Clinically silent infections in patients with oligoarthritis: Results of a prospective study

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Abstract
Oligoarticular synovitis of undetermined origin can closely resemble an incomplete form of reactive arthritis/Reiter’s syndrome. Eighty three patients with oligoarthritis of undetermined origin were studied prospectively to identify asymptomatic infections potentially triggering the inflammatory response in the synovial fluid. At the time of initial evaluation, 57 (69%) of the patients with oligoarthritis and 4/20 (20%) of the control subjects were carriers of clinically silent infections. Evidence for persistent or prior chlamydial infections was frequently and exclusively found in the study group (30/83 (36%) patients vs 0 controls), whereas undetected urogenital infections with mycoplasma were present in nine (11%) patients and four (20%) controls. Eleven (13%) of the patients carried cellular and humoral responses to Borrelia burgdorferi. The HLA-B27 haplotype represented a major risk factor for the development of oligoarthritis but not for development of saccroilitis. Re-evaluation after one year showed that the course and outcome of the oligoarticular disease did not correlate with a specific infectious organism and were not affected by antibiotic treatment sufficient to treat the carrier state.

Oligoarticular synovitis is of multifactorial origin with a broad spectrum of possible diagnoses. In many patients the nature of the underlying disease cannot be uncovered and the origin of the oligoartritic syndrome is categorised as undetermined. This patient group includes subjects with chronic synovitis presenting with an asymmetric distribution and predominantly affecting the leg joints, reminiscent of an incomplete form of reactive arthritis/Reiter’s syndrome (ReA/RS).1 These patients, however, do not fulfil the diagnostic criteria for ReA/RA2; in particular, they have no history of an initial infectious event. Our attempts to understand incomplete forms of ReA/RS have been limited by the fact that the relation between the preceding infection and the arthritic complication in patients with classical ReA/RA is not yet understood. A wide variety of infectious agents have been described as potential triggers of the seronegative spondyloarthopathies.1–3 The synovitis represents a complication of infection following invasion by infectious organisms in the preceding few weeks to a few months. The initial infection seems to be resolved by the time the inflammatory process in the synovial fluid causes clinical symptoms. The role of the triggering infectious agent in the chronicity of the sterile inflammatory synovitis remains to be elucidated. Despite dense infiltrates of inflammatory cells causing tissue destruction, significant numbers of infectious organisms cannot be found, suggesting that the immune response to minute amounts of antigen might be crucial in the pathogenesis of the disease. Another intriguing factor is the distance between the site of the initial mucosal infection and the site of the immune response in the synovial fluid of patients with ReA/RS, suggesting that asymptomatic forms of genital or intestinal infections might also have the potential to induce a chronic immune response in genetically predisposed subjects.

Patients and methods

PATIENTS
Eighty three patients were enrolled in the study between January 1987 and June 1988. All patients had clinical evidence of oligoarthritis affecting up to five joints, mainly knees and ankles. Patients with the following symptoms were excluded: evidence of a preceding infection, urethral discharge or diarrhoea, positive salmonella and yersinia antibody titres, clinical evidence of psoriasis, a personal or family history of psoriasis, inflammatory bowel disease, history suggestive of crystal induced arthropathy, or history of trauma. The study group partially overlapped with the group of patients in a recently published study, which compared the prevalence of positive serological tests for Borrelia burgdorferi in patients with reactive arthritis and oligoarthritis.4 Twenty normal subjects matched for age and sex who had no history of arthritis and no personal or family history of rheumatic disease were selected as controls. The following laboratory investigations were carried out at entry: complete blood cell count, erythrocyte sedimentation rate (ESR), platelet count, uric acid, rheumatoid factor, and antinuclear antibody. Patients with significant titres of rheumatoid factor or titres of antinuclear antibodies were excluded. HLA-B27 typing was performed in all patients. In addition, antero-posterior radiographs of the sacroiliac joints were taken. Radiographs were mixed with other radiographs unrelated to the study population.
and read 'blindly' by one observer. For the diagnosis of sacroiliitis, at least grade 2 abnormalities, according to the New York Classification, were required. Equivocal findings (grade 2 sacroiliitis) were confirmed by conventional or computed tomography.

