SPIROCHETAL ANTIGENS AND LYMPHOID CELL SURFACE MARKERS IN LYME SYNOVITIS

Comparison with Rheumatoid Synovium and Tonsillar Lymphoid Tissue

ALLEN C. STEERE, PAUL H. DURAY, and EUGENE C. BUTCHER

Using monoclonal antibodies to spirochetal antigens and lymphoid cell surface markers, we examined the synovial lesions of 12 patients with Lyme disease, and compared them with rheumatoid synovium and tonsillar lymphoid tissue. The synovial lesions of Lyme disease patients and rheumatoid arthritis patients were similar and often consisted of the elements found in normal organized lymphoid tissue. In both diseases, T cells, predominantly of the helper/inducer subset, were distributed diffusely in subsynovial lining areas, often with nodular aggregates of tightly intermixed T and B cells. IgD-bearing B cells were scattered within the aggregates, and a few follicular dendritic cells and activated germinal center B cells were sometimes present. Outside the aggregates, many plasma cells, high endothelial venules, scattered macrophages, and a few dendritic macrophages were found. HLA–DR and DQ expression was intense throughout the lesions. In 6 of the 12 patients with Lyme arthritis, but in none of those with rheumatoid arthritis, a few spirochetes and globular antigen deposits were seen in and around blood vessels in areas of lymphocytic infiltration. Thus, in Lyme arthritis, a small number of spirochetes are probably the antigenic stimulus for chronic synovial inflammation.

Lyme disease is a multisystem disorder caused by a tick-borne spirochete, *Borrelia burgdorferi* (1). In the US, arthritis occurs in approximately 60% of Lyme disease cases (2) and may become chronic, with erosion of cartilage and bone (3,4). Studies of hematoxylin and eosin–stained sections have shown the synovial lesions of Lyme arthritis to be similar to those of other chronic inflammatory arthritides, including rheumatoid arthritis (RA) (5). In markedly inflamed synovium with lymphoid follicles, the lesion may have features of a peripheral lymph node (6), the primary site where T cell–dependent immune responses are mounted against foreign antigens. Since the etiology of Lyme arthritis is now known with certainty, this illness provides an important opportunity to determine the pathogenesis of one form of chronic inflammatory arthritis.

In order to extend previous observations, we examined the synovial lesions of Lyme disease, using monoclonal antibodies to *B burgdorferi* and to lymphoid cell surface markers, and we compared these lesions with those from rheumatoid synovium and tonsillar lymphoid tissue. The synovial lesions of the 2 diseases were similar and often consisted of the elements found in tonsillar lymphoid tissue. In Lyme arthritis, a few spirochetes and globular antigen deposits were seen in and around blood vessels in areas of lymphocytic infiltration.

PATIENTS AND METHODS

Patient population. Synovial tissue was obtained from 12 patients with Lyme disease who underwent arthro-

From the Departments of Internal Medicine and Pathology, Yale University School of Medicine, New Haven, Connecticut, and the Department of Pathology, Stanford University School of Medicine, Veterans Administration Hospital, Palo Alto, California.

Supported in part by research grants AR-20358, RR-00125, AI-19957, and GM-37734 from the US Public Health Service and by the Arthritis Foundation. Dr. Butcher is an Established Investigator of the American Heart Association.

Allen C. Steere, MD (current address: Division of Rheumatology/Immunology, New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts); Paul H. Duray, MD; Eugene C. Butcher, MD.

Address reprint requests to Allen C. Steere, MD, Division of Rheumatology/Immunology, New England Medical Center, Tufts University School of Medicine, 750 Washington Street, Box 406, Boston, MA 02111.

Submitted for publication August 14, 1987; accepted in revised form October 28, 1987.

Table 1. Characteristics of patients with Lyme disease or rheumatoid arthritis*

	Lyme disease (n = 12)	Rheumatoid arthritis (n = 12)
Age, mean ± 1 SD	28 ± 16	42 ± 25
Males/females	6/6	2/10
Years of arthritis, mean ± 1 SD	1.3 ± 0.7	6.2 ± 6
ESR (mm/hour), mean ± 1 SD	30 ± 15	45 ± 20
RF titer ≥1:80†	–	9
Drug therapy		
NSAIDs	12	11
Remittive agents	0	8
Corticosteroids	0	3
Immunosuppressive agents	0	3

* ESR = erythrocyte sedimentation rate; RF = rheumatoid factor; NSAIDs = nonsteroidal antiinflammatory drugs.
† Determined in rheumatoid arthritis patients by latex agglutination. Not determined in Lyme disease patients.

scopic synovectomy between 1984 and 1986. Patient characteristics are shown in Table 1. In general, these patients were young adult men or women who had had arthritis for 1–2 years. They usually had moderately increased erythrocyte sedimentation rates (ESR), and all of them had markedly elevated antibody titers to *B burgdorferi*. Although they were not tested for rheumatoid factor, previous studies have shown that patients with Lyme arthritis rarely have positive findings on latex agglutination tests (7). All patients had received antibiotic therapy and nonsteroidal antiinflammatory drugs (NSAIDs) prior to arthroscopic synovectomy.

