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CHLAMYDIA TRACHOMATIS NUCLEIC ACIDS CAN BE FOUND IN THE SYNOVIUM OF SOME ASYMPTOMATIC SUBJECTS

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Objective. **The recent identification of antigens or nucleic acids of infectious agents in the joints of patients with reactive arthritis has raised questions about whether chlamydial or other infectious agent nucleic acids are also present in normal joints. We had the opportunity to study synovium from 30 asymptomatic volunteer subjects by use of polymerase chain reaction (PCR) for attempted identification of *Chlamydia* and other infectious agents.**

Methods. **All subjects had blind needle synovial biopsies with the Parker-Pearson needle. DNA was extracted and PCR performed using primers for *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Borrelia burgdorferi*, and pan bacterial 16S ribosomal RNA (rRNA).**

Results. **Two subjects were identified with nucleic acid for the 16S rRNA gene of *C trachomatis*. All other PCR reactions were negative except for the pan bacterial 16S rRNA in the *C trachomatis*–positive subjects. Both subjects, although symptom free, had some evidence of synovial reaction.**

Conclusion. ***C trachomatis*** **appears to occasionally be disseminated to joints without producing overt disease.**

The identification over the past few years of antigens or nucleic acids from *Chlamydia* (1–7) and several other organisms (see later) in the joints of patients with reactive arthritis has led to a reevaluation of the pathogenesis and treatment of these patients. Our studies and others have shown that evidence of bacterial components (most often *Chlamydia trachomatis*) in joints, although most common in reactive arthritis, can also occur in patients with other diagnoses (5,8).

Since it is known that many other organisms, such as mycobacteria, can be widely disseminated without producing symptoms, we thought that it was important to examine normal control subjects to begin to get some impressions about whether and how often *C trachomatis* and other bacteria associated with arthritis might be disseminated to joints without producing symptoms. This report describes the first study of apparently normal subjects, a survey of 30 asymptomatic volunteers who consented to blind needle biopsies of knee synovium, for evidence of chlamydial or other infectious agents in these joints.

SUBJECTS AND METHODS

Thirty normal volunteers gave their informed consent and were entered into a protocol approved by the Institutional Review Board at the clinical center at the National Institute of Arthritis and Musculoskeletal and Skin Diseases to study a number of aspects of the features of normal synovium. All subjects were required to have been totally free of any knee or other joint symptoms at any time and to have no objective signs of arthritis. All were also questioned about any antecedent infections that might have later been associated with arthritis, but subjects were not excluded on this basis.

All subjects had normal findings on knee radiographs. Results of physical examinations were normal except for mild, painless, retropatellar crepitus noted in 4 subjects. Subjects were age and sex matched to reflect the composition of an ongoing study our group is conducting concerning synovitis of recent onset. The study subjects were ages 22–66; there were 12 men and 18 women. Eight subjects were black and 22 were white.

In addition to radiographs, subjects were screened with complete blood cell counts, coagulation studies, rheumatoid

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factor, antinuclear antibody, erythrocyte sedimentation rate, serum creatinine, liver function tests, hepatitis screens, and urinalyses.

Each patient underwent needle synovial biopsies of 1 knee (usually the left) using the Parker-Pearson needle as previously described (9). Biopsies were performed on an outpatient basis. After skin infiltration with 1% lidocaine, the joint was aspirated to exclude any effusion that had been missed. After further lidocaine installation into the joint space, 20–30 small synovial specimens were removed over 20 minutes through the cannula, which was left in place throughout the procedure. Specimens were immediately placed into formalin for light microscopy, into 2% glutaraldehyde for electron microscopy, into sterile plastic tubes for quick freezing at −70°C for extraction of DNA for polymerase chain reaction (PCR), into OCT embedding medium for immunohistochemistry or in situ hybridization, and into culture tubes for routine and chlamydial cultures.

