Serum Reactivity against *Borrelia burgdorferi* OspA in Patients with Rheumatoid Arthritis

Yu-Fan Hsieh, Han-Wen Liu, Tsai-Ching Hsu, James C.-C. Wei, Chien-Ming Shih, Peter J. Krause, Gregory J. Tsay

Institute of Immunology, Department of Medicine, Chung Shan Medical University, Taichung, Taiwan; Department of Parasitology and Tropical Medicine, National Defense Medical Center, Taipei, Taiwan; and Department of Pediatrics, University of Connecticut School of Medicine, Farmington, Connecticut

Received 10 April 2007/Returned for modification 5 July 2007/Accepted 6 September 2007

Lyme arthritis and rheumatoid arthritis share common clinical features and synovial histology. It is unclear whether they also share similar pathogenesis. Previous studies have shown that the severity and duration of Lyme arthritis correlate directly with serum concentrations of antibody against outer surface protein A (OspA) of the causative pathogen *Borrelia burgdorferi*. We tested the sera of 68 subjects with rheumatoid arthritis, 147 subjects with other autoimmune diseases, and 44 healthy subjects who had never had Lyme disease, as well as sera of 16 patients who had Lyme disease, for reactivity against the *B. burgdorferi* OspA protein. The sera of about a quarter of the rheumatoid arthritis patients and a 10th of the autoimmune disease and Lyme disease patients reacted against OspA antigen. Of 50 rheumatoid arthritis patients who could be evaluated for disease severity, a 28-joint count disease activity score of >2.6 was noted for 11 of 15 (73%) patients whose sera reacted against OspA antigen and 13 of 35 (37%; *P* < 0.05) whose sera were nonreactive. Serum reactivity against OspA antigen is associated with the pathogenesis of rheumatoid arthritis.

Lyme disease is the most common vector-borne disease in North America and Europe and has a worldwide distribution (28). The illness may evolve in stages, beginning with erythema migrans and progressing through a stage of dissemination during which arthritic, neurological, and cardiac complications may occur. Some patients with Lyme arthritis experience recurrent episodes of joint inflammation for months or years. Although the pathogenesis of this condition is unclear, several lines of evidence suggest autoimmunity. The histology of synovial lesions in Lyme arthritis is similar to that for rheumatoid arthritis (RA) and includes hyperplasia, vascular proliferation, and lymphoid infiltrates (29). The majority of individuals with treatment-resistant Lyme disease have the HLA-DRB1*0401 or HLA-DRB1*0101 allele, alleles which also occur more frequently in patients with RA (16, 30). Furthermore, while *Borrelia burgdorferi* DNA can be detected in joint fluid of Lyme disease patients by PCR prior to treatment with antibiotics, it is unusual to detect such DNA in synovium or synovial fluid after antibiotic treatment, especially for patients experiencing recurrent Lyme arthritis (6, 20). These findings suggest that the pathogenesis of joint disease in chronic Lyme arthritis may be a result of antibody directed against a component of the *B. burgdorferi* spirochete that cross-reacts with synovial tissue.

* Borrelia* spirochetes are unique bacteria in the abundance of their surface-displayed lipoproteins, some of which play important roles in the pathogenesis of Lyme disease (27). One of these lipoproteins, outer surface protein A (OspA), is a significant virulence factor for *B. burgdorferi* colonization and transmission. OspA has been used in a first-generation Lyme disease vaccine for humans (21, 22, 32). Although antibody directed against OspA may protect against Lyme disease, some patients vaccinated with an OspA-containing formulation developed transient arthralgia (23). About a 10th of the patients who develop Lyme arthritis experience recurrent synovitis despite multiple courses of antibiotics, and these people often develop high titers of OspA-specific antibody during the late phase of disease (17, 31). Anti-OspA immunoglobulin G (IgG) antibody concentrations correlate directly with the severity and duration of Lyme arthritis (1). Sequence similarity exists between the OspA 165–173 epitope and leukocyte function-associated antigen 1α, amino acid positions 332 to 340 (LFA-1α 332–340) (14). These data suggest that immune reactivity triggered by OspA antibody or an antibody that cross-reacts against OspA antigen is involved in the development of joint disease in patients experiencing Lyme disease.

Because of the similarities between Lyme arthritis and RA, it may be that the sera of RA patients also are reactive against OspA antigen and that this reactivity is associated with disease severity. Accordingly, we tested the sera of patients with RA and other autoimmune diseases against an OspA fusion protein and compared the severity of disease in RA patients whose sera reacted against anti-OspA antigen with that of patients whose sera were nonreactive.