Synovial tissue was also obtained from 12 patients who had definite or classic RA according to the American Rheumatism Association criteria (8). Nine of these patients were seropositive (Table 1). Two of them underwent arthroscopic biopsy prior to total lymphoid irradiation (biopsy specimens provided by Dr. David Sherman, Stanford University, Palo Alto, CA), 4 underwent synovectomy or total joint replacement (tissue provided by Dr. Robert I. Fox, La Jolla, CA), and the remaining 6 had therapeutic joint procedures performed at Yale University (tissue provided by Dr. John Aversa, New Haven, CT). The RA patient group had a higher percentage of women, a longer mean disease duration, and a higher mean ESR than did the Lyme disease group; however, these differences were not statistically significant. In addition to NSAIDs, all of the RA patients had received remittive, corticosteroid, or immunosuppressive agents.

For comparison, samples of tonsils (the most accessible lymphoid tissue) were obtained from 4 patients who underwent tonsillectomy for benign tonsillar hyperplasia.

Preparation of tissue samples. In patients who underwent arthroscopy, 4 pieces of macroscopically inflamed synovium were taken from the knee using a biopsy forceps, under direct visualization. In those who underwent open procedures, 4 pieces of inflamed synovium or tonsil were selected from the surgical specimen. After washing in phosphate buffered saline (PBS), 1 piece was fixed in 10% phosphate buffered formalin, and the remaining 3 pieces were snap-frozen in optimal-temperature cutting compound (Miles Laboratories, Naperville, IL) by immersion in a mixture of dry ice and acetone. The frozen blocks were stored at −70°C.

Monoclonal antibodies. To produce monoclonal antibodies against *B burgdorferi*, spirochetes (strain 297, a cerebrospinal fluid isolate from a patient with Lyme meningitis) were grown in Barbour, Stoenner, Kelly medium (1). At late-log-phase growth, organisms were centrifuged at 10,000*g* for 20 minutes at 4°C, washed, and sonicated on ice (9). At 2-week intervals, each of 3 LEW/n rats was injected twice intraperitoneally with 1.5 mg of the sonicate, which consisted of at least 29 spirochetal polypeptides (10).

After the antibody response to this preparation was determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting (9,10), 1 rat with antibodies to the 18-, 21-, 31-, 34-, 41-, and 55-kd spirochetal polypeptides was given a third injection of the sonicate intravenously. Three days later, the spleen cells from that rat were fused with YB2/0 rat myeloma cells and cloned by limiting dilution. The hybridoma supernatants were screened by ELISA, and those producing antibody to *B burgdorferi* were tested by immunoblotting to determine their antigenic specificity. From this fusion, antibodies to the 31-kd outer membrane polypeptide (11) and the 41-kd flagellar antigen of the spirochete (12) were obtained. The antibodies to cell surface markers on T and B cells, plasma cells, macrophages, and endothelial cells were obtained commercially (Table 2).

Table 2. Primary antibodies used in immunoperoxidase staining

Antibody to*	Specificity
T cells	
Leu-4	Pan-T cell
Leu-3a	T helper/inducer cell
Leu-2a	T cytotoxic/suppressor cell
Leu-7 and Leu-11	Natural killer/killer cell
B cells	
6A4	Pan-B cell
IgD	IgD-bearing B cell
B532	Activated germinal center B cell
T05	Follicular dendritic cell, C3b receptor
Plasma cells: T10	Stem, thymocyte, and null cell activated antigen
Macrophages	
Leu-M1	Myelomonocytic antigen
Leu-M3	Monocyte/macrophage
Endothelial cells	
Factor VIII	Endothelial cells
D locus markers	
Leu-5	HLA–DR, nonpolymorphic antigen
Leu-10	HLA–DQ, nonpolymorphic antigen
Borrelia burgdorferi	
1D	31-kd outer membrane polypeptide
2A	41-kd flagellar-associated protein

* All of the antibodies are mouse monoclonal antibodies, except for 1D and 2A, which are rat monoclonal antibodies, and anti–Factor VIII, which is a rabbit polyclonal antibody. The Leu antibodies were from Becton Dickinson (Sunnyvale, CA); T05 and Factor VIII were from Dako (Santa Barbara, CA); T10 was from Ortho (Raritan, NJ). Antibodies 6A4 and IgD were kindly supplied by Dr. Ronald Levy, Stanford, CA; B532 was kindly provided by Dr. Dennis Frisman, San Diego, CA.

Staining procedures. On the day prior to staining, 6-μm sections were cut on a cryostat at −20°C; they were placed on glass slides coated with 0.01% poly-L-lysine (Sigma, St. Louis, MO), and were fixed in cold acetone for 10 minutes. Immunoperoxidase staining was done the next day by an avidin-biotin method. The primary antibodies (Table 2) were used at the titer that gave optimal staining, and were followed by biotinylated horse anti-mouse, anti-rat, or anti-rabbit IgG (1:100; Vector, Burlingame, CA), and then peroxidase-avidin complex (1:100; Vector). Each incubation was done for 40 minutes at room temperature, separated by 5-minute washes in PBS.

Color was developed using a solution of 0.05% 3,3-diaminobenzidine tetrahydrochloride (Sigma) and 0.003% hydrogen peroxidase in PBS, followed by immersion in 0.5% $CuSO_4$ diluted in 0.9*N* NaCl for 5 minutes. The sections were counterstained with hematoxylin, dehydrated in absolute alcohol, cleared in xylene, and coverslipped with Permount (Fisher Scientific, Fair Lawn, NJ). Formalin-fixed tissues were stained with hematoxylin and eosin.

