Prominent Expression of mRNA for Proinflammatory Cytokines in Synovium in Patients with Juvenile Rheumatoid Arthritis or Chronic Lyme Arthritis

Ref. 41

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ABSTRACT. ***Objective.*** To examine the cytokine profiles in synovium of patients with juvenile rheumatoid arthritis (JRA) or Lyme arthritis, 2 chronic inflammatory arthritides that affect children.

Methods. We used *in situ* hybridization with specific riboprobes to determine the expression of mRNA for proinflammatory or antiinflammatory cytokines in synovial samples from 5 patients with early, untreated JRA, 15 patients with late, treated JRA, and 9 patients with chronic Lyme arthritis. For comparison, synovia were examined from 6 patients with rheumatoid or psoriatic arthritis, and from 9 patients with various orthopedic conditions.

Results. Among the children with early, untreated JRA, a median of 3 to 8% of inflammatory cells in synovial samples expressed mRNA for the proinflammatory cytokines interleukin 1ß (IL-1ß), tumor necrosis factor-α (TNF-α), or interferon-γ (IFN-γ). Although a median of 3.9% of the cells expressed mRNA for the antiinflammatory cytokine IL-10, none had IL-4 mRNA. Most of the patients with late, treated JRA, chronic Lyme arthritis, rheumatoid, or psoriatic arthritis had mRNA for each of these proinflammatory cytokines in about 1% of the cells, whereas mRNA for the antiinflammatory cytokines was less frequent. The inflammatory cell density was much less in the synovium of patients with various orthopedic conditions, but about 1% of the infiltrating cells expressed mRNA for at least one of the proinflammatory cytokines.

Conclusion. Patients with early or late JRA or chronic Lyme arthritis have expression of mRNA in synovial tissue primarily for proinflammatory cytokines, with less expression of antiinflammatory cytokines. (J Rheumatol 2000;27:497–503)

Key Indexing Terms:
JUVENILE RHEUMATOID ARTHRITIS — LYME ARTHRITIS
BORRELIA BURGDORFERI — CYTOKINES — *IN SITU* HYBRIDIZATION

Juvenile rheumatoid arthritis (JRA) encompasses a heterogeneous collection of chronic inflammatory arthritides in children, which may be divided into pauciarticular, polyarticular, and systemic onset forms. Lyme arthritis was recognized as a separate entity in 1976 because of geographic clustering of children in Lyme, Connecticut, who were thought to have pauciarticular JRA[1]. Lyme arthritis, which is now known to be caused by the tick-borne spirochete *Borrelia burgdorferi*[2], is characterized by intermittent attacks of arthritis in a few large joints, especially the knees, in both children and adults[3]. However, in about 10% of the patients, chronic arthritis develops[3], which may have an autoimmune component[4]. The synovial lesion in these diseases, as in other forms of chronic inflammatory arthritis, shows synovial cell hypertrophy, vascular proliferation, and a lymphoplasmacellular infiltrate[5,6].

Cytokines are important mediators of inflammation and regulators of the immune response[7,8]. Proinflammatory cytokines, which constitute a Type 1 response, include interleukin 1ß (IL-1ß), tumor necrosis factor-α (TNF-α), produced primarily by macrophages, and interferon-γ (IFN-γ), produced by T cells[9,10]. Antiinflammatory cytokines, which constitute a Type 2 response, include IL-10 (produced both by macrophages and T cells) and IL-4 (produced by T cells). IL-6, which is secreted primarily by macrophages, stimulates the production of acute phase proteins and is a B cell differentiation factor. IL-2 is a T cell growth factor produced by T cells.

In children with JRA, initial studies reported high levels of IL-6 in joint fluid[11–14], intermediate levels of TNF-α[12,14], and undetectable levels of IL-2 and IFN-γ, as determined by ELISA assays[12]. More recently, using molecular techniques, mRNA for pro inflammatory cytokines, including TNF-α and IFN-γ, was often found among children with polyarticular or

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systemic onset JRA, whereas the antiinflammatory cytokine IL-4 was detected more frequently among those with pauciarticular onset disease[15,16]. In initial studies of patients with Lyme arthritis, peripheral blood T cells in culture produced primarily IFN-γ, as measured by ELISA[17,18]. Using molecular techniques, mRNA for IFN-γ was detected in synovial samples from all 5 patients with Lyme arthritis, whereas mRNA for IL-10 or IL-4 was found in 2 or 3 cases, respectively[19]. In an analysis of joint fluid samples from 11 patients with Lyme arthritis, the ratio of Th1 to Th2 cells correlated with the severity of joint inflammation, such that the higher the ratio, the larger the effusion[20]. Thus, proinflammatory cytokines usually predominate in synovial tissue or fluid in these diseases, but antiinflammatory cytokines may be found as well, which may have a disease restricting role.

