Detection of mycoplasmal infections in blood of patients with rheumatoid arthritis

J. Haier, M. Nasralla, A. R. Franco[1] and G. L. Nicolson

Institute for Molecular Medicine, 15162 Triton Lane, Huntington Beach, CA 92649-1041 and [1]Arthritis Center of Riverside, Riverside, CA 92501, USA

Abstract

Objective. Mycoplasmal infections are associated with several acute and chronic illnesses. Some mycoplasmas can enter a variety of tissues and cells, and cause system-wide or systemic signs and symptoms.

Methods. Patients (14 female, 14 male) diagnosed with rheumatoid arthritis (RA) were investigated for mycoplasmal infections in their blood leucocytes using a forensic polymerase chain reaction (PCR) procedure. Amplification was performed with genus- and species-specific primers, and a specific radiolabelled internal probe was used for Southern hybridization with the PCR product. Patients were investigated for the presence of *Mycoplasma* spp., and positive cases were further tested for infections with the following species: *M. fermentans*, *M. hominis*, *M. pneumoniae* and *M. penetrans*.

Results. The *Mycoplasma* spp. sequence, which is not entirely specific for mycoplasmas, was amplified from the peripheral blood of 15/28 patients (53.6%) and specific PCR products could not be detected in 13 patients (46.4%). Significant differences ($P < 0.001$) were found between patients and positive healthy controls in the genus test (3/32) and in the specific tests (0/32). Moreover, the incidence of mycoplasmal infections was similar in female and male patients. Using species-specific primers, we were able to detect infections with *M. fermentans* (8/28), *M. pneumoniae* (5/28), *M. hominis* (6/28) and *M. penetrans* (1/28) in RA patients. In 36% of the patients, we observed more than one *Mycoplasma* species in the blood leucocytes. All multiple infections occurred as combinations of *M. fermentans* with other species.

Conclusions. The results suggest that a high percentage of RA patients have systemic mycoplasmal infections. Systemic mycoplasmal infections may be an important cofactor in the pathogenesis of RA, and their role needs to be explored further.

KEY WORDS: Rheumatoid arthritis, Chronic infections, *Mycoplasma*, Polymerase chain reaction.

Mycoplasmas are the smallest self-replicating, pleotrophic bacteria that lack cell walls [1, 2]. The largest group of the class Mollicutes is divided into >100 *Mycoplasma* species, which are further subclassified into various strains. Mycoplasmas are often found as extracellular parasites attached to the external surfaces of host cells, but some species invade host tissues and cells, and replicate intracellularly. These microorganisms can produce a variety of effects on host cells and tissues. Besides affecting cell growth and morphology, mycoplasmas are able to alter metabolic, immunological and biochemical functions [3].

Mycoplasmas are commonly found in the oral cavity and as symbiotic gut flora. Formerly, mycoplasmas were considered as relative benign microorganisms with a low pathogenic potential. When they penetrate into blood vessels and colonize major organs, certain species can,

however, cause acute and chronic illnesses. Some mycoplasmas, such as *M. penetrans*, *M. fermentans* and *M. pirum*, can enter a variety of tissues and cells, and cause a broad spectrum of signs and symptoms [3]. Mycoplasmas have also been shown to have a complex relationship with the immune system [4]. They are very effective at evading host immune responses, and synergism with other infectious agents has been seen. The best known species is *M. pneumoniae*, which can cause atypical pneumonia [5, 6]. Mycoplasmal infections can be present as different clinical disorders with acute and chronic signs and symptoms. Although many of these signs and symptoms are non-specific, they seem to be related, in part, to immunological or autoimmunological responses. For example, using culturing techniques, *Ureaplasma urealyticum*, *M. pneumoniae* and *M. salivarium* have been localized in the joint tissues of patients with rheumatoid diseases [7]. Hoffman *et al.* [8] found serological evidence for active and inactive mycoplasmal infections in patients with rheumatoid arthritis (RA) and juvenile RA, but they could not detect mycoplasmal DNA in the synovial fluid of these

patients using the polymerase chain reaction (PCR). Other studies observed immunological evidence for mycoplasmal infections in RA patients [9, 10].