Specimens for PCR were transported on dry ice, and DNA was extracted the following day for testing by sensitive and specific assays, as described previously (10–12), for subsequent PCR for *C trachomatis* 16S ribosomal RNA (rRNA), and major outer membrane protein (MOMP) (10,12) and for several other organisms. *Chlamydia pneumoniae* 16S rRNA and MOMP were assessed using 2 sets of primers as described (13,14). Our data indicate that we can routinely find about 20 cells of these chlamydial species with the assays used. *Borrelia burgdorferi* outer surface protein A (OspA) was tested for using published primers for OspA and a genomic sequence (15) and using nested primers as described for OspA and OspB (16). Pan bacterial 16S rRNA was assessed using a conserved sequence present across all bacteria using published primers developed by Li et al (11) and by our group (17). For all assays for all organisms targeted, extreme care was taken to avoid crosscontamination of samples or assay preparations; that is, nucleic acids were prepared for analyses in a laboratory distant from that in which the PCR screening assays were prepared and run; separate sets of pipettors were used for DNA preparation and assay setup.

For all assays targeting specific organisms (i.e., *C trachomatis* or *C pneumoniae*) the identity of PCR products was confirmed by Southern blot hybridization using an internal-sequence probe specific to each organism.

RESULTS

All 30 subjects had adequate DNA for PCR study, as assessed by the demonstration of actin DNA. Two subjects who are described below had positive findings on tests for chlamydial DNA by PCR. Patient 1 was positive for both chlamydial 16S rRNA and MOMP, each of which was confirmed by hybridization. Patient 2 was positive for 16S rRNA with hybridization but had inconclusive hybridization for MOMP. Both were also positive with the pan bacterial 16S rRNA screen but were negative for all other bacteria studied. All other subjects were negative for any chlamydial DNA tested, and all those tested to date have also been negative for a pan bacterial 16S rRNA screening, for *Borrelia*, and for *C pneumoniae*. All routine and chlamydial cultures of the synovial tissue were negative.

Synovial biopsies were adequate for light microscopic histology and electron microscopy in 26 and 22 subjects, respectively. These findings have been presented previously (18) and are to be described elsewhere in detail. Findings in the 2 subjects with *Chlamydia* are presented below.

Subject 1 was a 30-year-old black woman. She had no history of genitourinary disease but had serologic evidence of a possible past chlamydial infection, with serum IgG antibodies to *C trachomatis* of 0.69 enzyme immunoassay (EIA) units (Specialty Laboratories, Santa Monica, CA). IgM antibodies to *C trachomatis* were negative. She had no serologic or other abnormal findings in the laboratory tests to suggest systemic rheumatic disease. Her synovial biopsy showed a normal lining layer but a few scattered deeper mononuclear cells. Electron microscopy showed no evidence of bacteria in the small pieces examined. She was lost to followup.

Subject 2 was a 51-year-old black woman who had a history of a tubal ligation for contraception 20 years earlier, had had unprotected sex with a number of men, and had undergone a total abdominal hysterectomy for uterine fibroids. She had no history of sexually transmitted disease, had been tested, and was negative, for human immunodeficiency virus, but had not had serologic testing for *Chlamydia*. She had recurrent sinusitis that had been treated with various antibiotics. She had a history of removal of ganglions from her right wrist 5 years earlier.

On examination of this subject, there was mild retropatellar crepitus but no joint tenderness, warmth, or swelling. Her other joints were all normal. She had an erythrocyte sedimentation rate of 28 mm/hour and otherwise totally normal findings on screening laboratory tests and autoimmune serology. Antichlamydial IgG antibodies were positive at 0.62 EIA units (normal or indeterminate 0–0.17). There were no IgM antibodies. Her synovial biopsy demonstrated prominent villi and clusters of perivascular mononuclear cells including some plasma cells (Figure 1). Electron microscopy was unsatisfactory because the specimen prepared for examination showed only fibrous tissue.