Studies of *B. burgdorferi* infection in inbred strains of mice support this concept[21-23]. C3H/HeJ mice, which carry *H-2*^{k} alleles, develop severe arthritis when infected with the spirochete, and their T cells produce primarily IFN-γ. In contrast, *B. burgdorferi* infected BALB/c mice, which carry *H-2*^{d} alleles, develop only mild arthritis. Although these mice initially secrete IFN-γ, they produce more IL-4, which accelerates the resolution of arthritis.

Our goal was to examine the cytokine profiles in synovium of patients with JRA or chronic Lyme arthritis using *in situ* hybridization with specific riboprobes, a molecular technique that has not been employed in these diseases. The major advantage of this method is that large collections of archival tissue may be utilized. We report here that synovia in both diseases had prominent mRNA expression for both macrophage and T cell derived, proinflammatory cytokines, with less antiinflammatory cytokine expression.

MATERIALS AND METHODS

Patients. For this study, archival synovial tissue specimens were utilized from 5 children with early untreated JRA, 15 children with long standing, treated JRA, and 4 children and 5 adults with chronic Lyme arthritis. The children with JRA met the American College of Rheumatology classification criteria for the diagnosis of JRA[24], and those with Lyme arthritis met the US Centers for Disease Control criteria for the diagnosis of Lyme disease[25]. For comparison, samples were obtained from 6 patients with rheumatoid or psoriatic arthritis, and 9 patients with orthopedic conditions — 3 had plica, 2 had congenital dislocation or subluxation of the hips, and 4 had recurrent patellar subluxation. Most of the specimens were paraffin embedded, formalin fixed samples that were obtained from the archival collection in the Pathology Department at New England Medical Center. However, 2 specimens from patients with JRA seen at the University of Cincinnati (a kind gift from Dr. Kevin Murray) were fresh frozen samples embedded in optimal temperature cutting compound and stored in liquid nitrogen. Of the 9 samples from patients with Lyme arthritis, which were obtained from the collection of one of us (ACS), 8 were fresh frozen samples and one was formalin fixed tissue.

Histopathology. Tissue sections (8–10 mm) were cut and mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA, USA). The slides with fresh frozen tissue were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) (pH 7.2) and washed with PBS; paraffin embedded tissue slides were deparaffinized in xylene and rehydrated in ethanol. All reagents were prepared with diethyl-pyrocarbonate treated water. For histopathologic determinations, sections were stained with hematoxylin and eosin (H&E).

Riboprobes. To make riboprobes detect cytokine mRNA, plasmid vectors were obtained that contained DNA inserts encoding the various cytokines (Table 1). If the insert was not already in the pBluescript SK+ vector (Stratagene, La Jolla, CA, USA), a fragment was excised from the original vector with 2 distinct restriction endonucleases and purified by agarose gel electrophoresis. The fragment was then ligated to pBluescript SK+ DNA, which had been digested with the appropriate enzymes using a 3:1 molar ratio of insert to vector[26,27]. Ligated DNA was transformed into competent *Escherichia coli* DH 10B cells (Gibco-BRL, Gaithersburg, MD, USA). Plasmid DNA was purified from transformants, digested with restriction endonucleases, and fractionated by agarose gel electrophoresis to screen for the presence of the appropriate insert.

Both anti-sense (complementary) and sense (non-complementary) RNA probes were synthesized by *in vitro* transcription of DNA in the presence of digoxigenin labeled uridine-triphosphate (digoxigenin-UTP), using the T7 and T3 promoters of pBluescript SK+ and the appropriate RNA polymerases. The incorporation of label was assessed by dot blot using a sheep anti-digoxigenin antibody conjugated to alkaline phosphatase (1:1500), according to the manufacturer's protocol (Genius IV kit, Boehringer Mannheim, Indianapolis, IN, USA). The size of the transcripts was determined by RNA agarose gel electrophoresis.