We have begun to examine patients with chronic illnesses for the presence of systemic mycoplasmal infections. In recent studies, we have shown that patients with chronic fatigue syndrome (CFS) and/or fibromyalgia syndrome (FMS) have a much higher incidence of mycoplasmal infections in their blood leucocytes than healthy controls without clinical signs and symptoms [11–13]. We hypothesized that chronic mycoplasmal infections might also be related to the pathogenesis of other chronic illnesses, such as RA.

Mycoplasmal infections are usually diagnosed by serological procedures or culture techniques [14, 15]. Both of these techniques are very limited in their sensitivity, and thus mycoplasmal infections are often under-diagnosed or misdiagnosed [16]. The introduction of *Mycoplasma*-specific primers in PCR enables sensitive and specific detection of mycoplasmal infections, and discrimination between different *Mycoplasma* species. Using PCR techniques, the presence of mycoplasmas was investigated in synovial fluids of patients with RA and other chronic arthritis. Schaeverbeke *et al.* [17] showed that *M. fermentans*, but not *M. penetrans*, was detectable in 20% of these patients and other types of arthritis of unknown causes, but not in patients with reactive, post-traumatic or chronic juvenile arthritis. Additionally, *M. genitalium* was found in some RA patients [18]; however, the sensitivity of the conventional PCR procedures was not satisfactory [19]. The forensic PCR method that we use to identify mycoplasmal infections is very sensitive and highly specific [11].

In this preliminary study, we report on the detection of mycoplasmas in blood leucocytes of patients with RA. Using a sensitive forensic PCR method with genus-specific primers, we investigated blood samples for the presence of any type of mycoplasmal infection. Using species-specific primers, we then tested for the presence of several *Mycoplasma* species.

Materials and methods

Patients

Blood samples from 28 patients (14 female, 14 male), diagnosed with RA, were investigated for mycoplasmal infections in their blood leucocytes. The American College of Rheumatology modified criteria were used for diagnosis [20]. All patients were examined by a rheumatologist (ARF) and all fulfilled the ACR classification criteria for RA. Patients' ages ranged between 22 and 65 yr (median 42 yr). The duration of RA history was 16–300 months (median = 149 months). All patients had had no antibiotic treatments for at least 6 weeks before the blood was drawn.

Specimens

Specimens were collected and treated as previously described [11]. Briefly, blood was collected in citrate-containing tubes and immediately brought to ice bath temperature. Samples were shipped refrigerated or on wet ice by overnight courier. Whole blood (50 μl) or blood leucocytes were used for the preparation of DNA using Chelex (Biorad) as follows. Blood cells were lysed with nanopure water (1.3 ml) at room temperature for 30 min. After centrifugation at 13 000 *g* for 2 min, the supernatants were discarded. Chelex solution (200 μl) was added, and the samples were incubated at 56°C and 100°C for 15 min each. Aliquots from the centrifuged samples were used immediately for PCR or stored at −70°C until use.

Amplification

Amplification of the target sequences (Table 1) was performed in a total volume of 50 μl PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 9) containing 0.1% Triton X-100, 200 μm each of dATP, dTTP, dGTP and dCTP, 100 pmol of each primer, and 0.5–1 μg of chromosomal DNA. Purified mycoplasmal DNA (0.5–1 ng of DNA) was used as a positive control for amplification. The amplification was carried out for 40 cycles with denaturation at 94°C. Annealing was performed at 60°C (genus-specific primers and *M. penetrans*) or 55°C (*M. pneumoniae*, *M. hominis* and *M. fermentans*). The extension temperature was 72°C in all cases. Finally, product extension was allowed at 72°C for 10 min [21–23]. Negative and positive controls were used in each experimental run.