MATERIALS AND METHODS

**Subjects.** Sera were obtained from patients with autoimmune disease from the Chung Shan Medical University Hospital, Taiwan, including 68 patients with RA, 67 patients with Sjogren’s syndrome (SS), 67 patients with systemic lupus erythematosus (SLE), and 13 patients with ankylosing spondylitis (AS). The diagnosis of autoimmune disease was made using standard criteria for RA (1987 revised criteria) (3), SS (American-European Consensus Group criteria) (35), SLE (1982 revised criteria) (33), and AS (1984 revised criteria) (34). None of our...
RA patients or patients with other autoimmune diseases had ever fulfilled criteria for the diagnosis of Lyme disease (7, 8). Sera also were obtained from 16 patients who had experienced Lyme disease and had been enrolled in previous studies that were carried out at the University of Connecticut School of Medicine and from 44 normal healthy people living in Taiwan (18). All subjects provided informed consent in accordance with institutional review board (Research Ethics Committee) guidelines of the Chung Shan Medical University Hospital, Taichung, Taiwan, and the University of Connecticut School of Medicine/Connecticut Children’s Medical Center. Disease activity for the RA patients was based upon the 28-joint count disease activity score (DAS28) using the disease activity measures of the World Health Organization/International League of Associations for Rheumatology core set (3). Using a conversion formula, the appropriate cutoff point for remission measured by DAS28 was 2.6 (12). Therefore, the cutoff point for disease activity was arbitrarily determined to be 2.6.

Preparation of B. burgdorferi strain B31 antigenic extracts. The genospecies strain B31 of B. burgdorferi sensu stricto was used in this study to prepare antigen for antibody assays because the Taiwan isolates are classified in the genospecies B. burgdorferi sensu stricto, the same strain that caused infection in our Lyme disease subjects (9, 26). Briefly, spirochetes were cultured at 34°C in a humidified incubator with 5% carbon dioxide and maintained in BSK-H medium (catalog no. B5528; Sigma Chemical Co, St. Louis, MO) supplemented with 6% rabbit serum (catalog no. R7136; Sigma Chemical Co, St. Louis, MO) as previously described (9). All cultures were examined weekly for the optimal growth of spirochetes by dark-field microscopy (model BX-60; Olympus Co., Tokyo, Japan).

Preparation of OspA fusion proteins. We constructed an OspA fusion protein in order to produce a sufficient amount of OspA to complete our experiments. The OspA cDNA (GenBank accession no. X14407) encoding amino acid residues (all from Novagen, Madison, WI) for detection and purification. The thioredoxin protein (109 amino acids [aa]), His-Tag (12 aa), and S-Tag (15 aa) sequences (all from Novagen, Madison, WI) for detection and purification. The BL21(DE3) strain of Escherichia coli, which contained a full-length cDNA of OspA, was used as the bacterial host for expressing the fusion proteins. When the optimal density of the culture at 600 nm reached 0.7 to 0.9, protein expression was induced by the addition of isopropyl-β-D-thiogalactoside at a concentration of 50 mmol/liter Na2CO3-NaHCO3 buffer, pH 7.2. The bacteria were harvested by centrifugation at 4,000 × g for 20 min and suspended in 20 ml phosphate-buffered saline (PBS) containing 20 μM phenylmethylsulfonyl fluoride. The cell suspension was sonicated (Heat Systems Ultrasonic) and centrifuged at 10,000 × g for 30 min. The pellet was washed with PBS containing phenylmethylsulfonyl fluoride and 0.1% Tween 20 and dissolved with 10 ml guanidium lysis buffer (6 M guanidine hydrochloride, 20 mM sodium phosphate, 500 mM NaCl, pH 7.8). The sample was loaded onto a 2-md Ni2+-nitrilotriacetic acid column (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom) and washed with PBS buffer (50 mM NaPO4, [pH 8.0]–0.25 mM EDTA–100 mM NaCl). Protein then was eluted with a 0.1 M imidazole gradient and dialyzed with PBS using 6 M guanidine hydrochloride as buffer of urea. Eluted fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot antibody assays. Recombinant OspA proteins for enzyme-linked immunosorbent assay (ELISA) were derived from cDNA clones as previously described (15).