**SEROLOGY AND CULTURES**

All patients and controls were analysed for chlamydial, mycoplasmal, and *B burgdorferi* infection. Urethral and cervical swabs were cultured for chlamydia using McCoy cells as detector cells. In addition, all swabs were screened for chlamydial antigen in an enzyme linked immunosorbent assay (ELISA) system (Abbott, Wiesbaden, West Germany). IgM and IgG antibodies to chlamydial antigens were determined by indirect immunofluorescence. IgG antibodies at a titre $\geq 1/256$ were considered significant. To detect *Mycoplasma hominis* and *Ureaplasma urealyticum* all swabs were cultured for mycoplasma. Antibodies specific to *B burgdorferi* were determined by indirect immunofluorescence and by an ELISA. To detect low titres of IgM antibodies the total IgM was purified by affinity column chromatography. To analyse T cell proliferation, specific for *B burgdorferi*, T cells and antigen presenting cells were purified from peripheral blood mononuclear cells by rosetting with 2-aminophenyl-sulpho-thiouronium bromide treated sheep red blood cells. Non-rosetting cells were pulsed with 100 $\mu$g/ml ultrasonicated *B burgdorferi* antigen for 20 minutes at 37°C. T cells (1x10^5/well) were cultured with 5x10^5 antigen pulsed antigen presenting cells in 96-well round bottom plates. Microcultures were pulsed with 37 kBq [^3]H]thymidine after 96 hours and harvested after an additional 12 hours.

**FOLLOW UP TREATMENT**

Patients with identified infections were treated with doxycycline, 200 mg/day, for four weeks. All patients were re-evaluated after two to three months. Patients who had a positive culture for either chlamydia or mycoplasma at the initial visit had repeated swabs. In addition, serological tests for antibodies to chlamydia and *B burgdorferi* were repeated in those patients who had evidence of chlamydia or borrelial infection.

All patients who showed no improvement after three months and had persisting oligoarthritis symptoms, despite anti-inflammatory treatment, were offered further treatment with sulphasalazine, 2 g/day. Of the initial 83 patients, 38 chose this option. All patients had a second re-evaluation one year after the initial visit. Based on the presentation, the course of the disease was defined as acute, acute remittent, and chronic persistent. The patients were classified as complete responders when they had no objective findings on physical examination and were free from any arthritic symptoms for the last eight weeks. Patients who showed marked improvement as determined by the patient's and doctor's assessment were classified as partial responders. All patients were assessed for the duration of significant disability (total number of days the patient was unable to work) during the past 12 months. Sacroiliac joint radiographs were repeated in patients who had had negative results at the initial visit but who had developed symptoms of spondyloarthropathy during follow up.

**Results**

Eighty three patients (40 male, 43 female) with oligoarthritis of undetermined origin were enrolled into the study. The mean age of both the male and female patients was 34.5 years. At study entry most patients had arthritis in a leg joint, the knee being the most commonly affected joint. Figure 1 summarises the results of chlamydial, mycoplasmal, and spongochaetal infection. Thirty (36%) of the patients had evidence of persistent or prior chlamydial infection. None of the controls had a positive chlamydial culture or significant antibody titres specific for chlamydial antigens. Nine (11%) patients carried a mycoplasmal infection of the urethra or cervix, which was similar to the 20% (4/20) mycoplasmal infection in the control group. In 11 (13%) of the study group we found cellular and humoral immune responses to *B burgdorferi*. None of the controls had evidence for a spongochaetal infection. Thus at the time of the initial evaluation 57 (69%) of the patients with oligoarthritis and four (20%) of the controls were carriers of an asymptomatic infection.

Table 1 gives details of the different study groups. Twelve patients (eight men, four women) carried significantly raised serum antibody titres to chlamydia antigens. The humoral response to antibiotic treatment was not consistent (data not shown). In some patients the start of antibiotic treatment was associated with an increase of antibodies to chlamydia. Other patients showed decreasing titres of antibodies to chlamydia.

Ten female and two male patients were found to carry mycoplasma or ureaplasma in the
urethra or cervix. Three of the patients were coinfected with chlamydia and were allocated to the chlamydia group for the follow-up evaluation. In all patients follow-up cultures were negative after antibiotic treatment. The ESR was only slightly raised, and the disability time was the shortest among the five groups. Six (50%) of the patients with a positive mycoplasma culture transiently produced low titres of rheumatoid factors. This phenomenon was not seen in any other patient group.

