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Chlamydia pneumoniae – a new causative agent of reactive arthritis and undifferentiated oligoarthritis

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Abstract

***Objective*—To examine whether reactive arthritis (ReA) known to occur after a urogenital infection with *Chlamydia trachomatis* can also follow an infection with *Chlamydia pneumoniae*, a recently described species of Chlamydiae that is a common cause of respiratory tract infections.**

***Methods*—Specific antibodies (micro-immunofluorescence test) and lymphocyte proliferation to *C trachomatis* and *C pneumoniae* in paired samples of peripheral blood and synovial fluid were investigated in 70 patients with either reactive arthritis (ReA) or undifferentiated oligoarthritis (UOA).**

***Results*—Five patients with acute ReA after an infection with *C pneumoniae* are reported. Three had a symptomatic preceding upper respiratory tract infection and two had no such symptoms. In all patients a *C pneumoniae*-specific lymphocyte proliferation in synovial fluid and a high specific antibody titre suggesting an acute infection was found.**

***Conclusion*—*C pneumoniae* needs to be considered a new important cause of reactive arthritis.**

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Among the most frequent causes of reactive arthritis (ReA) are infections of the urogenital tract with *Chlamydia trachomatis*.[1–3] In undifferentiated oligoarthritis, *C trachomatis* has also been identified as a triggering bacterial antigen.[4 5]

In addition to *C trachomatis*, two species of the genus *Chlamydia* are known, *Chlamydia psittaci* and *Chlamydia pneumoniae*. *C psittaci* which can infect humans has occasionally been reported as a cause of reactive arthritis.[6] *C pneumoniae*, formerly *C trachomatis* serovar TWAR, has only recently been recognised as a separate species of *Chlamydiae*. It is now recognised as a major cause of respiratory tract infections.[7] Many *C pneumoniae* infections are mild or asymptomatic and may therefore remain undiagnosed.[7] Specific antibodies against *C pneumoniae* were found in about 50% of the adult population in seven Western countries indicating a high rate of previous infection with this pathogen; five to 10 times more frequent than antibodies against *C trachomatis*.[7] The question that arises is whether *C pneumoniae* can also cause ReA after symptomatic or asymptomatic respiratory tract infections.

At present the microimmunofluorescence test is the only specific serological test for the diagnosis of infections with any of the *Chlamydiae* and in most human chlamydial infections there is little or no cross-reactivity between the different species.[8] Synovial lymphocyte proliferation to bacterial antigen has been used as a diagnostic test in ReA and undifferentiated oligoarthritis.[5 9–11] This method seems to be useful for the diagnosis of ReA, especially in chronic forms of arthritis where specific antibodies are often not detectable. In the past the cellular immune response to *C pneumoniae* has not been examined in detail, but differentiation of the proliferative response of lymphocytes to the three different *Chlamydia* species should be possible because the homology in DNA sequence among them is less than 10%.[7]

In this study we report that *C pneumoniae* may cause acute reactive arthritis following a symptomatic upper respiratory tract infection; it also can trigger undifferentiated oligoarthritis.

Patients and methods

PATIENTS

Paired samples of peripheral blood (PB) and synovial fluid (SF) were obtained from 70 patients (38 men and 32 women; mean age 32 years, range 17–65 years) with either reactive arthritis (n = 11) or undifferentiated oligoarthritis (n = 59). In all patients bacteria-specific antibodies (only in serum) and the lymphocyte proliferation induced by *C trachomatis* and *C pneumoniae* in PB and synovial fluid were investigated. The characteristics of the patient with a humoral and cellular immune response to *C pneumoniae* (patients 1–5) or *C trachomatis* (patients 6–7) are shown in detail in the table.

CONTROLS

Antibodies against *C trachomatis* and *C pneumoniae* were also measured in 58 healthy controls (26 men, 32 women; mean age 27 years, range 19–55) from the same area.

DIAGNOSTIC CRITERIA

ReA was defined as asymmetrical oligo- or monarthritis with a history of urethritis, gastroenteritis, or an upper respiratory tract infection in the previous four weeks.