Grading system. The slides were read separately, without knowledge of the diagnosis, by 2 of the authors (ACS and PHD). On slides stained with hematoxylin and eosin, the synovial lesion was evaluated for the presence of synovial cell hyperplasia, synovial lining hypertrophy, vascular proliferation, fibrin deposition, stromal edema, lymphoid infiltrates, and lymphoid aggregates. Each of these features was scored as 0 (absent), 1 (slight), 2 (moderate), or 3 (severe).

In preliminary readings of immunoperoxidase-stained slides, we attempted to enumerate each cell type in multiple fields. However, some of the monoclonal antibodies did not clearly distinguish individual cells (e.g., anti–Leu-3a and B532), and the number of cells varied greatly in different fields. Therefore, we decided to use the same semiquantitative system discussed above to estimate the number of cells bearing a particular surface marker. When the 2 observers assigned different scores to a given slide, it was reviewed by both observers together and a final score was agreed upon.

Table 3. Characteristics of the synovial lesions of Lyme disease patients versus those of rheumatoid arthritis patients*

	Lyme disease (n = 12)	Rheumatoid arthritis (n = 12)
Hematoxylin and eosin–stained slides		
General description		
Lymphoid infiltration with aggregates	8	6
Lymphoid infiltration without aggregates	2	5
Only slight lymphoid infiltration	2	1
Specific features		
Synovial cell hyperplasia	2.7	3
Synovial lining cell hypertrophy	2	2
Vascular proliferation	2.3	1.5
Obliterative vascular changes	1.5	0
Fibrin deposition	1.1	0.5
Stromal edema	1.7	1
Lymphoid infiltrates	1.9	1.8
Lymphoid aggregates	1.7	1.8
Immunoperoxidase-stained slides		
T cells		
Leu-4	2	1.8
Leu-3a:Leu-2a ratio	2.6:1	2.4:1
Leu-7+, Leu-11+	0	0
B cells		
6A4	1.7	1.6
IgD-bearing	1.2	1.2
B532	0.2	0.8
T05	0.8	1.3
Plasma cells: T10	2	2.2
Macrophages		
Leu-M1	1.3	1.5
Leu-M3	2.3	2.5
Endothelial cells: Factor VIII	2.4	2.7
D locus markers		
Leu-5	3	3
Leu-10	3	3

* "General description" values for hematoxylin and eosin–stained slides are the numbers of patients. All other values are the mean scores (0 = absent; 1 = slight; 2 = moderate; 3 = severe).

RESULTS

Organization of the cellular components in synovium. In hematoxylin and eosin–stained sections, the 12 Lyme disease and the 12 rheumatoid synovia showed moderate-to-marked synovial lining cell hypertrophy, synovial cell hyperplasia, and vascular proliferation (Table 3). In both patient groups, the intensity of lymphoid infiltration in the subsynovial lining areas varied greatly among individual patients; it ranged from heavy infiltration with apparent lymphoid follicles, to diffuse or patchy infiltration without follicles, to hypertrophied synovium with few lymphocytes. Within the stroma, fibrin deposition and edema tended to be greater in Lyme synovia, but could also be found in some rheumatoid lesions. When all patients in each group were considered, the overall grading of each of these features was similar in both Lyme and rheumatoid synovium.

In immunoperoxidase-stained sections, the organization of the cellular infiltrate and the numbers of each cell type, determined by monoclonal antibodies to lymphoid cell surface markers, were also similar in both Lyme and rheumatoid synovium (Figure 1 and Table 3). Leu-4–bearing T cells were found in a patchy or diffuse distribution in subsynovial lining areas. Leu-3 (helper/inducer) T cells predominated over Leu-2 (suppressor/cytotoxic) T cells, and Leu-7+ and Leu-11+ (natural killer) T cells were rare.

In all but 2 patients (1 with Lyme arthritis and 1 with RA), aggregates of tightly intermixed T and B cells were found amidst the more diffusely scattered T cells. In many instances, these B cell aggregates were