A followup examination was arranged 8 months after the original biopsy. For 1 month, she had been experiencing painful fingers. She had no other new health problems. On examination most proximal interphalangeal joints had bony enlargement but the left second and third proximal interphalangeal joints were

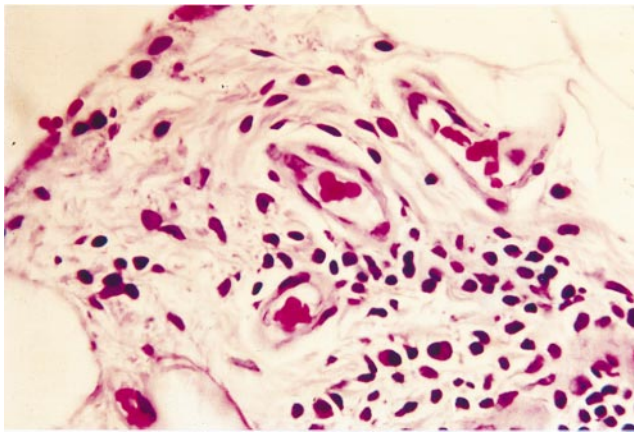

Figure 1. Synovial biopsy from subject 2, showing chronic inflammation in the totally asymptomatic knee from which chlamydial DNA was identified. Synovial lining cells are not increased. There is mild vascular congestion and surrounding mononuclear cell infiltration with plasma cells, lymphocytes, and macrophages. Hematoxylin and eosin stained; original magnification × 400.

also boggy and tender. The left knee was still asymptomatic but was warmer and slightly swollen without a definite bulge sign. There was no crepitus. She declined further studies.

DISCUSSION

This first study of apparently normal volunteers has shown that a small number of asymptomatic individuals harbor nucleic acids of *Chlamydia trachomatis* in their synovium. The 6.6% frequency of positive subjects (2 of 30 subjects studied) is much lower than the frequency of positivity among subjects we have studied with reactive arthritis (~50%) and subjects with rheumatoid or unclassifiable arthritis who have some evidence of synovial *Chlamydia* (10–20%) (8,19). We have not studied osteoarthritis (OA), but Bas et al (4) included 15 patients with OA in their series and reported 1 patient positive for *C trachomatis*. That patient had evidence of a higher than expected joint fluid leukocyte count, suggesting the presence of something other than isolated OA.

Both of our subjects, although asymptomatic, had at least some histologic evidence of a synovial inflammatory reaction to the organism. Whether these subjects will develop overt disease remains to be seen. On reevaluation of patient 2, there was some suggestion that clinical joint disease might be evolving. Studies of larger numbers of individuals would be needed to gain some notion of exactly how often *Chlamydia* disseminates to joints and whether it ever does so with absolutely no host response. Whether *Chlamydia* is ever disseminated and then cleared without the development of disease will also require further study. This background level of PCR positivity found in the synovium of asymptomatic subjects can begin to define a level against which various disease states can be compared. *Chlamydia* certainly is known to persist asymptomatically in the genital tract, and this is a likely site for further study as a possible source. Our initial short-term studies in experimental animals have so far shown that *Chlamydia* can spread from ocular and genital infections without overt disease but with some histologic changes (20).

These studies to date have not identified any other bacteria in asymptomatic joints, but the possibility of their existence is certainly not excluded. Whether our inability to demonstrate other organisms in asymptomatic joints is due to variation in sensitivity of techniques, small numbers of people sampled, or actual predilection for *Chlamydia* in joints is not yet clear. Studies by Wilbrink et al (21), our group (17), and other investigators have begun to accumulate evidence of bacterial 16S rRNA in a number of diseased joints in which infectious agents had not especially been suspected.

If findings such as ours are confirmed, implications for treatment or identification of bacterial DNA in asymptomatic joints or at other sites may need to be addressed in systematic studies. Parvovirus (22), and *Borrelia* (23), as well as *Chlamydia*, are examples of organisms that have been detected in inflamed joints without clinical evidence of antecedent disease at other sites by use of the newer sophisticated methods such as PCR. Whether nucleic acids of these other organisms occur in asymptomatic joints is not known, but could have important implications for studies on pathogenesis. Parvovirus B19 DNA has been reported in the synovium of joints arthroscoped after trauma (24).