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Characteristics of patients with reactive arthritis after an infection with Chlamydia pneumoniae (1–5) or Chlamydia trachomatis (6–7)

Patient no	*Age*	*Sex*	*Involved joints*	*Duration of arthritis**	*Symptomatic preceeding infection*	*B27*
1	45	m	Both knees	1 month	No	not done
2	23	f	Knee, elbow	2 months	Pharyngitis 2 weeks earlier	+
3	31	m	Knee, Achilles tendon	2½ weeks	No	+
4	17	f	Knee	1 week	Bronchitis 3 weeks earlier	–
5	66	m	Knee, wrist	2 days	Bronchitis 1 week earlier	–
6	31	f	Knee	2 weeks	Urethritis 2 weeks earlier; urethral smear Chlamydia positive	+
7	58	m	Knees, shoulder	4 weeks	no; urethral smear Chlamydia positive	+

*at the time of investigation.

Undifferentiated oligoarthritis was defined as arthritis involving four or less joints including the knee, with no history of antecedent infection or extra-articular features suggesting Reiter's syndrome. Other rheumatic diseases were excluded by investigations.

CELL SEPARATION AND CULTURE

Mononuclear cells (MNC) were separated as previously described[3] from paired samples of PB and SF by density gradient centrifugation (Lymphoprep, Nycomedas, Norway) and resuspended at 2×10^6/ml in tissue culture medium (TCM) comprising RPMI-1640 (Gibco, Paisley, UK) with 10% fetal calf serum (Gibco), penicillin/streptomycin (100 units/100 μg per ml; Biochrom KG, Berlin, Germany) and glutamine (2 mM/ml; Biochrom KG, Berlin, Germany). Cells were aliquoted into 96-well plates at 2×10^5/well and cultured for six days in a 5% CO_2 incubator.

Triplicate wells were stimulated with some or all of the following agents: TCM alone (background proliferation); *Chlamydia trachomatis* serovar L1 (5 μg/ml) and *Chlamydia pneumoniae* (5 μg/ml), grown and purified as previously described[12]; *Yersinia enterocolitica* 0·3 and 0·9 (3 μg/ml), grown in trypticase soya bouillon over 48 hours and washed in phosphate buffered saline (PBS); *Tetanus Toxoid* (TT, Behring, Marburg, Germany, 1 μg/ml); *Pokeweed Mitogen* (PWM, Sigma, Poole, UK, 1 μg/ml). Wells were pulsed with ^{3}H-thymidine (0·2 μCi per well) for the last 18 hours of culture and incorporation measured at day 6 as previously described.[3] Optimal dose and time for proliferation assays were investigated in preliminary experiments (data not shown). Stimulation was done with whole bacteria; all bacteria were heat-inactivated for one hour at 60°C. The stimulation index (SI) was defined as proliferation to antigen divided by background proliferation.

SEROLOGY

IgG, IgM and IgA chlamydial antibodies were measured by standardised micro-immunofluorescence as previously described.[13] The following serotypes were used as antigens: *Chlamydia trachomatis* (serotypes A–C, D–K, L1–L3), *Chlamydia pneumoniae* (TWAR; isolates TW- 183 and IOL-207) and a pool of *Chlamydia psittaci* isolates (human 33–L and IOL–395, cat 457–F, sheep abortion A–22, pigeon SDP–247).

Results

Chlamydia pneumoniae could be identified as the triggering agent of arthritis in five of 70 (7%) patients. These five patients (clinical details see table) had antibodies specific to *C pneumoniae* and also an antigen-specific synovial lymphocyte proliferation was found. Three of these patients had a preceding symptomatic infection of the upper respiratory tract and two did not. All patients had oligoarthritis predominantly of the lower limbs. Two of five patients were HLA B27 positive and two negative.

SPECIFIC HUMORAL IMMUNE RESPONSE TO *C pneumoniae*

Five patients had specific antibodies against *C pneumoniae* in their sera with either an IgM-titre >1/8 or an IgG-titre ≥1/512 indicating[7] acute infection (figs 1A, 2A). None of the other patients had antibody titres fulfilling these criteria. As can be seen in figure 1A there was no crossreactivity of the humoral response between *C pneumoniae* and *C trachomatis*. This was also true for patient 5 who had no anti-CT antibodies.

In comparison, none of 58 healthy controls had an IgG-titre >1/64 to any of the tested *Chlamydiae* and only three showed a low IgM-titre (1/8) to *C trachomatis*. Fifteen of these 58 (26%) and 18 of 58 (31%) had low titres of antibodies against *C trachomatis* serovar D–K or CPn respectively. No antibodies against *C psittaci* were detectable.