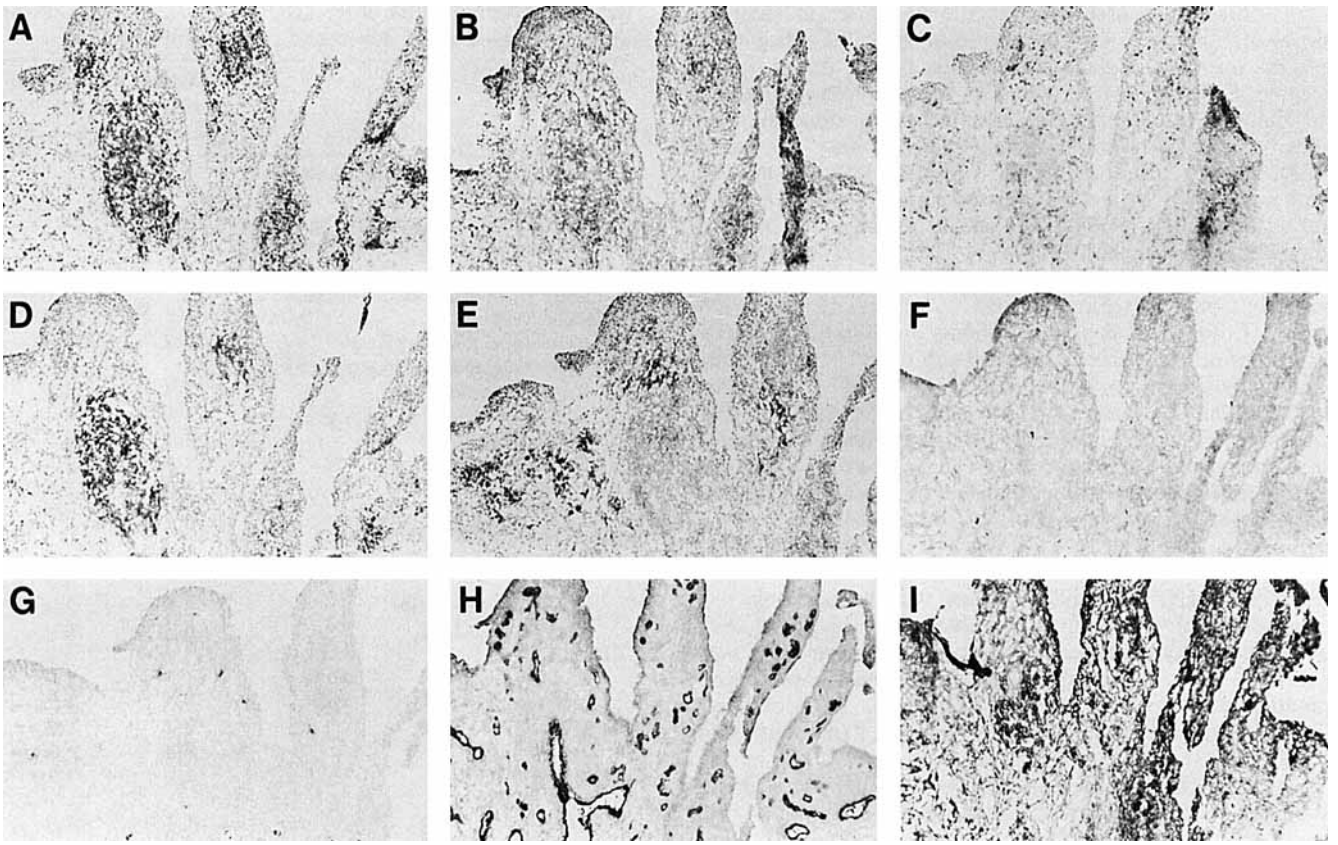


Figure 1. Organization of the cellular infiltrate in synovium from a patient with Lyme arthritis. **A,** Leu-4–bearing T cells. **B,** Leu-3 (helper/inducer) T cells. **C,** Leu-2 (suppressor/cytotoxic) T cells. **D,** 6A4 B cells. **E,** T10 plasma cells. **F,** Leu-M3–staining macrophages and synovial cells. **G,** Leu-M1–staining dendritic macrophages. **H,** Factor VIII endothelial cells. **I,** Ia (HLA–DR) expression on synovial lining cells and on many synovial and infiltrating cells in sublining areas. (Immunoperoxidase stained, original magnification × 100.)

small, and could not be spotted in hematoxylin and eosin–stained sections. Outside the aggregates, large numbers of plasma cells were clustered. Leu-M3–staining macrophages were located throughout the T cell areas, but usually not in the B cell aggregates, and many synovial cells also bore this marker. A few Leu-M1–staining dendritic macrophages were also scattered in T cell locations. In areas of marked lymphocytic infiltration, high endothelial venules were found, particularly around the aggregates. HLA–DR expression was intense on synovial lining cells and on many synovial and infiltrating cells in sublining areas. HLA–DQ receptors were also expressed on many of these cells, but the staining was not as intense (results not shown).

Comparison with tonsillar lymphoid tissue. The cellular constituents of tonsillar lymphoid tissue were often present in both Lyme and rheumatoid synovium, but their organization in synovium was distinct (Figure 2). In lymphoid tissue, the B cells aggregated in germinal centers around follicular dendritic cells; uncommitted B cells bearing IgD on their surface formed rings around the outer part of the follicles, and activated germinal center B cells were found in the center of the follicles. Although most Lyme and rheumatoid synovial tissues had B cell aggregates, they also contained many tightly intermixed T cells (Figures 1A and D). IgD-bearing B cells were often present, but they were scattered within the aggregate. Seven of 12 patients with Lyme disease and 7 of 10 with RA had few-to-moderate numbers of follicular dendritic cells within the aggregates, but only 2 of those with Lyme disease and 5 with RA had a few activated germinal center B cells. In both tonsillar and synovial tissue, T cells were located between aggregates, and plasma cells were clustered outside of the aggregates. How-

