Expression of Adhesion Molecules in Synovia of Patients with Treatment-Resistant Lyme Arthritis

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The expression of adhesion molecules in synovium in patients with Lyme arthritis is surely critical in the control of *Borrelia burgdorferi* infection but may also have pathologic consequences. For example, molecular mimicry between a dominant T-cell epitope of *B. burgdorferi* outer surface protein A and an adhesion molecule, human lymphocyte function-associated antigen 1 (LFA-1), has been implicated in the pathogenesis of treatment-resistant Lyme arthritis. Using immunohistochemical methods, we examined synovial samples for expression of adhesion molecules in 29 patients with treatment-resistant Lyme arthritis and in 15 patients with rheumatoid arthritis or chronic inflammatory monoarthritis. In Lyme arthritis synovia, endothelial cells showed intense expression of P-selectin and vascular adhesion protein-1 (VAP-1). Expression of LFA-1 was also intense on infiltrating cells, particularly in lymphoid aggregates, and intercellular adhesion molecule-1 (ICAM-1) was markedly expressed on synovial lining and endothelial and infiltrating cells. Moderate expression of vascular cell adhesion molecule-1 (VCAM-1) was seen on synovial lining and endothelial cells, and mild expression of its ligand, very late antigen-4, was apparent in perivascular lymphoid infiltrates. Except for lesser expression of VCAM-1 in Lyme synovia, the levels of expression of these adhesion molecules were similar in the three patient groups. We conclude that certain adhesion molecules, including ICAM-1 and LFA-1, are expressed intensely in the synovia of patients with Lyme arthritis. Upregulation of LFA-1 on lymphocytes in this lesion may be critical in the pathogenesis of treatment-resistant Lyme arthritis.

Lyme disease worldwide is caused by three genospecies of the tick-borne spirochete *Borrelia burgdorferi* sensu lato (11). In the United States, where the infection is caused by *B. burgdorferi* sensu stricto strains, intermittent or chronic oligoarticular arthritis primarily affecting large joints, especially the knees, is a prominent late manifestation of the illness (33–35). Although most patients with Lyme arthritis can be treated effectively with antibiotic therapy, about 10% of patients have persistent knee swelling for months to years after ≥ 2 months of oral antibiotics or ≥ 1 month of intravenous antibiotics. This condition has been termed antibiotic treatment-resistant Lyme arthritis.

Adhesion molecules in inflammatory foci have three important cellular functions: homing to lymphoid tissues, migration to inflammatory sites, and costimulation of cellular activation (23). There are four major structural classes of adhesion molecules (reviewed by Janeway et al. and McMurray [18, 21]). The selectins and vascular addressins mediate the initial phases of extravasation, which cause the tethering and rolling of leukocytes on endothelial surfaces (31). Leukocyte integrins, including lymphocyte function associated antigen-1 (LFA-1 [α L β 2]) and very late antigen-4 (VLA-4 [α 4 β 1]), bind to their ligands of the immunoglobulin superfamily, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), respectively (1, 18, 22). These interactions attach leukocytes firmly to endothelial surfaces. Binding of

these adhesion molecules also mediates intercellular communication in inflammatory foci. For example, the interaction of LFA-1 on T cells with its main ligand, ICAM-1, on macrophages anchors the cells together and provides a potent costimulatory signal for T-cell activation (18).

In addition to having standard functions, host adhesion molecules seem to have specific consequences in the pathogenesis of *B. burgdorferi* infection. The spirochete attaches to the platelet-specific integrin receptor (α _{IIb} β ₃), also known as the fibrinogen receptor, which is expressed only on activated platelets (9). This mechanism may aid the spirochete in homing to sites of endothelial cell injury. In addition, the spirochete binds the ubiquitous vitronectin (α v β 3) and fibronectin (α 5 β 1) receptors (10) and attaches to various proteoglycans, including decorin, which decorates the surface of collagen (15, 27). Attachment to these adhesion molecules may be critical in the spread and survival of *B. burgdorferi* in the joint. Furthermore, it has recently been proposed that autoimmunity develops within the proinflammatory milieu of the joints in genetically susceptible patients with Lyme arthritis because of molecular mimicry between a dominant T-cell epitope of outer-surface protein A (OspA) of *B. burgdorferi* and LFA-1 (14). Thus, the expression of adhesion molecules may have specific pathologic consequences in Lyme arthritis.