Southern blot confirmation

The amplified samples were run on a 1% agarose gel containing 5 μl/100 ml of ethidium bromide in TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0). After denaturing and neutralization, Southern blotting was performed as follows. The PCR product was transferred to a Nytran membrane. After transfer, UV cross-linking was performed. Membranes were pre-hybridized with hybridization buffer consisting of Denhardt's solution and 1 mg/ml salmon sperm as blocking reagent. Membranes were then hybridized with ^{32}P-labelled corresponding internal probe (10^7 c.p.m./bag) (see Table 1). After hybridization and washing to remove unbound probe, the membranes were exposed to autoradiography film for 7 days at −70°C.

Results

For the detection of mycoplasmal infections in blood leucocytes, we first used genus-specific primers. The *Mycoplasma* spp. sequence was amplified from DNA extracted from the peripheral blood of 15/28 (53.6%) patients, whereas specific PCR products were not detected in the 13 negative patients (46.4%). Results were similar in female and male patients. In 32 healthy controls without any clinical signs and symptoms, positive results were shown in three cases (9.4%) for *Mycoplasma* spp. test, but not for the other species-specific tests (0/32).

Specific primers for *M. fermentans*, *M. pneumoniae*, *M. penetrans* and *M. hominis* were used to detect species-

TABLE 1. Sequences from mycoplasmal DNA used for *Mycoplasma* genus-specific and species-specific PCR. The specificity of each primer was evaluated using the Blast-Search program on GenBank [44]

Sequence name	Sequence	Target	Size (bp)	Source
GPO1 primer	ACT CCT ACG GGA GGC AGC AGT A	16S mRNA	717	Van Kuppeveld
MGSO primer	TGC ACC ATC TGT CAC TCT GTT AAC CTC	Genus		*et al.*, 1992 [24]
UNI- probe	TAA TCC TGT TTG CTC CCC AC			
SB 1 primer	CAG TAT TAT CAA AGA AGG GTC TT	*tuf* gene	850	Berg *et al.*,
SB 2 primer	TCT TTG GTT ACG TAA ATT GCT	*M. fermentans*		1996 [23]
SB 3 probe	TTT TTC AGT TTC GTA TTC GAT G			
MP5-1 primer	GAA GCT TAT GGT ACA GGT TGG	unknown gene	144	Bernet *et al.*,
MP5-2 primer	ATT ACC ATC CTT GTT GTA AGG	*M. pneumoniae*		1989 [45]
MP5-4 probe	CGT AAG CTA TCA GCT ACA TGG AGG			
Mhom1 primer	TGA AAG GCG CTG TAA GGC GC	16S mRNA	281	Van Kuppeveld
Mhom2 primer	GTC TGC AAT CAT TTC CTA TTG CAA A	*M. hominis*		*et al.*, 1992 [24]
GPO1 probe	ACT CCT ACG GGA GGC AGC AGT A			
IMM-7 primer	GGA AAC GGG AAT GGT GGA ACA GAT	P35 gene	704	Nasralla *et al.*
IMM-5 primer	TTC TGC TAA TGT TAC AGC AGC AGG	(lipoprotein)		(submitted)
IMM-3 probe	AGG GAA TCT GTG ATC TTA TTC	*M. penetrans*		

specific mycoplasmal DNA by PCR. In 10/15 patients with a positive signal for *Mycoplasma* spp., we detected one or more *Mycoplasma* species, but in five positive patients we were unable to find at least one of the four tested species. The incidences of infections with *M. fermentans* (8/28), *M. pneumoniae* (5/28) and *M. hominis* (6/28) were similar. *Mycoplasma penetrans* was found in only one patient. In 36% of the patients who tested positive for the general mycoplasmal infection, we observed more than one species in the blood leucocytes. These multiple infections occurred as combinations of *M. fermentans* with other species. Single infections were found in five patients (*M. fermentans*, n = 2; *M. hominis*, n = 2; *M. pneumoniae*, n = 1), but were not observed with *M. penetrans*. All four species were detected in one patient.