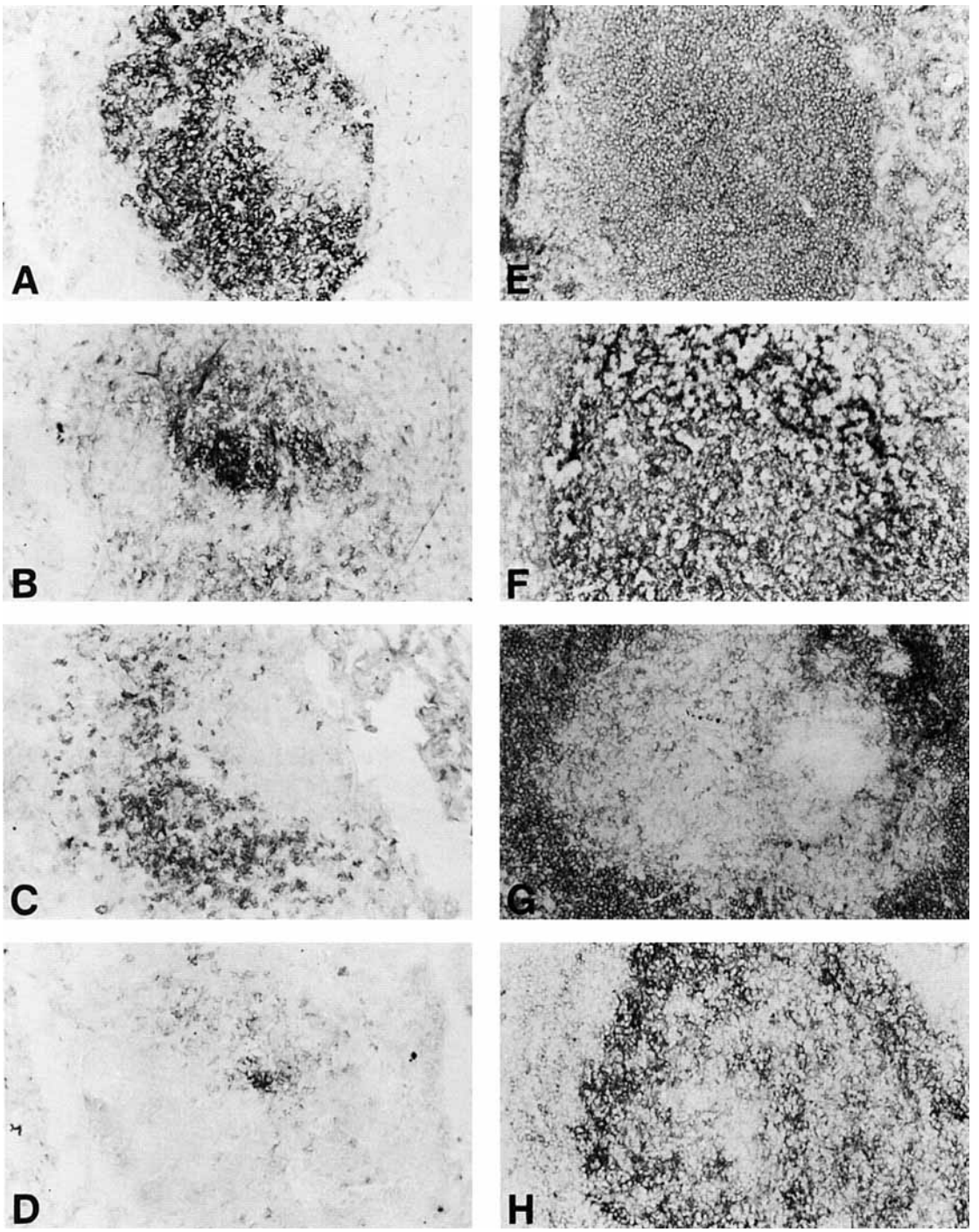
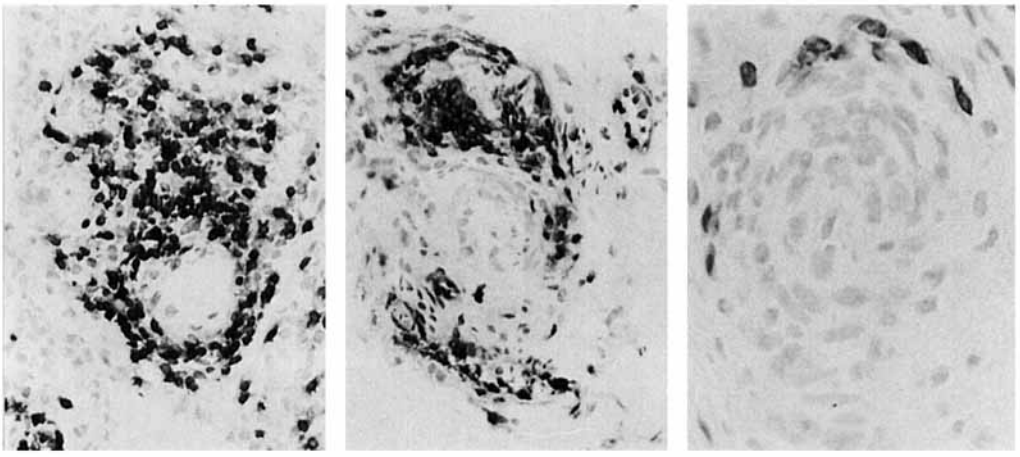


Figure 2. Comparison of a lymphoid aggregate in Lyme arthritis synovium (**A–D**) with a germinal center follicle in tonsillar lymphoid tissue (**E–H**). **A** and **E**, 6A4 B cells. **B** and **F**, T05 follicular dendritic cells. **C** and **G**, IgD-bearing B cells. **D** and **H**, B532 activated germinal center B cells. (Immunoperoxidase stained, magnification × 200.) See Figures 1A and D for the relationship between T cells and B cells in an aggregate.