The histopathological appearance of the synovial lesion in Lyme arthritis, which includes synovial hyperplasia, vascular proliferation, and lymphoid infiltrates, is similar to that seen in other chronic inflammatory arthritides, including rheumatoid arthritis (32). In rheumatoid arthritis, adhesion molecules, including P-selectin, LFA-1, ICAM-1, VLA-4, and VCAM-1, are up-regulated within the intense proinflammatory milieu of the

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TABLE 1. Adhesion molecules and cell surface markers included in this study

Family and/or adhesion molecule	Expression in synovium ^a	Monoclonal antibody	Source
Selectin (P-selectin)	EC, PLT	CD62P	Pharmingen, Burlingame, Calif.
Vap-1	EC	Vap-1	Courtesy of Sirpa Jalkanen, Turku, Finland
Integrins α4β1 αLβ2	Lymphocytes, monocytes, fibroblasts Leukocytes	CD29d CD11a	Chemicon International Inc., Temecula, Calif. Pharmingen
Immunoglobulin superfamily ICAM-1 VCAM-1	Leukocytes, DC, EC, fibroblasts, synoviocytes Activated EC, monocytes, synoviocytes	CD54 CD106	Pharmingen Pharmingen
Cell surface markers CD3 CD14 CD20	T cells Macrophages, DC, synoviocytes B cells	Dako-CD3 CD14 Dako-CD20	Dako, Carpinteria, Calif. Pharmingen Dako

^a Abbreviations used: EC, endothelial cells; PLT, platelets; DC, dendritic cells.

synovial lesion (16, 20, 36). In addition, in the murine model of acute Lyme arthritis, P-selectin, ICAM-1, and VCAM-1 are upregulated in *B. burgdorferi*-infected synovia (4, 29). However, there is currently no animal model of treatment-resistant

Lyme arthritis. We describe here the expression of adhesion molecules in synovial tissues from patients with treatment-resistant Lyme arthritis compared with that in patients with rheumatoid arthritis or chronic inflammatory monoarthritis.

FIG. 1. The cellular architecture and infiltrating cells are shown in serial frozen sections of synovial tissue from the knee joint of a patient with treatment-resistant Lyme arthritis. In hematoxylin-eosin-stained sections (A and B), villous hypertrophy, synovial cell hyperplasia, vascular proliferation, and infiltrating cells are seen. In sections stained with monoclonal antibodies to cell surface markers (brown color), CD14⁺ synovial cells of macrophage lineage and infiltrating macrophages are seen throughout the synovial sublining areas (C), CD3⁺ T cells are seen diffusely in synovial sublining areas (D) and in clusters (E), and CD20⁺ B cells are found primarily in lymphoid aggregates (F). Panels D to F are centered on the same aggregate. The nonspecific background stain is hematoxylin (purple color). Magnifications, ×100 (A and D), ×200 (C), and ×400 (B, E, and F).

TABLE 2. Comparison of synovial histologies and adhesion molecule expression levels in patients with Lyme arthritis, rheumatoid arthritis, or idiopathic, chronic inflammatory monoarthritis^a