Although the GPO1 and UNI sequences are capable of a few possible cross-reactions with *Mycoplasma*-related organisms, the conditions used yielded specific products for mycoplasmas as shown by van Kuppeveld *et al.* [24] and Dussurget and Roulland-Dussoix [25]. That the patients we examined had mycoplasmal infections was confirmed by species analysis using PCR. Using the *M. fermentans*-specific primers SB1 and SB2 from the *tuf* gene, we found a single band of 850 bp that hybridized only with the ³²P-labelled internal probe SB3. Similar results were obtained for the other *Mycoplasma* species (Fig. 1). To examine the reliability of the method, we performed multiple assays (repeated 3–7 times) on 40 samples with other diagnoses. All results were completely reproducible. In three cases, the sixth and seventh repeat of an initial positive result produced only a weak but positive signal due to degradation of DNA.

Fresh blood and immediate DNA preparation resulted in better results than blood that was processed after a period of time at room temperature. Six positive blood samples were divided into five aliquots each and stored at room temperature for different time intervals (processed immediately or after 1, 2, 4 or 7 days). Over time, the PCR signal decreased. In all samples that
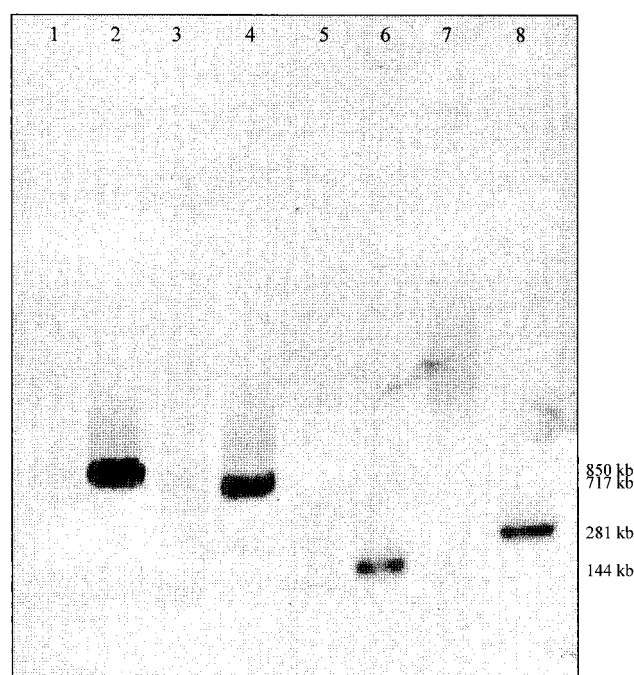

FIG. 1. Detection of different *Mycoplasma* species in control samples. Each sample was prepared as a positive control containing species-specific DNA and a negative control containing water. Control DNA was detected using primer pairs as described in Table 1. Electrophoresis was carried out on agarose gel containing ethidium bromide. Bands were visualized using UV light. Lane 1, *M. fermentans* (negative control); lane 2, *M. fermentans* (positive control); lane 3, *M. penetrans* (negative control); lane 4, *M. penetrans* (positive control); lane 5, *M. pneumoniae* (negative control); lane 6, *M. pneumoniae* (positive control); lane 7, *M. hominis* (negative control); lane 8, *M. hominis* (positive control). Inverted figure.

showed positive results in fresh DNA preparations, the PCR signal became weak after 2 days of blood storage at room temperature. After 4 days, negative results were obtained in four cases, whereas the other two samples showed very faint bands. No specific PCR product was

detectable after 1 week. Additionally, blood collected in tubes containing citrate gave better results than blood collected in acid-EDTA.