A B C

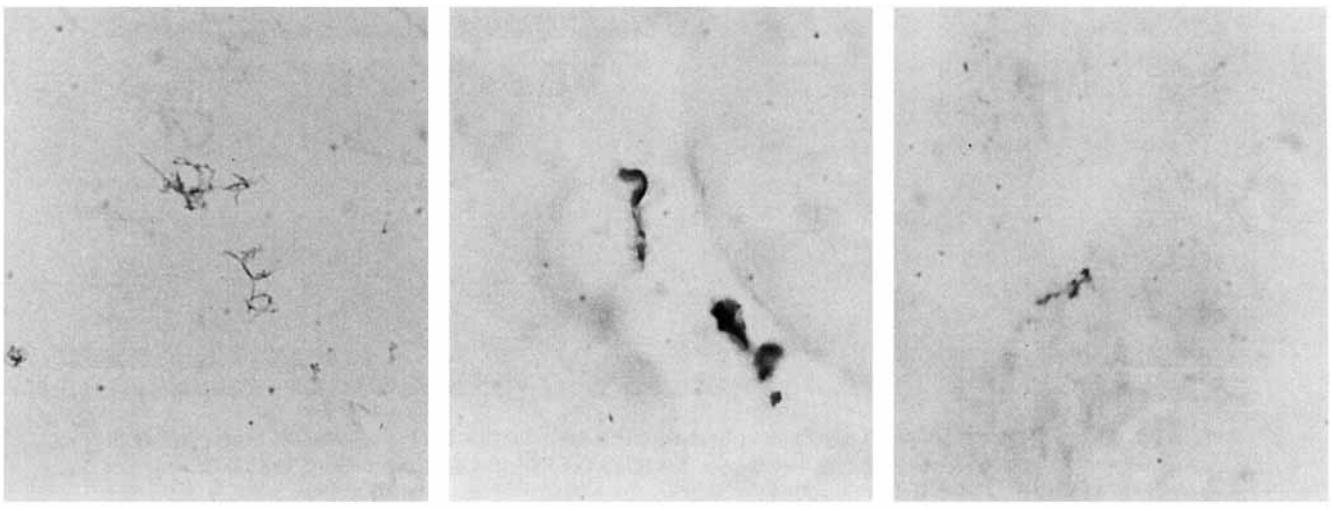
Figure 3. Obliterative microvascular lesions in Lyme arthritis synovium. **A,** Perivascular lymphoid aggregate. **B,** Lymphoid aggregate surrounding a vessel with mild proliferative changes. **C,** Obliterated vessel with only a few remaining lymphoid cells surrounding it. (Immunoperoxidase stained with anti–Leu-4, original magnification × 100.)

ever, the relative proportion of plasma cells was greater in synovium.

Distinctive features of Lyme synovium. Five of the 12 patients with Lyme arthritis had scattered perivascular lymphoid aggregates that partially surrounded or completely obliterated vessels (Figure 3). Using monoclonal antibodies to the 31- or 41-kd polypeptides of *B burgdorferi,* a few spirochetes and globular antigen deposits were seen in and around normal or injured blood vessels in areas of lymphocytic infiltration, in 6 of the 12 patients (Figure 4). No other evidence of spirochetal antigens was detected. Neither obliterative microvascular lesions nor evidence of spirochetal antigens were found in the 12 rheumatoid synovia.

DISCUSSION

We studied synovial tissue obtained from patients who had severe Lyme disease or rheumatoid arthritis. Those with Lyme disease had received anti-



A B C

Figure 4. *Borrelia burgdorferi* in the Lyme synovial villi shown in Figure 1. **A,** Control preparation of cultured *B burgdorferi* maintained in Barbour, Stoenner, Kelly medium. **B,** A spirochete and globular antigen deposit in a blood vessel in the synovium. **C,** Another spirochete in synovium from the same patient as in **B.** (Immunoperoxidase stained with monoclonal antibody 1D, directed against the 31-kd polypeptide of *B burgdorferi,* original magnification × 1,000.)

biotic therapy prior to synovectomy, and those with RA had received intensive antiinflammatory therapy, in many cases for years, prior to biopsy, synovectomy, or total joint replacement. Nevertheless, the synovial tissue findings in this study covered the range of synovial pathology seen in the chronic inflammatory arthritides. They included varying degrees of synovial cell villous hypertrophy, synovial lining cell hyperplasia, fibrin deposition, and diffuse, nodular, or perivascular mononuclear cell infiltration in the subsynovial lining area, sometimes with apparent germinal center formation.

Several previous studies have used monoclonal antibodies to lymphoid cell surface markers to assess synovial histology (13–17). Lindblad and coworkers emphasized that all forms of chronic inflammatory arthritis are characterized by thickened HLA–DR+, OKM1+ synovial lining cells of macrophage lineage, large numbers of HLA–DR+, OKM1− sublining synovial cells, and at least some infiltration of Leu-1+ T lymphocytes, predominantly of the Leu-3+ (helper/inducer) subset, which are in close contact with HLA–DR+ macrophage/dendritic cells (13).

