp. Neither serum nor synovial fluid antibacterial antibodies correlated with DNA findings or clinical diagnosis.

Conclusion. In this study, single and several combinations of bacterial DNA were identified in the synovial fluid of patients with long-term undifferentiated and definite juvenile onset SpA and adult onset SpA. Of relevance is that bacterial DNA corresponds to bacteria producing endemic disease in our population.

Key Words: Spondyloarthropathy, Synovial fluid, Juvenile onset, Adult onset, Rheumatoid arthritis.

Spondyloarthopathies (SpA) constitute a group of HLA-B27-associated clinical conditions of the entheses and synovium of peripheral and axial joints whose major representatives are ankylosing spondylitis (AS), reactive arthritis (ReA), Reiter’s syndrome, and a subgroup of undifferentiated forms. To a variable extent, the pathogenesis and clinical expression of SpA are influenced by genetic and infectious factors. The strong association between AS and HLA-B27 exemplifies the former and ReA exemplifies the infectious model of the disease. The fact that in some patients ReA and undifferentiated SpA may evolve into AS suggests that these diseases share a common pathway in their pathogenesis.

Attempts to identify bacterial structures in affected tissues have been carried out using several methods and with different results. Using the polymerase chain reaction (PCR), several studies have detected DNA from Chlamydia trachomatis [1, 2], Borrelia burgdorferi [3], Campylobacter sp. [4], Shigella sp. [4], Salmonella sp.
Patients and methods

Patient population

The core of this study was constituted by a group of patients with undifferentiated or definite SpA commencing before the age of 16 yr. The former included patients with undifferentiated disease or a syndrome of seronegative enthesopathy and arthropathy [15] and the latter patients with AS [16]. Additionally, we studied a group of adult onset patients with AS, undifferentiated SpA [17], and psoriatic arthritis and a control group of patients with rheumatoid arthritis (RA) who fulfilled the American College of Rheumatology (ACR) criteria [18]. Based on Taylor-Robinson et al.’s [1] detection rates (0.625 vs 0.0 cases and controls), we estimated an initial sample size of 8.65 per group (α = 0.05, β = 0.2), which was later increased to 22 SpA cases.

To enter the study, the patients in all groups had to have knee effusion of synovial fluid requiring drainage. Exclusion criteria were any puncture of the joint selected for the study to drain synovial fluid or injection of any medication in the previous 3 months and systemic administration of glucocorticoids within the last 4 weeks. The study protocol was approved by the Ethical and Research Committee of our institution and the patients voluntarily agreed to participate.

Procedures

Once selected for the study, the patients underwent an assessment of tender and swollen joints and entheses of both peripheral and axial joints as well as a careful investigation of clinical signs of intestinal, respiratory, or urinary infection. Then, 20 ml of synovial fluid from the knee and 10 ml of peripheral blood were simultaneously obtained for analysis. Synovial fluid was centrifuged at 1500 r.p.m. for 10 min. Synovial cells were stored at −70°C and residual fluid was stored at −20°C. Serum was stored at −20°C.

To avoid DNA contamination, every step of the process was carried out under sterile conditions. All samples and reagents were handled under laminar flow hoods. New disposable gloves were employed in every step. The PCR reaction mixture was prepared as a stock solution and divided into aliquots with separate micropipettes. A set of Gibson micropipettes was used when DNA-free reagents were added to the PCR reaction, and a different set of micropipettes was used when the DNA samples were added. Finally, disposable filters were used to avoid any contamination by aerosol. After adding DNA, the mixture was covered with two drops of sterile mineral oil and capped before preparing the next amplification mixture.

Patient sampling, sample processing, DNA extraction, DNA amplification, and hybridization were all performed on different days and in separate rooms. Likewise, the synovial fluid samples, PCR reagents, and PCR-amplified products were stored in different rooms. DNA extraction was performed with the Wizard® Genomic DNA Purification Kit under the manufacturer’s specifications (Promega, Madison, WI, USA) in one single procedure. Extracted DNA was stored at −20°C until amplification was carried out. Primers and internal probes were purchased from Bio-Synthesis (Lewisville, TX, USA). Southern blot hybridization was carried out by transferring amplified DNA products from the gel to a Nylkon membrane (Hybroid-N; Amersham, Amersham, UK) by overnight capillary transfer in 0.4 M NaOH. The membrane was washed, air-dried and then exposed to ultraviolet light for 3 min to bind the DNA. Hybridization was performed with a specific γ32P-labelled oligonucleotide directed against an internal sequence of PCR products. The conditions in every case followed the recommendations of source papers [2, 19–23].