The sensitivity of *Mycoplasma* detection by the described method was assessed by the detection of control *Mycoplasma* DNA and by internal Southern hybridization using *Mycoplasma*-specific probes. Using serial dilutions of *Mycoplasma* DNA, the method was able to detect as little as 10 fg of DNA [11]. In other experiments, *M. fermentans* was added to control blood samples at various concentrations. We were able to detect specific products down to 10 ccu/ml blood. Thus, with the use of specific Southern hybridization, this PCR procedure can result in specific test results of high sensitivity, down to the presence of approximately a single microorganism in a clinical sample.

In our experience, conventional PCR yields similar results to forensic PCR with extracellular *Mycoplasma*, but not with clinical samples that contain intracellular mycoplasmas. The reason for this is not known, but it could be due to inhibitors present in the clinical samples or to loss of *Mycoplasma* DNA in the conventional extraction procedures due to protein complexing.

Discussion

Although the underlying causes of RA are not known, RA and other autoimmune diseases could be triggered, at least in part, by infectious agents. The remarkable clinical and pathological similarities between certain infectious diseases in animal species and those of some human rheumatic illnesses, such as RA, have encouraged the search for a microbial aetiology for these syndromes. A long list of microorganisms, including aerobic and anaerobic intestinal bacteria, several viruses and mycoplasmas, have been proposed as important in these illnesses [26]. Although several initial findings on many aetiological agents were not corroborated by further studies, the concept of a microbial trigger for RA is attractive. Recently, there has been increasing evidence that mycoplasmas may, in part, play a role in the genesis of arthritis [27].

In the present pilot study, we detected several *Mycoplasma* species in blood leucocytes of patients suffering from RA. Although the patient numbers in these studies were not large, using a highly sensitive and specific PCR technique we were able to detect mycoplasmal DNA in >50% of patients. Mostly we detected *M. fermentans*, and *M. penetrans* was found in only one patient with multiple mycoplasmal infections. Recently, similar findings were published using synovial fluids and joint tissue specimens [17]. Additionally, we observed infections with *M. pneumoniae* and *M. hominis*. The presence of trace amounts of mycoplasmal antigens for these species or specific antibodies against *Mycoplasma* species were found in other studies using immunological methods [10, 14]. Interestingly, we detected multiple infections with several *Mycoplasma* species in a high percentage of our patients, but these multiple infections were seen only in combination with *M. fermentans*

infections. The UNI- and GPO1 primers are not totally genus specific. However, the conditions used for PCR yield amplification products with a high degree of specificity and sensitivity [24, 25]. To overcome the problems regarding the limited specificity, we confirmed the results for the *Mycoplasma* assay with highly species-specific assays. We were able to identify at least one *Mycoplasma* species in 10 of 15 patients where the general test was positive. In the remaining five patients, it is more likely that other *Mycoplasma* species were responsible for the positive amplification signal, such as *M. arthritidis*, rather than cross-reactions with other closely related microorganisms. However, the limited specificity of the general test cannot completely rule out such cross-reactivity. Future studies will include more mycoplasmal species using highly species-specific primers.

Since little is known about the possible involvement of mycoplasmas in the pathogenesis of chronic diseases, it remains uncertain whether our findings represent a causal agent, cofactor or secondary superinfection in patients with immune disturbances. However, mycoplasmas are able to induce immune dysfunctions and autoimmune reactions. Thus, mycoplasmal infections may, in part, be involved in the pathogenesis of RA.