Histologic characteristic or adhesion molecule	Score			P value
	Synovium in Lyme arthritis (n = 29)	Rheumatoid arthritis (n = 7)	Inflammatory monoarthritis (n = 8)	
Histology				
Synovial cell hyperplasia	2.62 ± 0.68	2.14 ± 0.90	2.63 ± 0.52	0.26
Lining cell hyperplasia	2.31 ± 0.76	2.29 ± 0.95	2.38 ± 0.92	0.97
Vascular proliferation	2.62 ± 0.78	2.43 ± 0.79	2.75 ± 0.46	0.70
Lymphoid infiltrates	1.80 ± 0.68	2.00 ± 0.82	1.33 ± 0.58	0.45
Lymphoid aggregates	2.40 ± 0.74	3.00 ± 0	2.80 ± 0.45	0.25
Cellular markers				
CD14	2.34 ± 0.81	2.71 ± 0.49	1.88 ± 1.13	0.16
CD3	2.10 ± 0.77	2.43 ± 0.79	1.62 ± 1.06	0.17
CD20	1.21 ± 1.08	2.00 ± 1.00	1.50 ± 1.31	0.24
Vascular markers				
Vap-1	2.55 ± 0.78	2.14 ± 1.21	2.71 ± 0.49	0.40
P-selectin	2.07 ± 0.88	2.57 ± 0.79	2.38 ± 0.74	0.32
Intercellular adhesion markers				
ICAM-1	2.55 ± 0.57	2.86 ± 0.38	2.50 ± 0.54	0.37
LFA-1	2.34 ± 0.72	2.57 ± 0.54	2.13 ± 0.99	0.52
VCAM-1	1.83 ± 0.80	2.71 ± 0.49	2.50 ± 0.54	0.006
VLA-4	1.28 ± 1.10	2.00 ± 0	1.38 ± 0.52	0.19

^a Expression of each adhesion molecule or histologic characteristic was scored on a scale of 0 to 3 as absent (0), mild (1), moderate (2), or marked (3). Results are expressed as mean values ± one standard deviation. The P value in bold is statistically significant by analysis of variance.

MATERIALS AND METHODS

Patients. For this study, we tested archival synovial tissue specimens from 29 patients with treatment-resistant Lyme arthritis obtained during a 20-year period (1978 to 1998). The patients met the Centers for Disease Control and Prevention (CDC) criteria for the diagnosis of Lyme arthritis. They had swollen knees and positive antibody responses to *B. burgdorferi* by enzyme-linked immunosorbent assay and Western blotting, interpreted according to the Centers for Disease Control-Association of State and Territorial Public Health Laboratory Directors criteria (6, 7). Their ages ranged from 10 to 66 years (median, 36 years); 15 were female, and 14 were male. The median duration from the onset of arthritis to synovectomy was 12 months (range, 6 to 96 months). All 29 patients were treated for their arthritis with antibiotic therapy, usually oral doxycycline and intravenous ceftriaxone for 30 days each. The median elapsed time from antibiotic treatment to synovectomy was 5 months. At that time, *B. burgdorferi* DNA was not detectable in the synovial samples (5). The comparison groups included synovial samples from eight patients with idiopathic, chronic inflammatory monoarthritis (the inflammatory arthritis group), six patients with rheumatoid arthritis, and one patient with juvenile rheumatoid arthritis (the rheumatoid arthritis group).

Histopathology. All tissue specimens were frozen in optimal-cutting-temperature compound (Tissue-Tek; Miles, Inc., Diagnostic Division, Elkhart, Ind.) and stored in liquid nitrogen. Serial 6-μm-thick cryostat sections from each patient were mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, Pa.); they were air dried and fixed in cold acetone for 10 min. The slides were then stored at -70°C until use. The first slide in the series was stained with hematoxylin-eosin to determine the general cellular architecture.

Monoclonal antibodies. We selected monoclonal antibodies that identify cell surface markers, selectins, integrins, and adhesion molecules of the immunoglobulin superfamily, as described in Table 1. In preliminary experiments, tonsillar tissue was used to determine the optimal concentration of each antibody that achieved maximal staining sensitivity with minimal background.

Immunohistochemistry. Immunoperoxidase staining was done with a Vectastain ABC kit (Vector Laboratories, Burlingame, Calif.). The slides were brought to room temperature, briefly soaked in phosphate-buffered saline, and then blocked for 20 min with normal horse serum. Subsequent incubations were carried out with primary monoclonal antibodies, a biotinylated secondary antibody, Vectastain ABC Reagent, and the chromogen substrate 3 diaminobenzidine-tetra-HCl (DAB, Acros, N.J.). After final rinsing with tap water, the slides were counterstained with hematoxylin (Fisher Scientific, Fair Lawn, N.J.) and mounted with Cytoseal (Stephens Scientific, Riverdale, N.J.). A negative control

section, stained with isotype-specific irrelevant primary antibody, was included on each slide.