DNA amplification was performed by PCR with 50 µl of sample DNA and 0.5 µM of each of the four dNTP (Gibco-BRL, New York, USA). Thirty picomoles of each sense and antisense primer and 2 units of Taq DNA polymerase (Gibco-BRL, New York, USA) were added to the reaction mixture. Amplified products were subjected to electrophoresis on 1% agarose gels and stained with ethidium bromide. We verified that the number of cycles was suboptimal for PCR amplification (i.e. that the plateau level for the PCR product was not reached at the end of the amplification). For each sample, DNA amplification was carried out at least three times. In every case, a positive control containing the specific bacterial DNA was amplified. Additionally, a constitutive gene (cyclophilin) was included in every reaction in order to exclude PCR inhibitors. To rule out any contamination of the
amplification cocktail, we included a negative control, which contained the amplification cocktail but no patient or bacterial DNA. In every case, the positive controls gave a bright detectable band, whereas no band was detected in the negative controls, which invariably was the last line on the gel, to discard carry over contamination.

Because several species of Shigella, Campylobacter, and Salmonella have been shown to be arthritogenic, we preferred the use of generic primers for amplification. For Yersinia, the primers chosen were sequences from Y. enterocolitica and Y. pseudotuberculosis and for C. trachomatis and M. tuberculosis the primers were species-specific. The sequences of the amplification and hybridization primers as well as the expected size of the PCR products are described in Table 1.

For C. trachomatis we used KL1’ and KL2’ that targeted a plasmid fragment sequence from a BamHI restriction site [2]. The amplified product gave a band of 190 bp. Hybridization was performed with KL2’ as the internal probe under the following conditions: pre-hybridization in 1× Denhardt’s solution, 1% sodium dodecyl sulphate (SDS) and 6× SSC for 2 h at 65°C; hybridization in 1× Denhardt’s solution, 1% SDS, 6× SSC, 100 μg/ml of denatured salmon sperm DNA and 100 μg of the probe at 60°C overnight. After hybridization, the membranes were washed twice for 10 min each with 2× SSC, 0.5% SDS at 48°C.

Regarding Y. enterocolitica and Y. pseudotuberculosis, the lcrE gene was the sequence targeted and AMV4 and AMV5 were the primers selected for the assay. DNA amplification was carried out as described previously [19]. The PCR product gave a band of 450 bp. Hybridization was performed with the AMV10 probe as recommended [19].

Shigella sp. detection was carried out with the sequence of a plasmid invasive-associated locus (ial) of 2.5 kbp from a HindIII restriction site. This sequence detects four species of Shigella (S. sonnei, S. boydii, S. dysenteriae and S. flexneri), but also enteroinvasive Escherichia coli [20]. The amplified product gave a band of 320 bp. Hybridization was performed with the ial probe as recommended [20].

Regarding Campylobacter sp., a 600 bp 16s-rDNA was amplified with CampC5 and P3MOD201C primers as described previously [21]. The amplified product gave a band of 600 bp. Hybridization was performed with the IVWC2 probe [21]. The targeted genes for Salmonella sp. were invA and invE [22]. The PCR product gave a band of 457 bp. Hybridization was performed with a 20-mer probe invA gene sequence [22]. For M. tuberculosis we targeted the genomic repetitive sequence IS6110 [23]. The amplified product gave a band of 123 bp. Southern blot hybridization was performed with an internal probe, this sequence is described in Table 1 (under the conditions described for C. trachomatis).