Mycoplasmal infections were reported in patients with various inflammatory diseases, such as endocarditis [28], pericarditis [29] or encephalomyelitis [30], where immunological or autoimmunological phenomena co-exist. Although the basis for these infections is not well understood, it is apparent that several species of pathogenic mycoplasmas are endowed with a sophisticated genetic machinery for altering their surface attributes. This surface phenotypic variation is thought to play a key role in the establishment and persistence of *Mycoplasma* infections by enabling evasion of host defences and by ensuring adaptation to the rapidly changing microenvironmental conditions encountered in the host [31]. Non-specific interactions between mycoplasmas and B lymphocytes have been implicated in disease pathogenesis, possibly leading to autoimmune reactions, modulation of immunity and/or promotion of lesion development [32]. The potential role of mycoplasmas in various joint diseases remains unknown, but they could be an important factor or cofactor. Thus, the complex relationship between mycoplasmal infections and the immune system of the host may, in part, be responsible for the pathogenesis of rheumatological inflammatory diseases. For example, *M. arthritidis*-related superantigens were found to compromise T cells [33], and they can trigger and exacerbate autoimmune arthritis in animal models. Furthermore, this *Mycoplasma* species releases substances that act on polymorphonuclear granulocytes, such as oxygen radicals, and chemotactic and aggregating substances [34]. Several studies have shown that mycoplasmal infections lead to increased levels of pro-inflammatory cytokines, such as interleukin-1, -2, -4 and -6 [35, 36]. Therefore, *M. arthritidis* and possibly other species may be responsible, in part, for autoimmune phenomena in the early stages of RA, and their progression. Deficient

or aberrant immune responses (or other underlying diseases) might be necessary for the development and progression of RA and other rheumatological illnesses.

Other microorganisms are still under investigation as causative agents or important cofactors for these chronic diseases. Reports about the detection of Epstein–Barr virus or cytomegalovirus in synovial specimens are controversial [37–39]. Furthermore, retroviruses and enteropathogenic bacteria continue to be intensively discussed as possible aetiological factors of RA [40, 41]. The identification of mycoplasmal infections in the leucocyte blood fractions of a rather large subset of RA patients supports the hypothesis that mycoplasmas, and probably other chronic infections as well, may be an important source of, or cofactor for, morbidity in these patients. Further investigation of the potential role of mycoplasmas in RA patients will require comparison with other forms of arthritis and chronic inflammatory diseases.

Recently, it was found that minocycline is an interesting new drug for the treatment of RA. Tetracycline compounds have long been used by rheumatologists, and their anti-rheumatic activity has been demonstrated [42]. The reason why minocycline alleviates the clinical signs and symptoms of RA is unclear, but the responses of some patients with RA to minocycline might be due to the susceptibility of mycoplasmas to tetracyclines [43].