LYMPHOCYTE PROLIFERATION TO *C pneumoniae* IN PERIPHERAL BLOOD (PB)

A high lymphocyte proliferation in PB was found in patients 1 and 2, but the stimulation index was always lower in PB compared with SF (fig 1B). In patients 3, 4 and 5 the lymphocyte proliferation to *C pneumoniae* in PB was small, similar or even lower than to control antigens (figs 1B, 2B).

SYNOVIAL LYMPHOCYTE PROLIFERATION TO *Chlamydia pneumoniae*

The same five patients who had specific antibodies against *C pneumoniae* showed a *C pneumoniae*-specific synovial lymphocyte proliferation. This was always higher than for *C trachomatis* (figs 1B, 2B) which elicited the second highest response in these patients.

In patient 5 a serial investigation of synovial fluid was possible because a relapsing knee

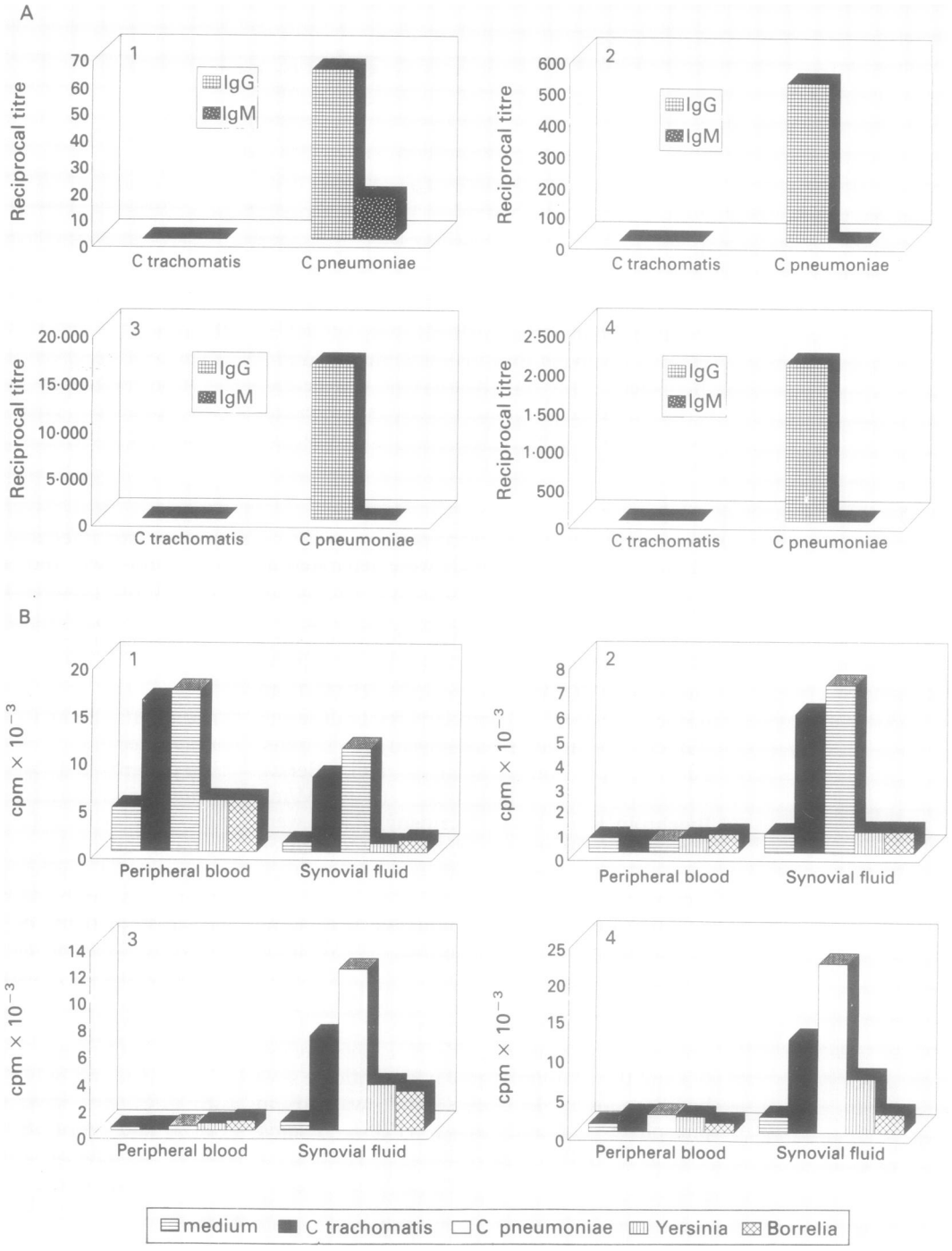

Figure 1 Humoral (A) and cellular (B) immune response to C pneumoniae and C trachomatis in patients with reactive arthritis after an infection with C pneumoniae. Numbers at the upper left corner of each graph identify each patient.

effusion required repeated arthrocentesis. As can be seen in fig 2A there was a reproducible antigen-specific synovial proliferation to *C pneumoniae*. Interestingly, the antibody response to *C pneumoniae* on day 7 and day 20 was negative or low, but a clear-cut local cellular immune response was already detectable.

SPECIFIC IMMUNE RESPONSE TO *C trachomatis*

Ten out of 70 (14%) patients showed an antigen specific proliferation to CT in synovial fluid. Only four of these, however, had high specific IgG- or IgM antibodies to *C trachomatis* indicating acute infection. Two of these patients (6 and 7) are presented in more detail (table, fig 3A and B) to demonstrate that the cellular immune response can differentiate between *C trachomatis* and *C pneumoniae*. Both patients had a high proliferation to *C trachomatis* in PB. The cellular immune response to *C pneumoniae* was the second highest (fig 3B) indicating that there is crossreactivity between *C pneumoniae* and *C trachomatis* in the cellular, but not in the humoral immune response (fig 3A).

SYNOVIAL LYMPHOCYTE PROLIFERATION TO OTHER ANTIGENS

Twelve of 70 (17%) patients showed an antigen specific lymphocyte proliferation to *Yersinia enterocolitica* and none to *Borrelia burgdorferi* (data not shown).