For the study of bacterial antibodies, Salmonella typhi, Campylobacter jejuni, Shigella flexneri, Klebsiella pneumoniae and Y. enterocolitica were first formalinized and then adjusted to 10⁵ cfu/ml. The presence and titles of specific anti-S. typhi, C. jejuni, S. flexneri, K. pneumoniae and Y. enterocolitica antibodies in adult sera were determined by indirect enzyme-linked immunosorbent assay (ELISA). Formalinized bacteria (10⁵ cfu/ml) were used to coat microtitre plates, and after serial dilution and incubation with sera, bound antibodies were demonstrated by reaction with anti-human IgA, IgG and IgM conjugated with horseradish peroxidase followed by the addition of enzyme substrate.

### Table 1. Selected bacterial genes, PCR primer and hybridization probe sequences and amplimer lengths

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Plasmid</th>
<th>Gene</th>
<th>Sequence</th>
<th>Amp (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia trachomatis</td>
<td>Plasmid</td>
<td>BamHI restriction site</td>
<td>CCT CTT CGT TGA CCG ATG TA&lt;br&gt;CCC AGA CAA TGC TCC AAG GA&lt;br&gt;AAG TCT TAA TGG AGA TT³</td>
<td>190</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>LcrE</td>
<td></td>
<td>CGG GGA GAA TCT GTG CAG&lt;br&gt;CAC GGA GCG GTG GCA GTG G&lt;br&gt;GAT GCC CTG AAA GGG CGC CCT Gº</td>
<td>450</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td></td>
<td>ial</td>
<td>CCA GCC CAA CAA TTA TTT CC&lt;br&gt;CTG GTA GAT GTG AGG&lt;br&gt;GTG TCC ATA AGA TTA TCT ACC³</td>
<td>320</td>
</tr>
<tr>
<td>Campylobacter sp.</td>
<td>600-bp 16s-rDNA</td>
<td></td>
<td>GCC TGA TCT AGC ATT ACT AGC GAT&lt;br&gt;GCG CGC ATT AGA TAC CCT AGT AGT CC&lt;br&gt;CTC AAC TTT CTA GAC AGC TAG CAC TCT CT³</td>
<td>600</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>Inv A/Inv E</td>
<td></td>
<td>TCG CTA CAA GCA TGA AAT GG&lt;br&gt;AAA CTG GAC CAC GGT GAC AA&lt;br&gt;CTG GTC GAT TTC CTG ATC GC³</td>
<td>457</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>IS6110</td>
<td></td>
<td>CCT GCG AGC GTA GGC GTC GG&lt;br&gt;CTC GTC CAG CGC CGC TTC GG&lt;br&gt;CGA ACC CTC CCC AGG TCG A³</td>
<td>123</td>
</tr>
</tbody>
</table>

*These sequences were used as the internal hybridization probe.
(H₂O₂) and chromogen (O-phenylenediamine; OPD). Antibody titres were determined as the reciprocal dilution, which gave a coefficient index.

Results

General characteristics

There were 24 synovial fluid cell samples from 22 patients with juvenile onset SpA having undifferentiated disease or AS included in the study (Table 2). As expected, the mean age and mean duration of the disease of patients with AS were higher than in patients with undifferentiated disease, but the range was certainly wide. Adult onset SpA included two patients with undifferentiated disease or psoriatic arthritis and one patient with AS. The RA group included nine females.

Bacterial DNA studies

The samples of nine patients with juvenile onset SpA, three with adult onset SpA and one with RA contained bacterial DNA. Of the patients with SpA, five juvenile and no adult onset samples had one single bacterial DNA detected. Two juvenile and three adult samples had DNA of two different bacteria, and two juvenile onset SpA had DNA of three different bacteria. Overall, Salmonella sp. DNA was detected in seven patients, Shigella sp. DNA, M. tuberculosis DNA, and Campylobacter sp. DNA were each detected in four samples and C. trachomatis DNA was detected in two. Although one of the assays allowed the identification of Yersinia DNA in five patients, further re-testing of these samples gave negative results. Because of these inconsistencies, we only considered as positive the samples in which we could demonstrate the existence of bacterial DNA in each of the three PCR amplification and hybridization procedures (Fig. 1).