Preparation of rabbit anti-OspA antibody. A female New Zealand White rabbit was immunized subcutaneously in the neck region with 0.5 mg of recombinant OspA fusion protein emulsified in Freund’s incomplete adjuvant at an interval of 2 weeks. SDs-PAGE and Western blot antibody assays. SDS-PAGE using a 12.5% acrylamide slab gel with a 5% acrylamide stacking gel was performed as previously described (15). Samples were reduced for 5 min in boiling water with 0.0625 M Tris–HCl buffer, pH 6.8, containing 2.3% SDS, 5% 2-mercaptoethanol, and 10% glycerol. Samples applied to the gel were run at 100 to 150 V for 1.5 h and then electrophoretically transferred to nitrocellulose. The nitrocellulose-transferred proteins were cut into strips and soaked in 5% nonfat dry milk in PBS for 30 min at room temperature. Antiserum diluted with 5% nonfat dry milk in PBS was reacted with the nitrocellulose strips and incubated for 1.5 h at room temperature. The strips were washed twice with PBS-Tween for 1 h and incubated with secondary antibody consisting of alkaline phosphatase-conjugated goat anti-human or mouse IgG antibodies. The substrate of nitroblue tetrazolium–5-bromo-4-chloro-3-indolyl phosphate was used to detect the reaction. OspA antibody ELISA. Screening for OspA antibody specificities was performed by ELISA (15). All sera were assayed at a dilution of 1/200. Briefly, microwell plates were coated overnight at 4°C with 2 μg/100 μl/well of OspA fusion protein in 50 mmol/liter Na2CO3–NaHCO3 buffer, pH 7.2. Wells were blocked with gelatin, washed with PBS-Tween, and sequentially incubated with human sera (1:200 dilutions) and peroxidase-conjugated goat anti-human Ig. The peroxidase-conjugated goat anti-human IgG was used at a dilution of 1/1,000. Substrate solution containing 1 mg/ml 2,2’-azino-di-(3-ethyl-benzthiazolin-6-sulfonic acid) and 0.005% hydrogen peroxide in 0.1 mol/liter Methylamine’s buffer was used for the peroxidase reaction. Each plate included three positive and three negative control sera. The normal value for absorbance was based on results for the 44 healthy controls (0.413 ± 0.410 [mean ± 2 standard deviations (SD)]). Values above 0.823 (normal value plus 2 SD) were regarded as showing reactivity against OspA antigen.

RF. Rheumatoid factor (RF) determination was performed using a commercial kit (Avitex-RF test; Omega Diagnostics).

Statistical analyses. Chi-square analysis was used to determine the significance between sets of categorical data.

RESULTS

Detection of anti-OspA antibody by ELISA in sera from study subjects. Sera reactive against OspA antigen were not found for patients with Lyme disease (19%) and RA (22%) and for those with AS (30%), SLE (13%), and SS (11%) (Fig. 1). The serum of patients with RA was more likely to contain IgG antibody that was reactive against OspA antigen than was that of the group of patients with other autoimmune diseases (P < 0.05). IgG antibody reactive against OspA antigen was found in the sera of patients with autoimmune diseases, including those with RA.

Detection of anti-OspA antibody by Western blotting in sera from study subjects. Serum IgG reactivity against the OspA fusion protein was confirmed by Western blotting with sera from representative patients with RA and Lyme disease as shown in Fig. 2. Sera reacting to the 52-kDa protein of OspA fusion protein included that from a rabbit immunized with OspA antigen (lane 1), from patients with Lyme disease (lanes 2 and 3), from patients with RA (lanes 4 to 7), and from healthy controls (lanes 8 and 9); results with anti-mouse histidine antibody IgG antibody also are shown (lane 10). Sera obtained from healthy control subjects were nonreactive against recombinant OspA by Western blotting (lanes 8 and 9).

Clinical severity of RA and serum reactivity against OspA antigen. Clinical severity of joint disease as measured by the DAS28 was available for 50 RA patients. The sera of 15 patients were reactive against OspA antigen, while those of the other 35 were nonreactive (Table 1). The male-female ratios and mean ages of the two groups were similar, as was the frequency of elevated erythrocyte sedimentation rate and antinuclear antibody. A higher percentage of RA patients who were seroreactive to OspA (73%) had DAS28 scores of >2.6 than was the case for nonreactive RA patients (37%; P < 0.02). Similarly, a higher percentage of OspA-seroreactive RA patients (60%) were RF positive than was the case for nonreactive RA patients (37%; P < 0.05).

DISCUSSION

We found that antibody directed against OspA antigen is detectable in the sera of some RA patients and that the presence of such antibody is associated with disease activity. Previous studies have shown that people who experience Lyme arthritis often develop OspA-specific antibody that correlates directly with the severity and duration of arthritis (1). Although joint inflammation in patients with Lyme disease and RA might result from the direct effect of OspA antibody, arthritis

Downloaded from http://cvi.asm.org/ on October 16, 2017 by guest
might also be due to antibody directed against joint antigens that cross-react with OspA antigen. One such antigen might be cytokeratin 10, which is present in synovial microvascular endothelium and which cross-reacts with OspA. Cytokeratin 10 has been shown to be a target ligand and a putative autoantigen in chronic, antibiotic treatment-resistant Lyme arthritis (13). Another cross-reacting antigen might be human leukocyte function-associated antigen 1. Molecular mimicry exists between the dominant T-cell epitope of OspA and human leukocyte function-associated antigen 1 (14). Sequence similarity has been shown to exist between OspA (165–173 epitope) and the leukocyte function-associated antigen (LFA-1α 332–340). Like human leukocyte function-associated antigen, OspA appears to have an adherence function and may help to retain *B. burgdorferi* within the tick gut (24). Antibodies directed against OspA epitopes of *B. burgdorferi* also share immune cross-reactivity with neural tissue in brain, spinal cord, and dorsal root ganglia (2). These data suggest that Lyme arthritis may be due in part to immunopathogenic mechanisms triggered by antibodies that are reactive against both joint tissue and the *B. burgdorferi* OspA lipoprotein.