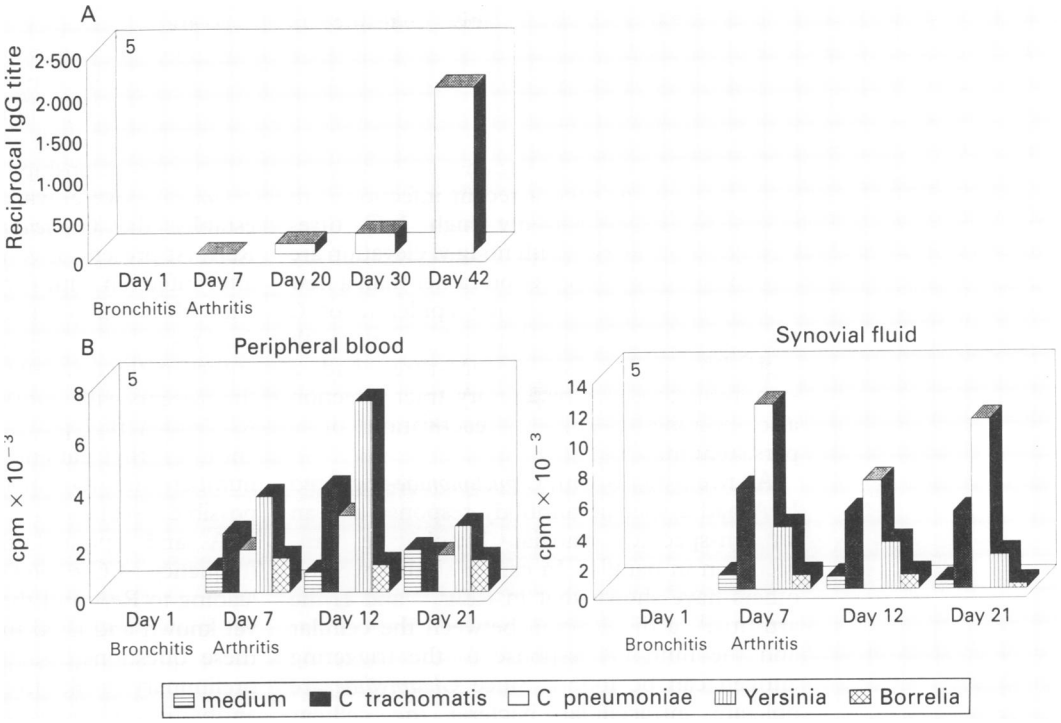

Figure 2 Humoral (A) and cellular (B) immune response to C pneumoniae and C trachomatis in one patient (patient 5) with a reactive arthritis after an infection with C pneumoniae at the beginning of the arthritis (one week after the preceding bronchitis) and at different times after. Antibodies to C trachomatis were not detectable. The number at the upper left corner at each graph identifies the patient.

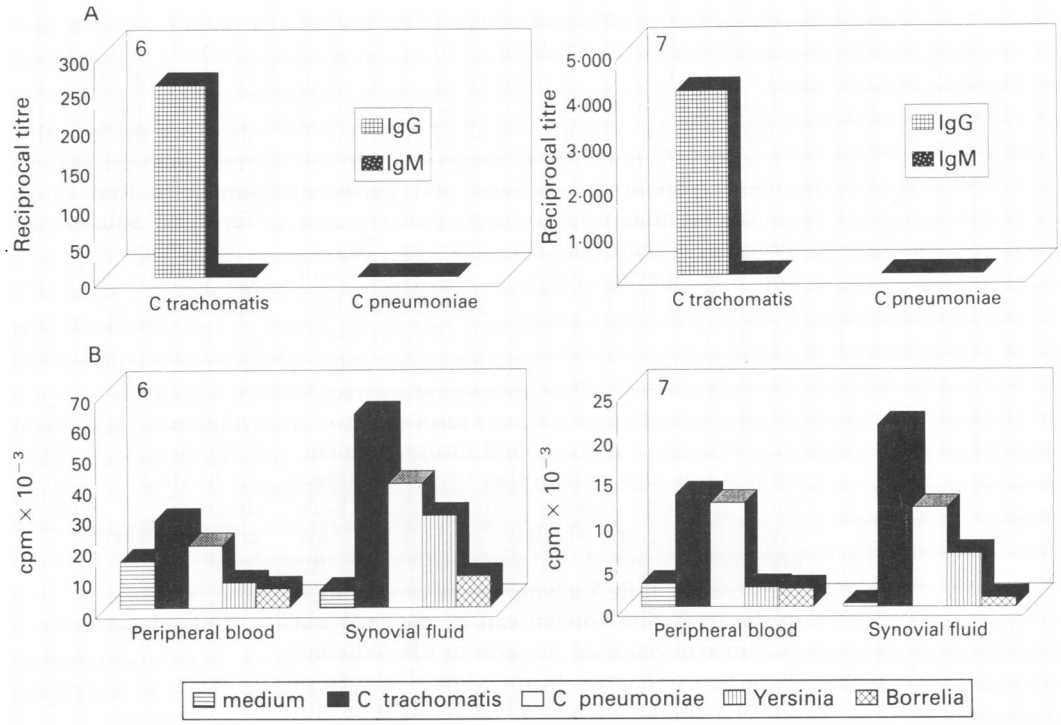

Figure 3 Humoral (A) and cellular (B) immune response to C trachomatis and C pneumoniae in two patients with a reactive arthritis after a urogenital tract infection with C trachomatis. Numbers at the upper left corner of each graph identify each patient.

Discussion

In this study we present five patients with arthritis occurring after an infection with *Chlamydia pneumoniae*, in three patients with a preceding symptomatic upper respiratory tract infection and in two patients without such symptoms. All five patients showed a *C pneumoniae*-specific cellular immune response demonstrated in the synovial lymphocyte proliferation assay and a specific humoral immune response with detection of IgM and IgG antibodies to *C pneumoniae*. This microbe therefore should now be considered a possible cause of reactive arthritis.