In situ hybridization protocol. For *in situ* hybridization, a modification of the method described by Lan, *et al*[28] was used. Briefly, slides were washed once in PBS containing 0.1 M glycine, once in PBS with 0.3% Triton X 100, and once again in PBS alone. The slides were placed in 0.01 M sodium citrate (pH 6.0) and heated in a microwave oven for 5 min. They were then incubated in 0.1 M Tris buffer (pH 8.0/0.05 M EDTA) with 5 mg/ml proteinase K at 37°C for 20 min and fixed again in 4% paraformaldehyde in PBS. After washing twice in 2 × SSC (1 × SSC = 0.15 M NaCl, 0.0015 M sodium citrate, pH 7.2)[26] for 5 min at room temperature, the sections were overlayed with 30 µl of a hybridization buffer [40% formamide, 10% dextran sulfate, 1 × Denhardt's

Table 1. The plasmid vectors, cytokine DNA insert length, and source of construct.

Cytokine	Plasmid Vector	Insert Length, bp	Source
IL-1ß	Yepsec 1	~600	ATCC 67024*
TNF-α	pFC54.t	~600	ATCC 53163
IL-6	Bluescript SK+	494	Dr. W. Giranek†
IL-2	pBR322	~940	ATCC 61391
IFN-γ	pBR322	~1100	ATCC 65949
IL-4	Bluescript SK+	455	Dr. B. Brody‡
IL-10	Bluescript SK+	996	Dr. B. Brody

bp: base pair. *American Type Culture Collection, Rockville, MD, USA. †Department of Orthopedics, Medical College of Virginia, Richmond, Virginia, USA. ‡Department of Pathology, University of Alabama Medical Center, Birmingham, Alabama, USA.

solution (Sigma, St. Louis, MO, USA), 4 × SSC, 0.01 M DTT, 1 mg/ml yeast t-RNA, and 1 mg/ml sheared salmon sperm DNA] and 5 ng of denatured digoxigenin labeled riboprobe[29]. The slides were incubated in a moist chamber overnight at 42°C. After washing twice with 2 × SSC at room temperature, and then with 0.1 × SSC at 42°C, single-stranded RNA was digested 5 min (for fresh-frozen sections) or 15 min (for paraffin embedded sections) at 37°C with 100 µg/ml RNase A in 2 × SSC. For detection of digoxigenin labeled hybridized probes, sections were blocked with 2% normal sheep serum for 20 min followed by incubation with alkaline phosphatase conjugated sheep anti-digoxigenin (1:1500) for 1 h at room temperature. The color was developed overnight in the dark at room temperature with the chromogenic agents nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate and 2 mM levamisole. The sections were then washed, counterstained with 70% hematoxylin, and mounted in an aqueous medium. For each tissue sample, hybridization was done with an anti-sense probe (to detect specific staining), a sense probe (to evaluate background staining), and with no probe (negative control).

Quantification of the cytokine producing cells. Quantification of cytokine mRNA expressing cells was performed according to methods developed by Simpson, *et al*[30] and Diaz-Cano, *et al*[31]. Briefly, 50 visual fields at × 400 (or all available fields if there were fewer than 50 fields) were assessed by a single pathologist (SD-C). Based on morphologic appearance, the number of infiltrating inflammatory cells in a given high power field (hpf) was calculated according to a method previously shown to be an accurate estimate of the actual number of cells per hpf[31]. First, the number of mononuclear inflammatory cells was counted that touched a line across the microscopic field diameter (n), and the total number of cells in that field (N) was calculated according to the formula, $N = (n \times \pi/4)^2$. Since the number of positive cells was considerably fewer than the total number of infiltrating cells, these cells were counted directly in each hpf. A cell was considered positive for a given cytokine mRNA if diffuse or perinuclear cytoplasmic staining was present. Finally, the ratio of positive cells to total inflammatory cells was determined for each hpf.

Statistical analysis. The proportion of cases that had at least one positive cell in any field for a given cytokine was compared among the various groups using chi-squared tests. The total number of inflammatory cells and the ratio of positive to total inflammatory cells were averaged across fields for each person who had positive cells. These measurements were then compared among the various groups using Kruskal-Wallis test. All p values are 2-tailed.