Table 1 Details of the study groups

<table>
<thead>
<tr>
<th>Chlamydia culture (n=17)</th>
<th>Antibodies to chlamydia (n=12)</th>
<th>Mycoplasma culture (n=12)</th>
<th>Anti-syphilitic T cells and antibodies (n=8)</th>
<th>No infection identified (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age (years)</td>
<td>33.5 (7.9)</td>
<td>38.2 (11.8)</td>
<td>33.1 (9.7)</td>
<td>33.0 (12.7)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>8/9</td>
<td>8/4</td>
<td>2/10</td>
<td>5/3</td>
</tr>
<tr>
<td>Mean (SD) ESR* (mm/h)</td>
<td>25 (23)</td>
<td>19 (14)</td>
<td>16 (13)</td>
<td>46 (31)</td>
</tr>
<tr>
<td>RF* positive (n)</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Mean (SD) sick time (months)</td>
<td>4.3 (5.1)</td>
<td>3.75 (5.1)</td>
<td>2.4 (2.3)</td>
<td>5.5 (5.2)</td>
</tr>
<tr>
<td>Co-infection with chlamydia (n)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Positive control culture after antibiotics (n)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*ESR = erythrocyte sedimentation rate; RF = rheumatoid factor.

Eleven patients, similar in age to any of the other subgroups, were found to have B cell and T cell responses specific for B burgdorferi. Eight of these patients (five male, three female) had enthesitic symptoms. Patients presented with moderately raised ESR and were unable to work for more than five months.

No infection was identified in 26 patients (13 male, 13 female). No significant differences were noted in the clinical and demographic characteristics of that subgroup compared with patients with quiescent infections. The ESR was only slightly raised, no rheumatoid factor was found, and the patients were out of work for an average of 3-7 months.

Positive cultures for chlamydia were grown from the specimens of eight male and nine female patients. In all patients with chlamydial infection proved by culture a follow-up culture was obtained two to four weeks after the end of antibiotic treatment. In three patients chlamydia was isolated again from the control culture despite antibiotic treatment of the patient and their partner. After a second course of antibiotic treatment, however, chlamydia was successfully treated in each of these three patients.

DIAGNOSIS OF CHLAMYDIAL INFECTION

Most bacterial diseases are diagnosed by isolation of the organism. Alternatively, the presence of antigen specific antibodies is used as indirect proof for a prior or present infection. In our study group identification of chlamydial infections through the isolation of the organism and the demonstration of antibodies to chlamydia seemed to be exclusive and not alternative. Figure 2 shows that patients with culture proved chlamydial infection did not carry specific IgG or IgM antibodies, with the exception of one patient. That patient greatly increased IgG antibodies to the organism at the time of a positive culture. All other subjects who were seropositive for antichlamydial reactivity had negative urethral and cervical cultures, including two patients who had significant IgM antibody titres.

INFECTION AGENT, HLA-B27, AND ENTHESOPATHY

Evidence of chlamydial, mycoplasmal, or spirochaetal infection allowed us to divide the study group into five subgroups. Clinically, the patients infected with mycoplasma could be distinguished from the four other subgroups. Many patients with chlamydial, borrelial, or no identified infection
presented with the clinical signs of an enthesopathy and an asymmetrical oligoarthritis preferentially affecting the legs. In the patients infected with mycoplasma we found no evidence of enthesitis, the arms were more affected than the legs, and at the time of re-evaluation the arthritis had a tendency to evolve into a symmetrically distributed polyarthritis.

Many reports have shown a high linkage between the presence of the HLA-B27 haplotype and sacroilitis. To the relation between HLA class I genes and the presence of sacroilitis in the study groups, all patients were typed for HLA-B27 and examined by radiography to diagnose sacroilitis. The HLA-B27 haplotype was clearly increased in the study group (fig 3). A high prevalence of patients with evidence of chlamydial infections and patients in whom we could not identify an infection carried the HLA-B27 haplotype. In the patient group with evidence for B burgdorferi infection and clinical evidence for enthesitis the HLA-B27 allele was the predominant HLA class I gene as well. Interestingly, the mycoplasma infected group had a prevalence of HLA-B27 similar to that of the normal population.

With the exception of the mycoplasma infected group, the prevalence of radiological changes of the sacroiliac joints (grades 2-4 according to the New York Classification) was amazingly high considering that these patients presented initially with an oligoarthritis and did not complain about lower back pain (fig 3). Many patients, however, recalled lower back pain and morning stiffness when specifically asked. The patient subset with antibodies to chlamydia included those with the highest prevalence of sacroilitis among the different study subsets. The prevalence of sacroilitis was lowest in the subset of patients in whom we did not identify an infection. Radiographical signs of sacroilitis were found in HLA-B27 positive and HLA-B27 negative subjects. In all clinical subsets the risk of developing enthesitis of the sacroiliac joints was almost as high for an HLA-B27 negative subject with oligoarthritis as for an HLA-B27 positive subject (table 2).