Grading system and statistical analysis. The series of slides from each patient were graded by two blinded observers. The expression of each adhesion molecule and cell marker was scored on a scale of 0 to 3 as absent (0), mild (1), moderate (2), or marked (3). When the two observers assigned different scores to a slide, it was reviewed by both observers together and a final score was agreed upon. The average scores for each adhesion molecule or cell marker tested were compared between the patients with Lyme arthritis and those with rheumatoid arthritis or chronic inflammatory monoarthritis by analysis of variance. Results are expressed as means ± one standard deviation.

RESULTS

Organization of the cellular components in synovium. In hematoxylin-eosin-stained sections, synovial samples from the 29 patients with treatment-resistant Lyme arthritis showed moderate-to-marked synovial lining cell hyperplasia, synovial cell hyperplasia, vascular proliferation, and infiltrates of mononuclear cells (Fig 1A and B). The intensity of mononuclear cell infiltration in the subsynovial lining areas varied greatly among individual patients. Fifteen of the 29 patients had lymphoid aggregates, whereas 10 patients had diffuse or scattered infiltrates without aggregates and 4 had hypertrophied synovium with few lymphocytes. Similarly, synovial samples from the seven patients with rheumatoid arthritis and the eight patients with chronic inflammatory monoarthritis showed moderate-to-marked synovial cell hyperplasia, vascular proliferation, and a range of lymphoid infiltration. The overall grades for each of these features were similar in the three patient groups (Table 2).

In immunoperoxidase-stained sections, the organizations of the cellular infiltrate and the numbers of each cell type, determined with monoclonal antibodies to cell surface markers, were also similar in synovia from patients with Lyme arthritis, rheumatoid arthritis, or chronic inflammatory monoarthritis

FIG. 2. Vascular markers (brown color) are shown in frozen tissue sections of synovial tissue from the knee joints of patients with treatment-resistant Lyme arthritis. The nonspecific background stain is hematoxylin (purple color). VAP-1 is seen on the cuboidal endothelium and deeper layers of high endothelial venules (A and B). P-selectin is seen exclusively on the endothelial cells in the capillaries and several larger vessels (C and D). Magnifications, $\times 100$ (A and C) and $\times 400$ (B and D).

(Table 2). CD14⁺ macrophages were scattered throughout the synovial sublining areas, and synovial cells of macrophage lineage also bore this marker (Fig. 1C). CD3⁺ T cells were found in a patchy or diffuse distribution in synovial sublining areas (Fig. 1D). Among the patients with lymphoid aggregates, tightly mixed clusters of CD20⁺ B cells and CD3⁺ T cells were seen (Fig. 1E and F). A few scattered B cells were sometimes seen in other locations.

Vascular adhesion molecules. The Lyme arthritis synovia showed vascular proliferation with moderate-to-marked expression of P-selectin and vascular adhesion protein-1 (VAP-1) (Fig. 2). P-selectin was expressed exclusively on platelets and endothelial cells in the microvasculature. Antibody to VAP-1 stained the smooth muscle fibers in the larger venules as well as the cuboidal endothelium and connective tissue in high endothelial venules, which resulted in a thicker appearance of the vessels. There were no significant differences among the three patient groups in the levels of expression of these vascular adhesion molecules (Table 2).