RESULTS

Patients with JRA. Of the 20 patients with JRA, 5 had pretreatment synovial biopsies that were obtained for diagnosis a median of 0.5 years after disease onset. These 5 patients were called the early, untreated JRA group. Of the 5 patients, 4 had pauciarticular onset disease, 3 had resolution of arthritis months to several years after the biopsy, and one had chronic erosive disease. The remaining patient in the early, untreated JRA group had polyarticular onset disease with a persistent course and destructive changes in joints. The other 15 patients had severe, persistent disease, which had been present for a median of 7.3 years at the time of joint surgery. In addition to nonsteroidal antiinflammatory agents, they were being treated with prednisone, gold, or methotrexate. These patients made up the late, treated JRA group. Of these 15 patients, 7 had polyarticular onset disease, 5 had systemic onset, and 3 had pauciarticular onset, which in 2 cases became polyarticular. In 12 of the 15 patients, synovial tissue was obtained during arthroplastic surgery of the hips or knees, and in 3 it was obtained during synovectomies of a knee or elbow.

Synovial samples in the 5 patients with early, untreated JRA showed marked synovial cell hypertrophy, vascular proliferation, and a median inflammatory cell density of 32 cells per hpf, consisting primarily of lymphocytes and macrophages. All 5 patients had mRNA expression for the proinflammatory cytokine IL-1ß (Table 2), and a median of 8% of their inflammatory cells expressed it (Table 3). In 3 patients, about 3% of the cells had mRNA for other proinflammatory cytokines, including TNF-α or IFN-γ. IL-6 mRNA, a cytokine that stimulates the production of acute phase proteins, was also found in about 3% of the cells. Only 2 patients had a few positive cells expressing mRNA for IL-2, a T cell growth factor. Although a median of 3.9% of the cells in the 5 patients

Table 2. Number of patients with inflammatory cells in synovium expressing mRNA for various cytokines.

	No. of Patients (%) with Positive Cells				
	Juvenile Arthritis		Chronic Lyme Arthritis,	RA	
Cytokine	Early Untreated, n = 5 (%)	Late Treated, n = 15 (%)	n = 9 (%)	or PsA, n = 6 (%)	Orthopedic Conditions, n = 9 (%)
Proinflammatory					
IL-1ß	5 (100)	12 (80)	8 (89)	6 (100)	6 (67)
TNF-α	3 (60)	13 (87)	8 (89)	4 (67)	5 (56)
IFN-γ	3 (60)	10 (67)	6 (67)	5 (83)	6 (67)
Acute phase protein stimulant and B cell differentiation factor					
IL-6	3 (60)	8 (53)	7 (78)	5 (83)	8 (89)
T cell growth factor					
IL-2	2 (40)	9 (60)	3 (33)	2 (33)	6 (67)
Antiinflammatory					
IL-10	5 (100)*	7 (47)	7 (78)	4 (67)	2 (22)
IL-4	0 (0)	8 (53)	2 (22)	4 (67)	1 (11)

*Early juvenile arthritis group vs the orthopedic group, $p \leq 0.005$.

Table 3. Percentage of inflammatory cells positive for mRNA for various cytokines.

Cytokine	Juvenile Arthritis: Early Untreated, n = 5	Juvenile Arthritis: Late Treated, n = 15	Chronic Lyme Arthritis, n = 9	RA or PsA, n = 6	Orthopedic Conditions, n = 9
Inflammatory cells[§]	32 (31–35)	48 (36–55)	62 (45–119)	61 (55–68)	8 (8–12)*
Proinflammatory**					
IL-1ß	8.0 (4.0–9.1)[†]	0.8 (0.6–2.1)	1.6 (1.2–3.0)	1.9 (0.9–2.3)	1.4 (0–1.7)
TNF-α	3.1 (0–3.4)	1.3 (0.9–1.9)	1.4 (1.2–2.4)	0.9 (0–2.7)	0.4 (0–1.0)
IFN-γ	2.5 (0–6.1)	0.6 (0–1.7)	1.1 (0–2.2)	1.6 (1.0–2.2)	1.6 (0–1.3)
Acute phase protein stimulant and B cell differentiation factor					
IL-6	3.4 (0–6.1)	0.5 (0–1.3)	1.3 (0.4–1.6)	1.5 (0.5–2.9)	0.9 (0.5–1.4)
T cell growth factor					
IL-2	0 (0–5.0)	0.6 (0–2.0)	0 (0–0.8)	0 (0–0.5)	0.8 (0–1.0)
Antiinflammatory					
IL-10	3.9 (1.6–4.1)[‡]	0 (0–1.2)	0.6 (0.1–0.8)	0.2 (0–0.05)	0 (0–0)
IL-4	0 (0–0)	0.1 (0–0.4)	0 (0–0)	0.4 (0–1.9)	0 (0–0)