The clinical appearance of these patients either with symptoms of a respiratory tract infection, such as, pharyngitis and bronchitis, or with no symptoms (table), reflects quite well the known clinical pattern of infections due to *C pneumoniae*.[7 8] One should be aware

therefore that ReA can possibly occur after an upper respiratory tract infection due to *C pneumoniae;* the preceding infection can either be symptomatic or not. In our patients without preceding symptoms a high IgG titre in one and a positive IgM titre against *C pneumoniae* in the other suggested a recent infection with this microbe. Reportedly high IgG titres against *C pneumoniae* with no IgM elevation are an indication of a reinfection rather than a new infection.[7] In a countrywide epidemic of *C pneumoniae* infection in Finland patients with high IgG-titres and no IgM-titres had a symptomatic upper respiratory tract infection also arguing against a reactivation of a persistent infection.[7]

All five patients with *C pneumoniae*-triggered ReA had a specific antibody response and an antigen-specific synovial lymphocyte proliferation in parallel. In earlier studies we and others have shown that in ReA[3 14] and Lyme arthritis,[15] a dissociation between the cellular and the humoral response to the triggering antigen can be found. Since *Chlamydiae* are obligate intracellular bacteria the cellular immune response is probably of major importance in *Chlamydia*-triggered arthritis due to the chronic persistence of this organism.[16] In the acute phase of infection the humoral immune response is also important because antibodies are able to prevent spreading of the bacteria. Thus the good correlation between the humoral and the local cellular immune response in the patients in this study could be explained by all of them having an acute infection with *C pneumoniae.* In one patient (patient 5) an early and persistent cellular immune response to *C pneumoniae* in the synovial fluid was detectable (fig 2B), at a time when specific antibodies (fig 2A) were still absent. Hypothetically, the bacteria could have spread from the site of the initial infection to the joint before the antibodies were able to confine the pathogen to the respiratory tract.

A low cellular immune response to *C pneumoniae* in PB was found in three patients while it was high in the other two. This agrees with previous results with other ReA-associated bacteria which we[5] and others[10] have previously reported. In some patients in the early course of ReA a lymphocyte proliferation to the triggering antigen can be detected not only in synovial fluid but also in PB. Whether there is a difference in the clinical course of arthritis in patients with a high compared with a low systemic immune response is not known.

We were able to show that the synovial lymphocyte proliferation differentiated between a cellular immune response to *C trachomatis* and *C pneumoniae.* This could also be demonstrated in two patients with *C trachomatis*-induced ReA (fig 3), but needs to be confirmed in a higher number of patients. In oligoarthritis without preceding symptoms typical for ReA we, and others, have shown that the specific synovial lymphocyte proliferation to ReA-associated bacteria can indicate an ongoing cellular immune response while the antibody response is negative.[5 14] The results of this study supports the argument that, in such patients, the lymphocyte proliferation assay is a valuable method for identifying triggering bacteria.

To date, only preliminary results and a case report based solely on antibodies about a possible relationship between ReA and *C pneumoniae* were available.[17–19] Our data now establish that *C pneumoniae* can indeed cause ReA. At present, it is not known whether ReA after infection with *C pneumoniae* is associated with HLA B27, spinal involvement or other features of spondylarthropathies like enthesopathy or daktylitis. In our study half of the patients with arthritis due to *C pneumoniae* were HLA B27 positive and one patient had enthesopathy (patient 3, table). However, our numbers are too small to speculate on the possible associations of *C pneumoniae*-triggered ReA and spondylarthropathies. Also, the frequency of *C pneumoniae*-induced infections leading to ReA and the clinical course are not yet known and need further study. Answering these questions could be of importance, as preliminary results from studies on long term antibiotic treatment of patients with *C trachomatis*-induced ReA are encouraging.[20]

The detection of *C pneumoniae*-antigen in the joint would certainly provide further evidence that this microbe causes ReA. Studies are in progress with a *C pneumoniae*-specific polymerase chain reaction to investigate synovial fluid pellets and synovial membrane biopsies from these patients for the presence of *C pneumoniae*-DNA. A positive result would also back the hypothesis that bacterial antigen in the joint drives the local immune response—similar to ReA caused by *C trachomatis,*[21 22] Yersinia,[23] Salmonella[24] and Borrelia.[25]

In conclusion, this study broadens the spectrum of arthritogenic bacterial agents by demonstrating that *Chlamydia pneumoniae* is a possible cause of ReA. Determination of specific antibodies and, where possible, measurement of the bacteria-specific synovial lymphocyte proliferation and a history of respiratory tract infection should be included in the diagnostic investigation and differential diagnosis of reactive arthritis and undifferentiated arthritis.

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