Young and colleagues divided rheumatoid tissue specimens into 3 groups according to the following patterns of lymphoid cell infiltrates: diffuse infiltration of T cells that surround clusters of germinal center B cells, diffuse T cell infiltration lacking germinal centers, and proliferation of subsynovial fibroblasts with relatively few lymphoid cells (16). Malone et al divided RA patients into two groups: one had a higher intensity of T cell and plasma cell infiltration, a higher ratio of Leu-3a:Leu-2a T cells, and many HLA–DR–bearing cells; the other had cells primarily of macrophage lineage, fewer infiltrating cells, a thin lining layer, and extensive fibrin deposition (17). Marked lymphocytic infiltration in synovium was associated with anergy to soluble recall antigens.

Our findings in rheumatoid synovium are consistent with these earlier reports regarding the numbers and spatial arrangement of T lymphocytes, macrophages, and DR expression. In addition, the current studies give a more detailed picture of synovial histopathology in Lyme disease than was previously available. These findings further show that the synovial lesions of Lyme disease are similar to those of the other chronic inflammatory arthritides, including RA.

It has been noted in the past that inflamed synovial tissue may have features of organized lymphoid tissues, such as lymph nodes or tonsils. In recent years, monoclonal antibodies have been used to further define the cellular constituents, architecture, and function of lymphatic tissue (18). Peripheral lymph nodes serve as a microenvironment for the support of B cell differentiation and secretion of first IgM and then IgG. Peripheral nodes also contain many T cells, predominantly of the helper/inducer phenotype, which regulate this response. T cells are located in paracortical areas, where there is a unique stromal element called the interdigitating cell; macrophages are scattered throughout the T cell areas, and B cells form follicles in the subcapsular cortex.

Uncommitted B cells bearing IgD on their surface form a mantle around the outer part of the follicle, and activated B cells cluster around follicular dendritic cells, which form germinal centers in the middle of the follicle. Some of the activated B cells are thought to become memory B cells, some travel to the medullary areas of the node where they become mature plasma cells, and others leave the node to seed other tissues of the body. In active nodes, B cells, macrophages, some activated T cells, and some endothelial cells express Ia antigens on their surfaces.

In this study, both Lyme disease and rheumatoid synovial lesions often contained the cellular elements of tonsillar lymphoid tissue, but their organization in synovium was distinct. Germinal center follicles in lymphatic tissue consisted primarily of B cells, while nodular aggregates in synovium contained many T cells tightly intermixed with B cells. Although a few follicular dendritic cells and activated germinal center cells were sometimes present in the aggregates, their numbers were considerably less than in the germinal center of a lymph node. In addition, relatively few IgD-bearing B cells were scattered throughout the synovial aggregates, whereas in lymphatic tissue, these cells formed a ring around the outside of the follicle. As in tonsils, large numbers of plasma cells were located outside of the synovial aggregates, but their numbers were relatively greater in synovium.

Cells that appear morphologically similar to lymphoid high endothelial venules (HEV), to which lymphocytes bind to enter lymph nodes (19), have been seen in synovium (20,21). In recent functional studies of synovial HEV (22,23), we found that these cells supported the binding of normal peripheral blood lymphocytes in vitro, and the characteristics of this binding were similar to those of binding in lymph nodes. This included a requirement for calcium ions, a dependence on metabolic activity, and a preferential adherence of circulating lymphocytes as opposed to immature thymocytes. However, the binding of lym-

phocytes to synovial HEV was not inhibited by monoclonal antibodies that block lymphocyte binding to lymph node or mucosal HEV, and synovial HEV did not bind either lymph node HEV–specific or mucosal HEV–specific B lymphoblastoid cells. Thus, just as the architecture of the synovial lesion is reminiscent of, but distinct from, lymphatic tissue, the endothelial cell recognition system in synovium also seems to be distinct from that in lymph nodes.

In a previous study of Lyme synovia, 5 of 17 patients were noted to have a distinctive microvascular lesion in which scattered vessels were sometimes partially or completely obliterated due to arteriolar muscle cell proliferation and concentric adventitial fibroplasia (5). In addition, using the Dieterle silver stain, a few spirochetes were seen in and around blood vessels in the specimens from 2 patients (5). These findings implied that the Lyme spirochete may survive for years in affected synovium and may be directly responsible for the microvascular injury. In the present study, we confirmed these distinctive features of Lyme synovia. Obliterative microvascular lesions at various stages of development were seen in 5 of the 12 Lyme synovial specimens. In addition, using monoclonal antibodies against the 41-kd flagellar antigen of the spirochete (12) or the 31-kd outer membrane component (11), a few *B burgdorferi* were seen in and around normal or injured blood vessels in areas of heavy lymphocytic infiltration, in 6 of the 12 specimens.

Although background staining was minimal, it was still very difficult to find spirochetes or spirochetal antigen in the tissue. Several high power fields of many sections had to be examined to find these. It is likely that the numbers of organisms were reduced by previous antibiotic therapy, and only whole organisms located completely within the plane of the tissue section could be identified as spirochetes. Small globular antigen deposits were sometimes seen near whole spirochetes. Perhaps these were parts of organisms located in different planes or proteins from partially degraded organisms. It is still possible that monoclonal antibodies directed against other components of the spirochete may provide evidence of cross-reactive antigens that were not apparent with the antibodies available for use here.