Intercellular adhesion markers. The Lyme arthritis synovia showed moderate-to-marked expression of LFA-1 on infiltrating cells, especially on lymphocytes in clusters or perivascular locations. In addition, widespread expression of its ligand, ICAM-1, was seen in endothelium, synoviocytes, and lympho-

cytes (Fig. 3). Levels of expression of these two adhesion molecules were similar among the three patient groups (Table 2). Most of the Lyme arthritis patients also had mild-to-moderate expression of VLA-4, particularly in perivascular lymphoid infiltrates and less often in the synovial lining (Fig. 3E and F). However, 7 of the 29 synovial samples (24%) from patients with Lyme arthritis had no discernible expression of VLA-4 whereas all 15 patients in the comparison groups showed mild-to-marked staining of this adhesion molecule. Therefore, the mean score for VLA-4 expression among Lyme arthritis patients tended to be less than those for the other two groups ($P = 0.19$). VCAM-1, the ligand of VLA-4, was most prominently expressed in synovial lining cells (Fig. 3G and H). However, in some patients, the staining was more diffuse and included endothelial cells and inflammatory cells. The mean expression of VCAM-1 was significantly less in Lyme arthritis patients than in the comparison groups by analysis of variance ($P = 0.006$) (Table 2).

DISCUSSION

In this study, we describe the inflammatory infiltrate, cellular markers, and adhesion molecules in the synovia of patients with treatment-resistant Lyme arthritis or other chronic in-

FIG. 3. Similar levels of expression of intercellular adhesion molecules (brown color) in frozen tissue sections of synovial tissue from patients with treatment-resistant Lyme arthritis (left panels) and rheumatoid arthritis (right panels). The nonspecific background stain is hematoxylin (purple color). LFA-1 expression is marked on infiltrating cells, especially in lymphocyte clusters in the synovial sublining and perivascular areas (A and B); ICAM-1 expression is widespread in synoviocytes, lymphocytes, and vessel endothelium (C and D); VLA-4 is expressed most in

flammatory arthritides. In an initial histopathologic study (19), we described the synovial lesions in patients with Lyme arthritis compared with those in patients with many other types of arthritis. A marked similarity was noted in the synovial architecture and cellular infiltrates in the chronic inflammatory arthritides, including Lyme arthritis and rheumatoid arthritis. In addition, Lyme synovia showed striking deposition of fibrin and a form of endarteritis obliterans, which was not seen in other synovial diseases. Subsequently, we identified lymphoid cell surface markers in Lyme synovia compared with those in rheumatoid synovia and tonsillar lymphoid tissue (32). Again, the synovial lesions in both Lyme and rheumatoid synovia were similar and often consisted of the elements found in normal organized lymphoid tissue. In both diseases, T cells, predominantly of the CD4⁺ helper cell subset, were distributed diffusely in the subsynovial lining areas, often with nodular aggregates of tightly intermixed T and B cells. HLA-DR and DQ expression was intense throughout the lesions. Later, using in situ hybridization with cytokine-specific riboprobes, we examined the expression of cytokine mRNA in synovia from patients with chronic Lyme arthritis, rheumatoid arthritis, or juvenile rheumatoid arthritis. Patients in each group had prominent expression of proinflammatory cytokines, with less intense expression of anti-inflammatory cytokines (17). This proinflammatory response may be critical in the development of autoimmunity within the joint.

In this study of patients with treatment-resistant Lyme arthritis, rheumatoid arthritis, or chronic inflammatory monoarthritis, P-selectin was highly expressed on capillary vessels in synovia in each of the three patient groups. This adhesion molecule, which binds Sialyl-Lewis^x protein and P-selectin glycoprotein ligand-1 on leukocyte cell membranes, is critical in the homing of monocytes to synovial tissue (28, 31). Another vascular marker, VAP-1, which is a unique sialoglycoprotein on vascular endothelium and smooth muscle cells, was expressed intensely in high endothelial venules, which are critical in leukocyte extravasation. VAP-1 has been implicated in the homing of small lymphocytes to synovia in patients with reactive arthritis or inflammatory bowel disease-associated arthritis (2, 28). We do not yet know from functional studies whether these adhesion molecules play the same role in lymphocyte and monocyte homing in Lyme arthritis as in reactive arthritis, but both molecules are highly expressed in Lyme synovia.