[§]Number of cells per high power field, median (quartile 1–quartile 3). **Percentage of inflammatory cells positive per high power field, median (q1–q3). *Orthopedic group vs each of the other 4 groups, $p \leq 0.005$. [†]Early juvenile arthritis group vs each of the other 4 groups, $p \leq 0.005$. [‡]Early juvenile group vs the orthopedic group, $p \leq 0.005$.

expressed mRNA for the antiinflammatory cytokine IL-10, none had IL-4 mRNA.

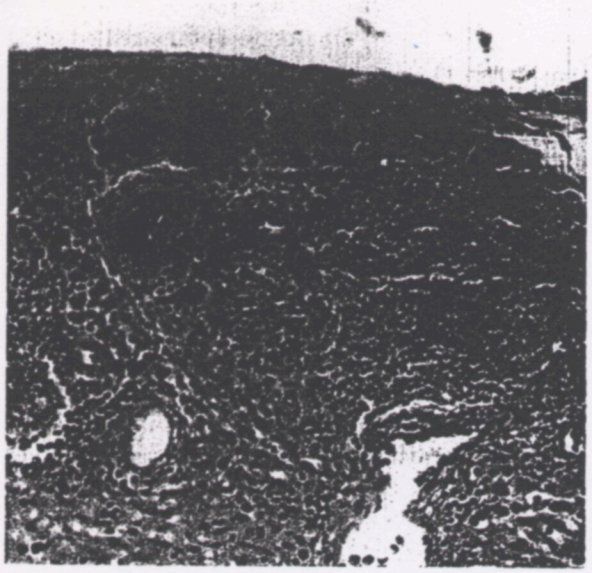
Compared with the patients with early, untreated JRA, the inflammatory infiltrates in the 15 patients with late, treated JRA had a median of 48 inflammatory cells per hpf consisting of a greater proportion of macrophages, and these cells were often found in deeper areas of the synovial tissue. Most of these 15 children had cells that expressed mRNA for each of the proinflammatory cytokines (Table 2). However, only 0.5% to 1.3% of the cells were positive for each cytokine, and only half of the patients had a few cells positive for IL-10 or IL-4 (Table 3). Although the numbers of patients with pauciarticular, polyarticular, or systemic onset was small, the cytokine profiles were not significantly different according to the pattern of JRA. The difference between the early and late JRA groups in the percentage of inflammatory cells positive for IL-1ß (8% vs 0.8%) was statistically significant ($p < 0.005$). In addition, early patients tended to have a greater percentage of cells with IL-10 mRNA expression, but these differences were not statistically significant. No positive cells were found in control sections that were stained with sense (noncomplementary) RNA probes.

Patients with chronic Lyme arthritis. In the 4 children and 5 adults with chronic Lyme arthritis, the synovial tissue was obtained after a mean duration of arthritis of 1.6 years. Except for one patient, they had previously received multiple courses of antibiotic therapy. Despite this treatment, they had persistent synovitis, and arthroscopic synovectomy was done for therapeutic reasons[32]. The synovial specimens in these patients showed synovial cell hypertrophy, vascular proliferation, and a lymphoplasmacellular infiltrate, in 3 instances with pseudolymphoid follicles (Figure 1A). The inflammatory cell density in these patients was as high as 119 cells per hpf (Table 3). In 8 of the 9 patients, mRNA was found for IL-1ß (Figure 1B), IL-6 (Figure 1C), TNF-α, or IFN-γ (Table 2), and 1.1% to 1.6% of the cells expressed mRNA for each cytokine (Table 3). Although 7 of the patients had mRNA expression of IL-10, only 0.6% of cells expressed it, and only 2 adult patients had a few positive cells for IL-4. Otherwise, a similar cytokine pattern was found in both children and adults.