The scarcity of organisms in the synovial lesions of Lyme arthritis is reminiscent of the findings in lesions of tertiary syphilis or tuberculoid leprosy. In these diseases, it is very difficult to detect organisms in the lesions, but the small number present are able to persist and trigger a florid, chronic lymphoplasmacytic immune response. Similarly, the antigenic stimulus in Lyme arthritis would appear to be a small number of live spirochetes, demonstrated here by monoclonal antibodies, which may persist in the synovial lesion for years.

ACKNOWLEDGMENTS

We thank Drs. David Sherman, Robert I. Fox, and John Aversa for providing tissue specimens; Drs. Ronald Levy and Dennis Frisman for providing monoclonal antibodies; Laurel Bolin, Chris Radzicka, Robert Bargatze, and Adrian Duijvestijn for technical assistance; Dr. Robert Rouse for review of slides; and Robert Specht for photographic assistance.

REFERENCES

1. Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, Schmid GP, Johnson E, Malawista SE: The spirochetal etiology of Lyme disease. N Engl J Med 308:733–740, 1983
2. Steere AC, Schoen RC, Taylor E: The clinical evolution of Lyme arthritis. Ann Intern Med 107:725–731, 1987
3. Steere AC, Gibofsky A, Patarroyo ME, Winchester RJ, Hardin JA, Malawista SE: Chronic Lyme arthritis: clinical and immunogenetic differentiation from rheumatoid arthritis. Ann Intern Med 90:286–291, 1979
4. Lawson JP, Steere AC: Lyme arthritis: radiologic findings. Radiology 154:37–43, 1985
5. Johnston YE, Duray PH, Steere AC, Kashgarian M, Buza J, Malawista SE, Askenase PW: Lyme arthritis: spirochetes found in synovial microangiopathic lesions. Am J Pathol 118:26–34, 1985
6. Poppema SA, Bahn K, Reinherz EL, McCluskey RT, Schlossman SF: Distribution of T cell subsets in human lymph nodes. J Exp Med 153:30–41, 1981
7. Kujala GA, Steere AC, David JS IV: IgM rheumatoid factor in Lyme disease: correlation with disease activity, total serum IgM, and IgM antibody to Borrelia burgdorferi. J Rheumatol 14:772–776, 1987
8. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA: 1958 revision of diagnostic criteria for rheumatoid arthritis. Bull Rheum Dis 9:175–176, 1958
9. Craft JE, Grodzicki RL, Steere AC: The antibody response in Lyme disease: evaluation of diagnostic tests. J Infect Dis 149:789–795, 1984
10. Craft JE, Fischer DK, Shimamoto GT, Steere AC: Antigens of Borrelia burgdorferi recognized during Lyme disease: appearance of a new IgM response and expansion of the IgG response late in the illness. J Clin Invest 78:934–939, 1986
11. Barbour AG, Tessier SL, Todd WJ: Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect Immun 41:795–804, 1983
12. Barbour AG, Hayes SF, Heiland RA, Schrumpf ME, Tessier SL: A Borrelia-specific monoclonal antibody binds to a flagellar epitope. Infect Immun 52:549–554, 1986
13. Lindblad S, Klareskog L, Hedfors E, Forsum U, Sund-

ström C: Phenotypic characterization of synovial tissue cells in situ in different types of synovitis. Arthritis Rheum 26:1321–1332, 1983
14. Førre O, Thoen J, Lea T, Dobloug JH, Mellbye OJ, Natvig JB, Pahle J, Solheim BG: In situ characterization of mononuclear cells in rheumatoid tissues using monoclonal antibodies: no reduction of T8-positive cells or augmentation in T4-positive cells. Scand J Immunol 16:315–319, 1982
15. Duke O, Panayi GS, Janossy G, Poulter LW: An immunohistologic analysis of lymphocyte subpopulations and their microenvironment in the synovial membrane of patients with rheumatoid arthritis using monoclonal antibodies. Clin Exp Immunol 49:22–30, 1982
16. Young CL, Adamson TC III, Vaughan JH, Fox RI: Immunohistologic characterization of synovial membrane lymphocytes in rheumatoid arthritis. Arthritis Rheum 27:32–39, 1984
17. Malone DG, Wahl SM, Tsokos M, Cattell H, Decker JL, Wilder RL: Immune function in severe active rheumatoid arthritis: a relationship between peripheral blood mononuclear cell proliferation to soluble antigens and synovial tissue immunohistologic characteristics. J Clin Invest 74:1173–1185, 1984
18. Butcher EC, Weissman IL: Lymphoid tissues and organs, Fundamental Immunology. Edited by WE Paul. New York, Raven Press, 1984, pp 109–127
19. Gowans JL, Knight EJ: The route of recirculation of lymphocytes in the rat. Proc R Soc Lond [Biol] 159:257–282, 1964
20. Freemont AJ, Jones CJP, Bromley M, Andrews P: Changes in vascular endothelium related to lymphocyte collections in diseased synovia. Arthritis Rheum 26:1427–1433, 1983
21. Iguchi T, Ziff M: Electron microscopic study of rheumatoid synovial vasculature: intimate relationship between tall endothelium and lymphoid aggregation. J Clin Invest 77:355–361, 1986
22. Jalkanen S, Steere AC, Fox RI, Butcher EC: A distinct endothelial cell recognition system that controls lymphocyte traffic into inflamed synovium. Science 233: 556–558, 1986
23. Jalkanen S, Bargatze RF, Toyos J, Butcher EC: Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85–95 kd glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. J Cell Biol 105: 983–990, 1987