While most of the DNA-positive samples from patients with juvenile onset undifferentiated SpA contained Shigella sp. DNA, most of the positive samples from those with juvenile onset AS contained Campylobacter sp. DNA (Table 3). Similarly, Salmonella sp. DNA was found in three adult onset patients. Remarkably, one Salmonella sp.-positive DNA sample from these patients belonged to a patient with psoriatic arthritis. Mycobacterium tuberculosis DNA was found in two juvenile and one adult onset patients with undifferentiated SpA and psoriatic arthritis. In RA, one positive patient had Campylobacter sp. DNA in the synovial fluid. Double and triple positive samples showed several combinations (Table 3). With an interval of at least 4 months apart, two patients were sampled twice. One of these patients first gave a positive result for both Salmonella sp. and Shigella sp. DNA, but was then negative when a second sample was analysed. In the second patient, both samples were negative. Bacterial DNA findings did not correlate with age or disease characteristics such as clinical diagnosis, severity, or duration of symptoms (Table 4).

Antibacterial antibodies

The levels of serum antibodies against Salmonella, Campylobacter, Shigella, Yersinia, and Klebsiella were similar to those found in the synovial fluid of all groups. The comparison between juvenile onset and adult onset SpA and RA levels of serum and synovial fluid antibodies showed only slight differences. More than 90% of patients with SpA and RA controls had both serum and synovial fluid IgG antibodies against

<table>
<thead>
<tr>
<th>Table 2. Patients’ general characteristics and specific diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
</tr>
<tr>
<td>Jo-U-SpA</td>
</tr>
<tr>
<td>Jo-AS</td>
</tr>
<tr>
<td>AS</td>
</tr>
<tr>
<td>U-SpA</td>
</tr>
<tr>
<td>PsA</td>
</tr>
<tr>
<td>RA</td>
</tr>
</tbody>
</table>

Jo-U-SpA, juvenile onset undifferentiated spondyloarthropathy (or a syndrome of seronegative enthesopathy and arthropathy); Jo-AS, juvenile onset ankylosing spondylitis; U-SpA, undifferentiated spondyloarthropathy; AS, ankylosing spondylitis; PsA, psoriatic arthritis; RA, rheumatoid arthritis.
onset ankylosing spondylitis; U-SpA, undifferentiated spondyloarthropathy; AS, ankylosing spondylitis; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Patient, sex</th>
<th>Diagnosis</th>
<th>Age (yr)</th>
<th>Disease duration (yr)</th>
<th>Bacterial DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, male</td>
<td>Jo-U-SpA</td>
<td>18</td>
<td>5</td>
<td>Salmonella sp., Shigella sp., M. tuberculosis</td>
</tr>
<tr>
<td>2, male</td>
<td>Jo-U-SpA</td>
<td>16</td>
<td>1</td>
<td>C. trachomatis, Salmonella sp., Shigella sp.</td>
</tr>
<tr>
<td>3, female</td>
<td>Jo-U-SpA</td>
<td>23</td>
<td>8</td>
<td>Shigella sp.</td>
</tr>
<tr>
<td>4, male</td>
<td>Jo-U-SpA</td>
<td>15</td>
<td>8</td>
<td>Salmonella sp., M. tuberculosis</td>
</tr>
<tr>
<td>5, male</td>
<td>Jo-U-SpA</td>
<td>16</td>
<td>5</td>
<td>Campylobacter sp.</td>
</tr>
<tr>
<td>6, male</td>
<td>Jo-AS</td>
<td>22</td>
<td>9</td>
<td>Salmonella sp., Campylobacter sp.</td>
</tr>
<tr>
<td>7, male</td>
<td>Jo-AS</td>
<td>23</td>
<td>15</td>
<td>Campylobacter sp.</td>
</tr>
<tr>
<td>8, female</td>
<td>Jo-AS</td>
<td>19</td>
<td>9</td>
<td>Campylobacter sp.</td>
</tr>
<tr>
<td>9, male</td>
<td>Jo-AS</td>
<td>35</td>
<td>19</td>
<td>Salmonella sp.</td>
</tr>
<tr>
<td>10, female</td>
<td>U-SpA</td>
<td>24</td>
<td>1</td>
<td>Salmonella sp., Shigella sp.</td>
</tr>
<tr>
<td>11, male</td>
<td>AS</td>
<td>23</td>
<td>5</td>
<td>Salmonella sp., M. tuberculosis</td>
</tr>
<tr>
<td>12, male</td>
<td>PsA</td>
<td>40</td>
<td>18</td>
<td>Campylobacter sp.</td>
</tr>
<tr>
<td>13, female</td>
<td>RA</td>
<td>33</td>
<td>13</td>
<td>Campylobacter sp.</td>
</tr>
</tbody>
</table>