Seroreactivity against *B. burgdorferi* antigens for RA patients has been inconsistently reported. About a 10th of RA patients...
were found to be seroreactive against whole *B. burgdorferi* sonicated lysate as determined by ELISA, while half were seroreactive to multiple *Borrelia* antigens as determined by Western blotting (36). In another study, sera from patients with early RA were not cross-reactive against *B. burgdorferi* antigen (10). Engstrom et al. found that the sera of only 1 of 16 RA patients contained IgG and IgM antibodies against whole *B. burgdorferi* sonicated lysate by Western blot analysis, but they emphasized that RA was associated with the presence of multiple antibodies to *B. burgdorferi* (11). These disparities in experimental results may be due to differences in patient selection, antigen preparation, or both. No seroreactivity to recombinant OspA was noted for seven RA patients with use of *B. burgdorferi* strain N40 OspA (19). In contrast, we noted seroreactivity in about a quarter of our RA patients with use of *B. burgdorferi* strain B31 OspA. We used strain B31 of *B. burgdorferi* sensu stricto in this study to prepare antigen because it is in the same genospecies (*B. burgdorferi* sensu stricto) that infected our study subjects experiencing autoimmune disease and Lyme disease (9, 26). Although the B31 and N40 strains of *B. burgdorferi* share similar OspA antigens, structural differences may exist between these OspA proteins. Analysis of sequence homology shows that there are slightly more clusters of nucleotides shared between LFA-1 and B31 (13.2% identity) than between LFA-1 and N40 (13.0% identity). Different *B. burgdorferi* strains have been shown to display different patterns of protein migration on SDS-PAGE and various reactivities with monoclonal antibody (4).

We found that some patients with AS, SLE, and SS reacted against OspA antigen. Our RA subjects and those with other autoimmune diseases were residents of Taiwan, where Lyme disease has only recently been detected and spirochete isolates belong to the *B. burgdorferi* sensu stricto genospecies (9, 25, 26). Lyme disease is uncommon in Taiwan, however, and none of our autoimmune disease subjects had experienced this infection. Our data suggest that serum reactivity against OspA antigen may play a role in the pathogenesis of RA. Further study of the structure and function of OspA antigen and putative autoantigens in joint tissue may help clarify the pathogenesis of RA and other autoimmune diseases.

ACKNOWLEDGMENTS

This study was supported by grants from the Center for Disease Control, R.O.C. (Taiwan) (grant DOH92-DC-1003 to G.T.), and the National Institutes of Health (grant AI 42042 to P.K.J.).

REFERENCES


8. Schoen, R. T., F. Meurice, C. M. Brunet, S. Cretella, D. Christianson, J. E. Edmonds, J. S. Smolen, N. Khaltaev, and K. D. Muirden. 2005. Antibodies against OspA epitopes of *Borrelia burgdorferi* sensu stricto genospecies (9, 25, 26). Lyme disease is uncommon in Taiwan, however, and none of our autoimmune disease subjects had experienced this infection. Our data suggest that serum reactivity against OspA antigen may play a role in the pathogenesis of RA. Further study of the structure and function of OspA antigen and putative autoantigens in joint tissue may help clarify the pathogenesis of RA and other autoimmune diseases.


12. Schoen, R. T., F. Meurice, C. M. Brunet, S. Cretella, D. Christianson, J. E. Edmonds, J. S. Smolen, N. Khaltaev, and K. D. Muirden. 2005. Antibodies against OspA epitopes of *Borrelia burgdorferi* sensu stricto genospecies (9, 25, 26). Lyme disease is uncommon in Taiwan, however, and none of our autoimmune disease subjects had experienced this infection. Our data suggest that serum reactivity against OspA antigen may play a role in the pathogenesis of RA. Further study of the structure and function of OspA antigen and putative autoantigens in joint tissue may help clarify the pathogenesis of RA and other autoimmune diseases.

ACKNOWLEDGMENTS

This study was supported by grants from the Center for Disease Control, R.O.C. (Taiwan) (grant DOH92-DC-1003 to G.T.), and the National Institutes of Health (grant AI 42042 to P.K.J.).