Both LFA-1-ICAM-1 and VLA-4-VCAM-1 interactions are important in the firm attachment of leukocytes to endothelial surfaces and in cell signaling in inflamed synovium. Moreover, the binding of LFA-1-ICAM-1 and VLA-4-VCAM-1 help to regulate each other (8, 26), an "integrin cross talk" which is necessary for the multistep process of leukocyte migration into inflamed tissues (31). LFA-1 is constitutively expressed on all circulating leukocytes, whereas ICAM-1 is found on synovial cells of macrophage lineage and on vascular endothelium cells and leukocytes (23). Antibodies against these molecules strongly inhibit leukocyte migration into synovial

tissue (13, 36). Both LFA-1 and ICAM-1 were intensely expressed in synovia from patients with the three diseases studied here.

Although VLA-4 and its ligand VCAM-1 were also strongly expressed in rheumatoid and chronic monoarthritis synovia, VCAM-1 expression was less in Lyme synovia than in the other two chronic inflammatory arthritides. VLA-4 is constitutively expressed on lymphocytes, monocytes, and eosinophils, whereas VCAM-1 is expressed on endothelial cells and fibroblast-like synovial lining cells. In addition to interacting with VCAM-1, VLA-4 binds to fibronectin, a matrix protein (1, 12, 24). Perhaps VLA-4-fibronectin interactions, rather than VLA-1-VCAM-1 interactions, account for the fact that VLA-4 expression seemed greater than VCAM-1 expression in Lyme synovia. Alternatively, this finding may reflect certain differences in cytokine profiles in these diseases. Tumor necrosis factor alpha up-regulates the expression of ICAM-1 and VCAM-1, whereas gamma interferon up-regulates only the expression of ICAM-1 (3, 25, 30). Using in situ hybridization, mRNA for gamma interferon was prominent in Lyme synovia, which may be less the case in rheumatoid synovia (17). Finally, this difference may be a reflection of the duration of synovitis, which was about 1 year in the Lyme arthritis group. Although the duration of arthritis was probably longer among patients in the comparison groups, clinical information was not available for these patients.

We were particularly interested in the expression of LFA-1 in synovium because of the recent postulate that molecular mimicry between a dominant T-cell epitope of OspA₁₆₅₋₁₇₃ of *B. burgdorferi* and LFA-1 α ₃₃₂₋₃₄₀ may induce autoimmunity in genetically susceptible patients with Lyme arthritis (14). An important component of the postulate is that LFA-1 on T cells is up-regulated within the inflamed joint, so that phagocytosis of apoptotic T cells by macrophages may lead to presentation of the LFA-1 α ₃₃₂₋₃₄₀ peptide. Some of the OspA₁₆₅₋₁₇₃-reactive T cells may then be stimulated not only by the OspA peptide, but also by the cross-reactive LFA-1 epitope. After the elimination of *B. burgdorferi* from the joints with antibiotics, OspA-primed T cells may remain activated for some time by the presentation of the cross-reactive LFA-1 peptide. The present study demonstrates the intense expression of LFA-1 on T cells among patients with antibiotic treatment-resistant Lyme arthritis. However, it is not yet possible to demonstrate whether major histocompatibility complex class II molecules in the lesion actually present the LFA-1 α ₃₃₂₋₃₄₀ peptide.

In summary, certain adhesion molecules, including LFA-1 and its main ligand, ICAM-1, were intensely expressed in synovial samples from patients with antibiotic treatment-resistant Lyme arthritis. Except for the lesser expression of VCAM-1 in Lyme synovia, the levels of expression of adhesion molecules were similar in the three patient groups. Up-regulation of LFA-1 on lymphocytes in Lyme synovia may be critical in the pathogenesis of treatment-resistant Lyme arthritis.

perivascular lymphoid infiltrates and some synoviocytes (E and F); and VCAM-1 expression on fibroblast-like synoviocytes is moderate in patients with treatment-resistant Lyme arthritis (G) and marked in patients with rheumatoid arthritis (H). Note that the extent of VCAM-1 expression is limited to synoviocytes in panel G but includes the endothelium of the vessel in the upper right-hand corner of panel H. Magnifications, $\times 200$ (A to D and G to H) and $\times 400$ (E and F).

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