Comparison groups. As a positive comparison group, synovial tissue was examined from the 6 patients with RA or psoriatic arthritis. The cytokine profiles in synovial samples from these patients were similar to those in the patients with chronic JRA or Lyme arthritis. In most of the patients, about 1% of the inflammatory cells expressed mRNA for each of the proinflammatory cytokines, whereas only 0.2% or 0.4% of the cells had mRNA for the antiinflammatory cytokines (Table 3). As a negative control, synovial tissue was examined from the 9 patients with various orthopedic conditions, including plica and subluxation of the hips or patellae. The median inflammatory cell density in the synovia of these patients was only 8 cells per hpf compared with 32 to 62 cells per hpf in the groups with inflammatory arthritis ($p \leq 0.005$). However, about two-thirds of the patients with orthopedic conditions had cells that expressed mRNA for at least one of the proinflammatory cytokines, and 0.4% to 1.6% of the cells expressed them. Only 2 patients had a few cells that expressed IL-10 or IL-4 mRNA.

DISCUSSION

As reported recently with other tissues[33], this study shows that cytokine mRNA may be detected by *in situ* hybridization in formalin fixed or fresh-frozen synovial tissue, making it pos-



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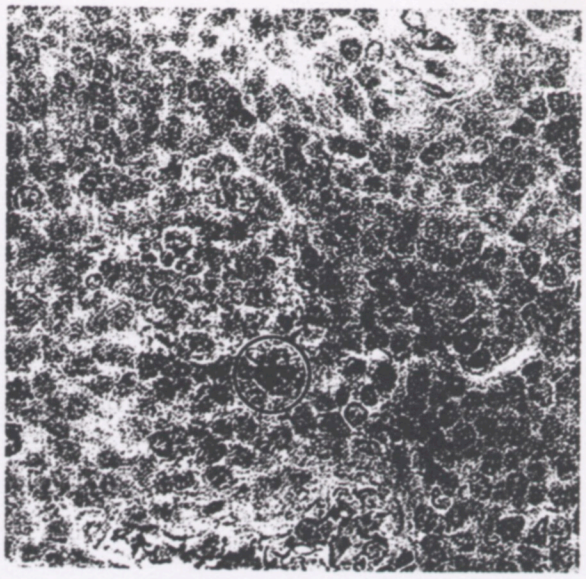

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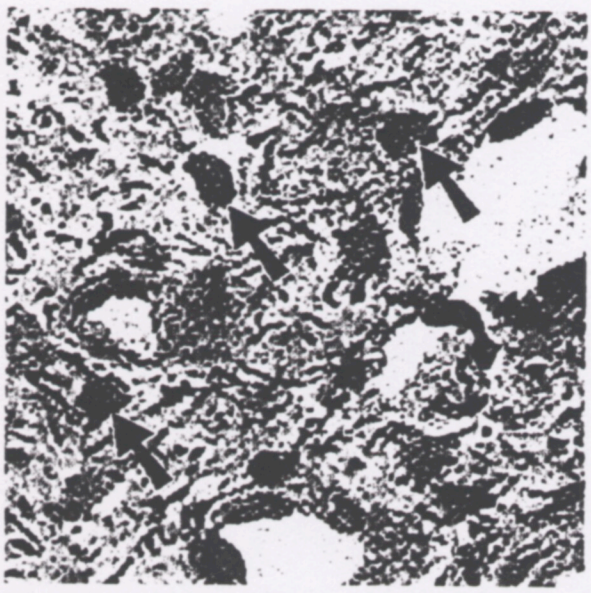

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Figure 1. Paraffin embedded synovial tissue from the knee joint of a 15-year-old boy with chronic Lyme arthritis. A. The histologic picture shows synovial hypertrophy, vascular proliferation, and an inflammatory infiltrate composed primarily of lymphocytes, plasma cells, and macrophages (H&E: original magnification × 200). B. *In situ* hybridization (ISH) using an anti-sense riboprobe for IL-1ß shows occasional cytoplasmic staining in a small number of inflammatory cells (encircled) (× 400). C. ISH using an anti-sense probe for IL-6 shows multiple inflammatory cells with cytoplasmic staining (arrows) (× 400).

sible to utilize large collections of archival specimens. Moreover, with this method, the location and number of cytokine-producing cells can be identified and distinguished from potential background expression in normal tissue. However, the method has several limitations. First, the sensitivity may be somewhat less than with other methods. In an early study, Firestein, *et al* enzymatically dispersed fresh synovial cells from 7 patients with various chronic inflammatory arthritides, and stained the cells with cytokine-specific riboprobes [34]. With this method, about 19% of the cells expressed IL-6 mRNA, 10% expressed IL-1ß, 6% had TNF-α, and none bound the IFN-γ probe. In comparison, a median of 1% to 8% of the cells in the current study patients with inflammatory arthritis had mRNA for these cytokines. Moreover, the sensitivity may be less with formalin fixed tissue than with fresh-frozen samples. Finally, the morphology of the cells in archival material may not allow a clear distinction of the type of inflammatory cell producing a given cytokine. For this reason, we did not report the type of inflammatory cell producing each cytokine although it appeared that IL-1ß, TNF-α, and IL-10 were secreted primarily by macrophages, whereas IFN-γ, IL-2, and IL-4 were produced primarily by T cells. Despite these limitations, the relative proportions of cells producing particular cytokines can be compared accurately with the method used here.