**Infectious agent, course, and outcome of the disease**

We considered whether the type of infection influenced the disease onset and the course of the disease (fig 4A). Three patterns of disease onset and course were defined in the study group: patients with an acute onset of the disease who went through one episode of the disease and did not relapse within 12 months; patients who had waxing and waning disease with more than one disease episode during the evaluation period; and patients with chronic disease with persistent symptoms throughout the study.

The proportion of acute disease onset was highest in the group infected with B burgdorferi. Only a minority of patients from whom we cultured mycoplasma or in whom we could not identify an infectious agent experienced an acute onset of the disease. The subset of patients infected with mycoplasma was characterised by chronic disease.

Patients were categorised as complete responders, partial responders, and non-responders at the end of the study period (fig 4B). The patient group with evidence of B burgdorferi infection included the highest

**Table 2: Effect of HLA-B27 on the development of sacroilitis**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Incidence of sacroilitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA-B27 positive</td>
</tr>
<tr>
<td>Chlamydia (culture)</td>
<td>6/10</td>
</tr>
<tr>
<td>Chlamydia (antibodies)</td>
<td>5/6</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>0/1</td>
</tr>
<tr>
<td>B burgdorferi</td>
<td>4/6</td>
</tr>
<tr>
<td>No infection</td>
<td>5/13</td>
</tr>
</tbody>
</table>

Figure 4: Clinical course and outcome in 83 patients with oligoarthritis with distinct clinically silent infections. The patient group was initially evaluated, allocated to the different subsets according to the infection identified, and re-evaluated after one year. As shown in fig 4A a subgroup of patients had acute disease with sudden onset and one major disease episode; some patients developed relapsing synovitis, and another subgroup had chronic, persistent disease. Results of the outcome evaluation are shown in fig 4B. Patients were classified as either complete responders, partial responders, or non-responders according to their clinical presentation. Three patients co-infected with C trachomatis not included.
Clinically silent infections in patients with oligoarthritis

proportion of patients who felt that they were almost back to normal; only a minority of these patients continued to be symptomatic at the re-evaluation date. In contrast, many patients remained symptomatic and required continuous analgesic treatment in the chlamydia and mycoplasma infected groups and in the subset in which no infection was identified. The worst outcome, with the highest proportion of patients only partially improved or not improved at all, was found in the patient group carrying antibodies to chlamydia.

Discussion
Sterile inflammatory polyarthritis in patients with ReA/RS occurring within a month of an acute urethritis or enteritis has been accepted as a disease model defining infectious agents as aetiological factors in chronic rheumatic diseases. Characteristically, attempts to isolate live microorganisms from the inflamed synovia have been unsuccessful, suggesting that the affected tissue may not be the preferred site of multiplication for the organism. In other patients with chronic synovitis the relation between a potentially causative infectious agent and the joint disease has not been established as definite but the potential role of infections has been considered. To study the hypothesis that in a subset of patients with oligoarthritis the relation between the infectious organism and the persistent synovitis remains unclear owing to the asymptomatic course of the infection, we prospectively evaluated patients presenting with oligoarthritis of undetermined origin. Here we report that about two thirds of patients consecutively evaluated for oligoarticular disease could finally be classified as having an incomplete form of ReA/RS. This high percentage is surprising considering that the patients who entered into the study did not have a history of preceding infection. A second important finding is that many patients were carrying an active infection of the urethra or cervix at a time when the arthritic complication had been present for many weeks. In 32% of the study group we could grow chlamydia or mycoplasma, and another 13% had evidence for a B burgdorferi infection.