Does persistence of nucleic acids definitely play a role in reactive or other arthritis? Might persistence more likely only cause disease in the presence of certain types of host response? Our current subjects have not been treated, but the subject whom we were able to contact has been advised of our findings and has been offered the opportunity of receiving followup care. Treatment of patients with known *Chlamydia*-associated reactive arthritis with antibiotics has not been proven to be invariably successful (25,26). Host factors may need evaluation and manipulation. Neither of our subjects had any clear clinical suggestion of host defects in handling infections, although 1 patient had recurrent sinusitis.

In our histologic study of normal synovium obtained by needle synovial biopsy, we have seen some other subjects with findings that appear outside of the usual normal range. Episodic or chronic bacterial or other infectious insults could be a factor in some of these changes. Bacteria and other particles reaching the circulation can readily, or perhaps even preferentially, localize to joints or entheses (27). Possibly more important than whether agents ever reach the joint is how the host responds to their presence.

REFERENCES

1. Schumacher HR Jr, Magge S, Cherian PV, Sleckman J, Rothfuss S, Clayburne G, et al. Light and electron microscopic studies on the synovial membrane in Reiter's syndrome: immunocytochemical identification of chlamydial antigen in patients with early disease. Arthritis Rheum 1988;31:937–46.
2. Keat A, Dixey J, Sonnex C, et al. Chlamydia trachomatis and reactive arthritis: the missing link. Lancet 1985;1:72–4.
3. Nikkari S, Puolakkainen M, Yli-Kerttula U, Luukkainen R, Lehtonen O-P, Toivanen P. Ligase chain reaction in detection of Chlamydia DNA in synovial fluid cells. Br J Rheumatol 1997;36: 763–5.
4. Bas S, Griffais R, Kvien TK, Glennås A, Melby K, Vischer TL. Amplification of plasmid and chromosome Chlamydia DNA in synovial fluid of patients with reactive arthritis and undifferentiated seronegative oligoarthropathies. Arthritis Rheum 1995;38: 1005–13.
5. Rahman MU, Cheema MA, Schumacher HR, Hudson AP. Molecular evidence for the presence of Chlamydia in the synovium of patients with Reiter's syndrome. Arthritis Rheum 1992;35:521–9.
6. Taylor-Robinson D, Gilroy CB, Thomas BJ, et al. Detection of Chlamydia trachomatis DNA in the joints of reactive arthritis patients by polymerase chain reaction. Lancet 1992;340:81–2.
7. Bas S, Ninet B, Delaspre O. Evaluation of commercially available tests for Chlamydia nucleic acid detection in synovial fluid of patients. Br J Rheumatol 1997;36:198–202.
8. Pando JA, Yarboro C, Ellaban A, Saaibi D, Kanik K, Villalba L, et al. Prevalence of Chlamydia trachomatis by PCR in the synovium of patients with early rheumatoid arthritis [abstract]. Arthritis Rheum 1995;38 Suppl 9:S287.
9. Schumacher HR, Kuika JP. Needle biopsy of the synovial membrane, experience with the Parker-Pearson technique. N Engl J Med 1972;286:416–9.
10. Branigan PJ, Gérard HC, Hudson AP, Schumacher HR Jr. Comparison of synovial tissue and synovial fluid as the source of nucleic acids for the detection of Chlamydia trachomatis by polymerase chain reaction. Arthritis Rheum 1996;39:1740–6.