Jo-U-SpA, juvenile onset undifferentiated spondyloarthropathy (or a syndrome of seronegative enthesopathy and arthropathy); Jo-AS, juvenile onset ankylosing spondylitis; U-SpA, undifferentiated spondyloarthropathy; AS, ankylosing spondylitis; PsA, psoriatic arthritis; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

Klebsiella, Shigella, Salmonella, Campylobacter and Yersinia. Regarding serum IgA and IgM antibodies, the proportion of RA patients having IgM antibodies against Campylobacter and IgA antibodies against Shigella was significantly higher in comparison with SpA. Except for a higher proportion of patients with SpA having synovial fluid IgM antibodies against Campylobacter, there were non-significant differences between the different groups.

Discussion

The results of this study demonstrate the existence of bacterial DNA in synovial fluid cells of patients with juvenile onset SpA. Specifically, 40% of the patients included in our study had DNA from Salmonella sp., Shigella sp., Campylobacter sp. and/or C. trachomatis, all implicated in ReA with DNA from M. tuberculosis. Similarly, samples from patients with adult onset SpA, including AS and psoriatic arthritis, contained DNA from some of these bacteria. The relevance of these findings relies on the identification of bacterial DNA in synovial fluid cells or the synovial membrane of patients with undifferentiated or chronic SpA in patients from a population where bacterial infections are a major cause of morbidity and mortality.

Previous studies have demonstrated bacterial DNA in synovial fluid cells or the synovial membrane of patients with adult onset ReA, Reiter’s syndrome, or undifferentiated oligoarthritis and in various forms of juvenile arthritis, but not juvenile onset SpA. Remarkably, bacterial DNA has also been found in patients with osteoarthritis as well as RA and even in healthy controls. The identification of DNA in such studies has been achieved by using genus- or species-specific primers or eubacterial primers. Using the former, DNA and less frequently RNA from C. trachomatis have been widely identified [1, 2, 24]. A few other studies have been able to detect Campylobacter, Salmonella and B. burgdorferi DNA [3–6]. The approach using eubacterial primers and then oligonucleotide sequencing for bacterial identification has led to the identification of a wide range of bacteria, including some species constituting the normal flora and some not previously associated with ReA [8, 25]. Wilkinson et al. [7] found DNA from several bacteria, including Y. enterocolitica, in patients with various forms of acute or chronic arthritides in a study that required a 95% oligonucleotide sequence homology. In another study, Wilbrink et al. [25] found a number of non-arthritogenic bacteria in patients with undifferentiated SpA.

Our approach was carried out using genus- or species-specific primers selected from the literature. The C. trachomatis and Y. enterocolitica primers were similar to those used in previous studies of patients with SpA and other arthritides. The Shigella sp., Salmonella sp., Campylobacter sp. and M. tuberculosis primers selected for this study have not been previously used for DNA identification in rheumatic patients. Because Shigella sp. and E. coli genomes share a high degree of homology, it is possible that some cross-reactivity could have been detected. Given the arthritogenic potential of Shigella sp. which contrasts with that of E. coli we have attributed our DNA findings to the former bacteria. This drawback has been previously recognized.
With regards to *C. trachomatis*, there were only two positive amplifications for *Yersinia* in our samples was confirmed by three amplification in concomitant infection. The existence of multiple DNA of *M. tuberculosis* and various arthritogenic bacteria, including findings in this and other studies and controls and the lack of correlation with DNA of seroconversion for all enterobacteria in both cases antibodies is limited and may account for the high rate of one previous study [6] detecting *Salmonella* sp. DNA in synovial fluid cells of patients with ReA, no previous studies have found *Shigella* sp. DNA in patients with SpA, only in patients with undifferentiated oligoarthritis. Likewise, the number of samples having *Campylobacter* sp. DNA exceeds that reported by Braun et al. [4]. The importance of these findings is enhanced by the fact that the group of patients in this study may be part of a population highly exposed to *Salmonella* as well as *Shigella* and *Campylobacter* infections. A multinational study carried out in Latin American patients has previously found *Salmonella* sp. DNA in patients with ReA [6]. These data suggest that the individuals’ exposure to arthritogenic bacteria in certain countries may influence the rate of DNA detection in synovial fluid cells and perhaps the disease pattern.