Epidemiological studies have clearly defined the role of chlamydia in Reiter’s syndrome after urethritis and of Gram negative bacteria in arthritis after enteritis. In our study all patients were explicitly asked for signs of infections and denied any clinical manifestations characteristic of infections of the genitourinary tract. Nevertheless, we were able to culture chlamydia or mycoplasma from about one third of the study subjects. Genital mycoplasma and chlamydia can be isolated from the genital mucosa of sexually active asymptomatic adults. Therefore, their relation with rheumatic diseases is difficult to evaluate, and the mere presence of the organism does not necessarily denote that they are the cause of a chronic inflammatory arthropathy. Although the control group was small, the results of the urethral and cervical cultures showed that chlamydial infection was uncommon in the area from which the patients and controls were recruited. The prevalence of chlamydial infection is very low in the study area. Even in high risk groups, like prostitutes, active infection is as low as 10–20% (data not shown). In contrast, the prevalence of mycoplasmal infection was similar in the patient and control groups. Further support for a potential relation between a chronic carrier state with genital chlamydia or mycoplasma and the oligoarthritis comes from two observations: the patient groups identified as chlamydia and mycoplasma carriers could be disected according to the clinical presentation and the presence of the genetic risk factor HLA-B27. Patients carrying chlamydia in the cervix or the urethra were frequently HLA-B27 positive and many had sacroilitis. The group infected with mycoplasma, however, was characterised by a low prevalence of HLA-B27 and none of the patients had sacroilitis or other clinical evidence of enthesitis. Thus the clinical presentation suggested that mycoplasma infection might be associated with an arthritic complication that is distinct from chlamydia induced ReA/RS. These findings indicate that at least two major factors play a crucial part in determining how the host’s immune system handles mucosal infections and their sequelae: the host/HLA molecules seem to influence the subject’s risk of developing an oligoarthritic syndrome, and the nature of the invading organism determines the pattern of articular disease complicating the mucosal infection.

A subset of patients carried high titres of antibodies against chlamydia. We were intrigued by the finding that a positive culture result was associated with an antichlamydial humoral response in only one patient (fig 2). These data suggest as one possibility that the host’s humoral immune response prevents mucosal infection or interferes with the process of culturing the infectious organism from a mucosal specimen. A second possible interpretation is that, at least in some subjects, a long lag period is required before seroconversion can be shown. These data also indicate that measurement of serum antibodies is insufficient to diagnose chronic chlamydial infection of the genital mucosa.

Two distinct factors were extremely common in four of the five patient subsets: the expression of the HLA-B27 gene and radiological changes characteristic of sacroilitis. Previous data have shown that the genetic background of the patient is critical for the pattern of the disease, with an extensive risk of HLA-B27 positive patients with psoriasis or inflammatory bowel syndrome developing spinal disease. Our finding that HLA-B27 negative subjects also have a high risk of developing sacroilitis suggests that the presence of HLA-B27 haplotype and sacroilitis might be two independent variables. In the patient group reported here HLA-B27 represents a risk factor for the development of oligoarthritis but not for the development of sacroilitis.

A current model suggests that chronic persistent synovitis can be caused by a peculiar immunological reaction of a genetically predisposed patient to infection. Therefore, the eradication of the microorganism might inter-
rupt the pathogenetic mechanism and treatment of the infection should imply treatment of the arthritis. All patients in whom we identified infection were treated with antibiotics for a minimum period of four weeks. We were successful in treating the existing infections, although three of the 17 patients with positive chlamydial cultures required a second cycle of antibiotic treatment. Although the study was not designed as a treatment study, the data presented here do not provide evidence that successful treatment of the infection is linked to successful treatment of the oligoarthritis disease. Most patients initially identified as chlamydia or mycoplasma carriers had only partially improved or not improved at all after one year of the study. Most of the patients chose to take sulphasalazine 2 g daily for at least six months despite antibiotic treatment. Only 40% of the patients were back to normal when they were re-evaluated.

Although many different infectious organisms have been identified as potential triggering factors of ReA/RS, the causative relation between the microorganisms and the chronic inflammatory response in the enthesis is still not proved. Demonstration of antigenic material in joints of patients with reactive arthritis might explain why these patients have a continuing immune response. The failure to culture live microorganisms from synovial fluid or synovial biopsy specimens suggests as one possibility that the antigenic material deposited in the synovial fluid is not produced locally but is transported from a distant site of infection into the synovial fluid. T lymphocytes are activated when they recognise a short antigenic fragment embedded into the HLA molecule. Thus continuous T cell activation does not require the presence of live microorganisms but only the presence of an immunogenic complex of antigen and an HLA molecule. A persistent infection, of which the infected subject was unaware, might be sufficient to provide antigenic material for a continuing immune response distant from the infection itself.

With the identification of more and more infectious agents which have the potential to trigger ReA/RS in a genetically predisposed subject, a model emerges in which the host's immune responsiveness to a number of microorganisms might include the potential to lead to the sequela of chronic synovitis. Although we feel that the significance of the HLA-B27 molecule as an important risk factor has been established and the intimate link between infection and the development of reactive arthritis has been demonstrated, there are several questions which remain to be answered. The risk of the host might be linked to the susceptibility to acquire an infection and failure to eliminate it rapidly. An HLA-B27 positive subject might be prone to disseminate antigenic material from the original site of infection, or the interaction of the HLA-B27 molecule when exposed to products of microorganisms might result in chronic stimulation of the host's immune response.

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References