11. Li F, Bulbul R, Schumacher HR Jr, Kieber-Emmons T, Callegari PE, von Feldt JM, et al. Molecular detection of bacterial DNA in venereal-associated arthritis. Arthritis Rheum 1996;39:950–8.
12. Gerard HC, Whittum-Hudson JA, Hudson AP. Genes required for assembly and function of the protein synthetic system in Chlamydia trachomatis are expressed early in elementary to reticulate body transformation. Mol Gen Genet 1997;255:637–42.
13. Gaydos CA, Quinn TC, Eiden JJ. Identification of Chlamydia pneumoniae by DNA amplification of the 16S rRNA gene. J Clin Microbiol 1992;30:796–800.
14. Balin BJ, Gerard HC, Arking EJ, Appelt DM, Abrams JT, Branigan PJ, et al. Identification and localization of Chlamydia pneumoniae in the Alzheimer's brain. Med Microbiol Immunol 1998;187:23–42.
15. Bayer ME, Zhang L, Bayer MH. Borrelia burgdorferi DNA in the urine of treated patients with chronic Lyme disease symptoms: a PCR study of 97 cases. Infection 1996;24:347–53.
16. Williams WV, Callegari P, Freundlich B, Keenan G, Eldridge D, Shin H, et al. Molecular diagnosis of Borrelia burgdorferi infection (Lyme disease). DNA Cell Biol 1992;11:207–13.
17. Gerard H, Hudson A, Williams W, Yarboro C, Klippel J, Branigan P, et al. Screening synovial fluid and tissue using PCR for pan bacterial 16S rRNA [abstract]. Arthritis Rheum 1997;40 Suppl 9:S227.
18. Arayssi T, Duray P, Crane M, Lee J, Klippel J, Schumacher HR. The spectrum of normal synovial histology [abstract]. Arthritis Rheum 1997;40 Suppl 9:S251.
19. Schumacher HR, Arayssi T, Branigan PJ, Gerard HC, Klippel JH, Pando J, et al. Surveying for evidence of synovial Chlamydia trachomatis by PCR: a study of 411 synovial biopsies and synovial fluids [abstract]. Arthritis Rheum 1997;40 Suppl 9:S270.
20. Whittum-Hudson JA, Gerard HC, Clayburne G, Schumacher HR, Hudson AP. A non-invasive murine model of Chlamydia induced reactive arthritis. Rev Rhum Engl Ed. 1999;66 Suppl:505–65.
21. Wilbrink B, van der Heijden IM, Schouls LM, van Embden JDA, Hazes JMW, Breedveld FC, et al. Detection of bacterial DNA in joint samples from patients with undifferentiated arthritis and reactive arthritis, using polymerase chain reaction with universal 16S ribosomal RNA primers. Arthritis Rheum 1998;41:535–43.
22. Takahashi Y, Murai C, Shibata S, Munakata Y, Ishii T, et al. Human parvovirus B19 as a causative agent for rheumatoid arthritis. Proc Natl Acad Sci U S A 1998;95:8227–32.
23. Priem S, Burmester GR, Kamradt T, Wolbart K, Rittig MG, Krause A. Detection of Borrelia burgdorferi by polymerase chain reaction in synovial membrane, but not in synovial fluid from patients with persisting Lyme arthritis after antibiotic therapy. Ann Rheum Dis 1998;57:118–21.
24. Soderlund M, von Essen R, Haapasaari J, Kiistala U, Kiviluoto O, Hedmank. Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. Lancet 1998;349:1063–5.
25. Lauhio A, Leirisalo-Repo M, Lähdevirta J, Saikku P, Repo H. Double-blind, placebo-controlled study of three-month treatment with lymecycline in reactive arthritis, with special reference to Chlamydia arthritis. Arthritis Rheum 1991;34:6–14.
26. Beutler AM, Hudson AP, Whittum-Hudson JA, et al. Chlamydia trachomatis can persist in joint tissue after antibiotic treatment in chronic Reiter's syndrome/reactive arthritis. J Clin Rheumatol 1997;3:125–30.
27. Schumacher HR. How micro-organisms are handled to localize to joints and within joints. Scand J Rheumatol 1995;24 Suppl 101: 199–202.