In our country, enteric and respiratory infections are endemic diseases and represent important causes of morbidity and mortality among children. Arthritogenic bacteria colonize the gastrointestinal tract of children at a very early age [31–40]. Regardless of clinical status, nearly one-third of the faecal samples of children from birth to the age of 2 yr may have various arthritogenic bacteria [31, 33, 38]. Regarding *Shigella*, for example, up to 55% of positive faecal samples taken on a monthly basis come from children less than 2 yr old with no symptoms of intestinal infection. In the case of *Campylobacter*, 25–66% of children in rural areas develop asymptomatic, short-lasting infections in the first year of life [37, 40–42]. On the other hand, 98% of children from non-urban populations have at least one episode of diarrhoea within the first year of life [38]. *Salmonella* is implicated in 5–20% of diarrhoeal episodes in children [32, 33, 35, 38], *Shigella* in 10–35% [32, 33, 38, 39] and *Campylobacter* in 5–15% [32, 35–37, 39]. In this context, the usefulness of measuring antibacterial antibodies is limited and may account for the high rate of seroconversion for all enterobacteria in both cases and controls and the lack of correlation with DNA findings in this and other studies [4, 7].

Most positive samples in our study contained DNA of various arthritogenic bacteria, including *M. tuberculosis* DNA, yet most patients had no evidence of recent or concomitant infection. The existence of multiple DNA in our samples was confirmed by three amplification and hybridization processes. Inconsistencies such as five positive amplifications for *Yersinia* and six for *Campylobacter* in the first assay, but negative for the former in two subsequent assays and two discordant cases for the latter were regarded as negative despite positive controls giving positive results.

Except for one patient having both *Salmonella* and *Shigella* DNA who was unsuccessfully treated with ciprofloxacin and then with azithromycin because of a severe relapse of her disease 6 months after the study, no other patients, including those with *M. tuberculosis* DNA-positive detection, received any form of treatment based on DNA findings. As shown in Table 4, the DNA results did not correlate with clinical findings. Patients with positive samples for *M. tuberculosis* were followed for up to 18 months and developed no clinical signs or radiographic evidence of active tuberculosis. The presence of *M. tuberculosis* in synovial fluid could not be confined to patients with active disease. Recently, Van der Heijden et al. [8] reported the existence of *M. tuberculosis* DNA in the synovial fluid of patients with various forms of arthritis, but no tuberculosis. On the other hand, sequential sampling of a few patients rendered discordant results in samples taken 2 months apart. Previous studies have shown the coexistence of one to four different bacterial DNAs in single samples of patients with various arthritides as well as *Mycobacterium* DNA in some others and *B. burgdorferi* [3] or *C. trachomatis* DNA in serial samples [43].

The significance of these findings is to be determined [44]. Because various studies have found bacterial DNA in patients with other arthritides and even in healthy controls, the specificity of findings in patients with SpA has been challenged. On the other hand, there is no definite evidence suggesting that the DNA findings represent the existence of viable bacteria in the joints, other than *Chlamydia* and *Borrelia* thus far. The implications of these data in the context of some of the characteristics of SpA in our population are interesting. In particular, the relatively high frequency of juvenile onset SpA in comparison with juvenile RA and adult onset SpA and on the other hand the clinical pattern and severity of disease may result from recurrent as well as single or multiple infections by arthritogenic bacteria in children [11–14]. In this context, the possibility that SpA might represent an important health problem in developing countries should be considered.

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