References

1. Razin S. Molecular biology and genetics of mycoplasmas (Mollicutes). Microbiol Rev 1985;49:419–55.
2. Razin S, Freundt EA. The Mycoplasmas. In: Krieg NR and Holt JG, eds. Bergey's manual of systematic bacteriology, Vol. 1. Baltimore: Williams and Wilkins, 1984:740–93.
3. Baseman JB, Tully JG. Mycoplasmas: sophisticated, re-emerging, and burdened by their notoriety. Emerg Infect Dis 1997;3:21–32.
4. Rawadi FA, Roman S, Castredo M *et al.* Effects of *Mycoplasma fermentans* on the myelomonocytic lineage. J Immunol 1996;156:670–80.
5. Balassanian N, Robbins FC. *Mycoplasma pneumoniae* infection in families. N Engl J Med 1967;277:719.
6. Fernald GW, Collier AM, Clyde WA Jr. Respiratory infection due to *Mycoplasma pneumoniae* in infants and children. Pediatrics 1975;55:327–35.
7. Furr PM, Taylor-Robinson D, Webster ADB. Mycoplasmas and ureaplasmas in patients with hypogammaglobulinemia and their roll in arthritis: microbiological observation over twenty years. Ann Rheum Dis 1994;53:183–7.
8. Hoffman RW, O'Sullivan FX, Schafermeyer KR *et al.* Mycoplasma infection and rheumatoid arthritis: analysis of their relationship using immunoblotting and an ultrasensitive polymerase chain reaction detection method. Arthritis Rheum 1997;40:1219–28.
9. Lee AH, Levinson AI, Schumacher HR Jr. Hypogammaglobulinemia and rheumatic disease. Semin Arthritis Rheum 1993;22:252–64.
10. Clark HW, Coker-Vann MR, Bailey JS, Brown TM. Detection of mycoplasmal antigens in immune system complexes from rheumatoid arthritis synovial fluids. Ann Allergy 1988;60:394–8.
11. Nicolson GL, Nasralla M, Haier J, Nicolson NL. Diagnosis and treatment of chronic mycoplasmal infections in fibromyalgia and chronic fatigue syndromes: Relationship to gulf war illness. Biomed Ther 1998; 16:266–71.
12. Nicolson GL, Nicolson NL, Nasralla M. Mycoplasma infections and chronic fatigue illness (Gulf War Illness) associated with deployment to Operation Desert Storm. Intern J Med 1998;1:80–92.
13. Nicolson GL, Nicolson NL. Diagnosis and treatment of mycoplasmal infections in Persian Gulf War Illness-CFDIS patients. Int J Occup Med Tox 1996;5:69–78.
14. Cimolai N, Malleson P, Thomas E, Middleton PJ. *Mycoplasma pneumoniae* associated arthropathy: confirmation of the association by determination of the anti-polypeptide IgM response. J Rheumatol 1989;16:1150–2.
15. Barile MF, Yoshida H, Roth H. Rheumatoid arthritis: new findings on the failure to isolate or detect mycoplasmas by multiple cultivation or serological procedures and a review of the literature. Rev Infect Dis 1991;13: 571–82.
16. Stephenson J. Studies suggest a darker side of 'benign' microbes. J Am Med Assoc 1997;278:2051–2.
17. Schaeverbeke T, Gilroy CB, Bebear C, Dehais J, Taylor-Robinson D. *Mycoplasma fermentans*, but not *M. penetrans*, detected by PCR assays in synovium from patients with rheumatoid arthritis and other rheumatic disorders. J Clin Pathol 1996;49:824–8.
18. Taylor-Robinson D, Gilroy CB, Horowitz S, Horowitz J. *Mycoplasma genitalium* in the joints of two patients with arthritis. Eur J Clin Microbiol Infect Dis 1994;13: 1066–9.
19. Schaeverbeke T, Renaudin H, Clerc M *et al.* Systematic detection of mycoplasmas by culture and polymerase chain reaction (PCR) procedures in 209 synovial fluid samples. Br J Rheumatol 1997;36:310–4.
20. Hochberg MC, Chang RW, Dwosh I, Lindsey S, Pincus T, Wolfe F. The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. Arthritis Rheum 1992;35: 498–502.
21. Erlich, HA, Gelfand D, Sninsky JJ. Recent advances in the polymerase chain reaction. Science 1991;252:1643–51.
22. Kwok S, Higuchi. R. Avoiding false positive with PCR. Nature 1989;339:237–8.
23. Berg S, Lueneberg E, Frosch M. Development of an amplification and hybridization assay for the specific and sensitive detection of *Mycoplasma fermentans* DNA. Mol Cell Probes 1996;10:7–14.
24. Van Kuppeveld, FJM, Van der Logt JTM, Angulo AF *et al.* Genus- and species-specific identification of mycoplasmas by 16S rRNA amplification. Appl Environ Microbiol 1992;58:2606–15.
25. Dussurget O, Roulland-Dussoix D. Rapid, sensitive PCR-based detection of mycoplasmas in simulated samples of animal sera. Appl Environ Microbiol 1994;60:953–9.
26. Midtvedt T. Intestinal bacteria and rheumatic disease. Scand J Rheumatol 1987;64(suppl.):49–54.
27. Schaeverbeke T, Vernhes JP, Lequen L, Bannwarth B, Bebear C, Dehais J. Mycoplasmas and arthritides. Rev Rheum Engl Ed 1997;64:120–8.
28. Prattichizzo FA, Simonetti I, Galetta F. Carditis associated with *Mycoplasma pneumoniae* infection. Clinical aspects

and therapeutic problems. Minerva Cardioangiol 1997; 45:447–50.