Consistent with the results using other methods[11–16], mRNA for proinflammatory cytokines, including IL-1ß and TNF-α, was prominent in the synovial tissue of the current

patients with JRA, Lyme arthritis, or adult RA. However, 2 recent studies suggest that cytokine profiles may be different in the various forms of childhood arthritis[15,16]. In synovial samples from 38 patients with JRA, TNF-ß, which was not assessed in this study, was present in greater amounts immunohistochemically in polyarticular compared with pauciarticular JRA, whereas TNF-α was similar in all groups[15]. In synovial fluid mononuclear cell preparations or synovial tissue samples from 50 patients with JRA, IL-4 mRNA was identified by RT-PCR significantly more often in patients with pauciarticular onset than in those with polyarticular onset[16].

In contrast, our study was a retrospective analysis of archival specimens. Synovial biopsies were obtained for diagnosis from several patients early in the course of the illness, most of whom had pauciarticular onset, whereas the other samples were obtained during joint surgery from patients with severe disease of years' duration, most of whom had polyarticular or systemic onset. However, the children in each group had arthritis for at least months and usually years after biopsy or reconstructive surgery, and regardless of the JRA subtype, their synovial samples showed a prominent proinflammatory response. Thus, the ability to show different cytokine profiles according to JRA subtype may depend on when in the illness the samples are obtained.

Although a proinflammatory response was more prominent, all 5 patients with early untreated JRA and many patients with late JRA or chronic Lyme arthritis had cells expressing mRNA for IL-10, the cytokine that initiates the antiinflammatory response. In an *in vitro* cell culture system, the addition of exogenous IL-10 (but not IL-4) to *B. burgdorferi* stimulated synovial fluid mononuclear cells of patients with Lyme arthritis suppressed, in dose dependent manner, the production of Type 1 cytokines IFN-γ and TNF-α[19], suggesting that the production of IL-10 may be insufficient *in vivo* to downregulate the Th1 response. A similar "relative deficit" in IL-10 production and lack of saturation of the IL-10 receptor system was shown in synovial membrane cultures from patients with RA[35]. Thus, it is likely that diminished or minimal IL-10 production contributes to the dominance of proinflammatory cytokines in the synovial membranes of patients with JRA or Lyme arthritis.

In Lyme arthritis, we believe that a dominant proinflammatory response is critical in the development of autoimmunity in synovial tissue. In patients with Lyme arthritis who have RA alleles, such as the HLA-DRB1*0401 allele, the dominant T cell epitope of outer-surface protein A (OspA) of *B. burgdorferi* presented by these class II MHC molecules has molecular mimicry with human leukocyte function associated antigen-1 (hLFA-1)[4]. After months within the proinflammatory cytokine milieu of the joint, OspA reactive T cells from patients with treatment resistant Lyme arthritis may develop reactivity with this autoantigen. A cross reactive T cell response between OspA and hLFA-1 would provide an amplification mechanism to explain more severe and prolonged Lyme arthritis. By analogy, we would postulate that in other chronic inflammatory arthritides, a prolonged proinflammatory response in synovium may be necessary for the development of autoimmunity.

In an effort to develop a negative control group, synovial tissue was also examined from patients with various orthopedic conditions. As expected, the number of inflammatory cells was markedly less in these conditions than in the chronic inflammatory arthritides. However, in the orthopedic cases, mRNA expression for proinflammatory cytokines was still found in about 1% of the infiltrating cells. Thus, mild inflammation may be a part of what we ordinarily think of as "non-inflammatory orthopedic conditions."

In summary, most patients with early or late JRA or chronic Lyme arthritis had prominent expression of mRNA for proinflammatory cytokines in synovial tissue, with less anti-inflammatory cytokine expression. This proinflammatory response may be critical in the development of autoimmunity within the joint.

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