29. O'Connor CM, Campbell PT, Van Trigt P, Corey GR. Mycoplasmal pericarditis: evidence of invasive disease. Clin Infect Dis 1993;17(suppl. 1):S58–62.

30. Kumada S, Kusaka H, Okaniwa M, Kobayashi O, Kusunoki S. Encephalomyelitis subsequent to mycoplasma infection with elevated serum anti-Gal C antibody. Pediatr Neurol 1997;16:241–4.

31. Citti C, Rosengarten R. Mycoplasma genetic variation and its implication for pathogenesis. Wien Klin Wochenschr 1997;109:562–8.

32. Simecka JW, Ross SE, Cassell GH, Davis JK. Interactions of mycoplasmas with B cells: antibody production and nonspecific effects. Clin Infect Dis 1993;17(suppl. 1): S176–82.

33. Cole BC, Griffith MM. Triggering and exacerbation of autoimmune arthritis by the *Mycoplasma arthritidis* super-antigen MAM. Arthritis Rheum 1993;36:994–1002.

34. Kirchhoff H, Binder A, Runge M, Meier B, Jacobs R, Busche K. Pathogenic mechanisms in the *Mycoplasma arthritidis* polyarthritis of rats. Rheumatol Int 1989;9: 193–6.

35. Mühlradt PF, Quentmeier H, Schmitt E. Involvement of interleukin-1 (IL-1), IL-6, IL-2 and IL-4 in generation of cytolytic T cells from thymocytes stimulated by a *Mycoplasma fermentans*-derived product. Infect Immun 1991;59:3962–8.

36. Quentmeier H, Schmitt E, Kirchhoff H, Grote W, Mühlradt PF. *Mycoplasma fermentans*-derived high-molecular weight material induces interleukin-6 release in cultures of murine macrophages and human monocytes. Infect Immun 1990;58:1273–80.

37. Fox RI, Luppi M, Pisa P, Kang HI. Potential role of Epstein-Barr virus in Sjögren's syndrome and rheumatoid arthritis. J Rheumatol 1992;32(suppl.):18–24.

38. Takei M, Mitamura K, Fujiwara S, Horie T, Ryu J, Osaka S et al. Detection of Epstein-Barr virus-encoded small RNA 1 and latent membrane protein 1 in synovial lining cells from rheumatoid arthritis patients. Int Immunol 1997;9:739–43.

39. Tsai YT, Chiang BL, Kao YF, Hsieh KH. Detection of Epstein-Barr virus and cytomegalovirus genome in white blood cells from patients with juvenile rheumatoid arthritis and childhood systemic lupus erythematosus. Int Arch Allergy Immunol 1995;106:235–40.

40. Aoki S, Yoshikawa K, Yokoyama T, Nonogaki T, Iwasaki S, Mitsui T et al. Role of enteric bacteria in the pathogenesis of rheumatoid arthritis: evidence for antibodies to enterobacterial common antigens in rheumatoid sera and synovial fluids. Ann Rheum Dis 1996;55:363–9.

41. Krause A, Kamradt T, Burmester GR. Potential infectious agents in the induction of arthritides. Curr Opin Rheumatol 1996;8:203–9.

42. Trentham DE, Dynesius Trentham RA. Antibiotic therapy for rheumatoid arthritis. Scientific and anecdotal appraisals. Rheum Dis Clin North Am 1995;21:817–34.

43. Tilley BC, Alarcon GS, Heyse SP et al. Minocycline in rheumatoid arthritis. A 48-week, double-blind, placebo-controlled trial. MIRA Trial Group. Ann Intern Med 1995;122:81–9.

44. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403–10.

45. Bernet C, Garret M, de Barbeyrac B, Bebear C, Bonnet J. Detection of *Mycoplasma pneumoniae* by using the polymerase chain reaction. J Clin Microbiol 1989;27: 2492–6.