Quantitative Analysis of Immunohistologic Features of Very Early Rheumatoid Synovitis in Disease Modifying Antirheumatic Drug- and Corticosteroid-Naïve Patients

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ABSTRACT. ***Objective.*** To describe the immunohistochemical features of very early rheumatoid synovitis in disease modifying antirheumatic drug- and corticosteroid-naïve patients.

Methods. Eight patients presenting with oligoarthritis or polyarthritis, who later met American Rheumatism Association criteria for rheumatoid arthritis (RA), underwent needle synovial biopsies of a knee joint within the first 6 weeks after onset of disease symptoms. Using antibodies to CD3, CD8, L26, CD68, and von Willebrand factor, a detailed quantitative immunohistochemical analysis was done.

Results. CD3+ T lymphocytes, CD8+ T lymphocytes, L26+ B lymphocytes, and CD68+ macrophages were seen in 8/8 (100%), 7/8 (87%), 4/8 (50%), and 6/6 (100%) of synovial biopsies stained with the respective marker. There was a wide variation in number of positive cells between patients. CD3+ and CD8+ T cells were seen predominantly in perivascular areas, less often in a diffuse distribution, and not in aggregates. L26+ B lymphocytes were found in much smaller numbers compared with T lymphocytes. A mean of 67 vessels/mm^2 was noted. No lymphoid aggregates were seen. In all cases, infiltration of macrophages and lymphocytes was limited mainly to relatively superficial parts of synovium, i.e., within 1 to 2 high power fields of the surface.

Conclusion. An absence of lymphoid aggregates or dramatic vascularity and a predominantly superficial infiltrate consisting mainly of perivascular T cells with few B cells characterized our patients with very early RA of < 6 weeks' duration. Thus, there appear to be some potentially important differences from findings reported in well established disease. (J Rheumatol 2004;31:1281–5)

Key Indexing Terms:
RHEUMATOID ARTHRITIS VERY EARLY SYNOVITIS
QUANTITATIVE IMMUNOHISTOCHEMISTRY

Synovial tissue in chronic rheumatoid arthritis (RA) is characterized histologically by proliferation of synovial lining cells, infiltration of lymphocytes, plasma cells and macrophages, increased vascularity, and fibrin deposition[1]. Although a number of studies have examined RA synovium during the first year of disease, few studies have reported histological features of very early rheumatoid synovitis, i.e., RA of less than 4 to 6 weeks' duration[2-5]. The studies did not include immunohistology.

Immunohistological features have been described in synovitis of less than 3 months' duration, and 6 months to one year[6-8]. Study of early disease can help us understand the initiating or triggering factors in RA; it may also be possible to determine if such factors are the same as those responsible for disease propagation and chronic disease.

The definition of "early disease" in RA has varied in studies, including less than 4 to 6 weeks[2-5], less than 3 months[6], or less than 6 months to one year[7,8]. We describe the histopathological changes of very early rheumatoid synovitis, defined as disease duration of 6 weeks or less, using immunohistochemistry to identify cellular infiltrates and vessels.

MATERIALS AND METHODS

Eight patients with oligoarthritis or polyarthritis of 6 weeks' or less duration, as measured from the onset of the first symptom or sign of arthritis, who later met American College of Rheumatology (ACR) criteria for diagnosis of RA[9], underwent a synovial biopsy of an affected joint. The

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Institutional Review Board of the University of Pennsylvania approved the study protocol.

Under sterile conditions and local anesthesia with 1% lidocaine, multiple synovial tissue samples were obtained from the affected joint (knee joint in all patients) with a Parker Pearson needle, as described[10]. No complications were noted in any of the patients.

The samples obtained for histological examination were formalin fixed and paraffin embedded. Immunohistochemical stains were performed using standard avidin-biotin complex technology and a robotic immunostaining system (Biotek 1000, Ventana, Tucson, AZ, USA). Immunohistochemical studies were performed for CD3+ T cells, CD8+ T cells, B cells (L26), macrophages (CD68), and von Willebrand factor (VWF). All primary antibodies were obtained from Dako Corp., Carpinteria, CA, USA. Since some synovial tissue was damaged during processing, some stains are not reported on all specimens.

All sections with synovial lining cells were read twice in a blinded fashion by a single observer (JS) using a Nikon Optiphot microscope (eyepiece diameter 18 mm). Only cells with distinct cytoplasmic staining were counted. The size of high power field (hpf, original magnification 400×) was calculated by using a stage micrometer (with 100 gradations of 0.01 mm each) and found to be 0.159 mm^2, thus generating a conversion formula of cells/mm^2 = cells/hpf × 0.159^{-1}. All high power fields (original magnification 400×) were evaluated for every patient, numbers of positively staining cells and blood vessels were counted, and results were expressed as positive cells/mm^2. Lymphocytic aggregates were defined as infiltrates of at least 50 T cells with or without presence of germinal center B cells.

In addition to mean and standard deviation (SD), median and range are given for various cell subpopulations. Since a wide range in values was observed, in most cases median may be a better measure of central tendency than mean.

RESULTS

Clinical and demographic characteristics of patients. The group consisted of 6 women and 2 men with a mean age of 41 years (SD 9). Mean disease duration was 3.6 weeks (SD 1.5, range 2–6). Patients had 11 mean swollen (SD 7) and 15 mean tender joint counts (SD 6). Rheumatoid factor was positive in 6/8 patients (75%), but none (0/8) had rheumatoid nodules at presentation. Six of 8 patients were taking various nonsteroidal antiinflammatory drugs (NSAID) at the time of biopsy. No patient was taking corticosteroids or disease-modifying agents before synovial biopsy.

Immunohistochemical features. A mean of 35 high power fields (median, 37 hpf) of synovium were examined for each patient, and an average of 4 or 5 biopsy specimens were examined for each immunohistochemical stain. Tables 1 and 2 show the wide range of immunohistochemical abnormalities seen in synovial tissue of these 8 patients: almost twice as many CD3+ cells as CD8+ cells (Table 2); relatively higher numbers of both CD3+ and CD8+ T lymphocytes distributed perivascularly than diffusely (Table 1); and a wide variation in the numbers of CD3+, CD8+, and CD68+ cells among patients (Table 2). In addition to their diffuse and perivascular distribution, CD68+ macrophages were seen in the surface lining layer (Figure 1A and Table 2). L26+ B lymphocytes were seen in only 4/8 patients with both perivascular and diffuse distribution (3/4 patients), but these cells were in much lower numbers compared to T lymphocytes and macrophages.

Table 1. Number of synovial specimens staining positive for the respective cells.

	CD3+ T Cells	CD8+ T Cells	L26+ B Cells	CD68+ Macrophages
Overall (any location)	8/8	7/7	4/8	6/6
Perivascular	7/8	6/7	4/8	5/6
Diffuse	8/8	7/7	3/8	6/6
Both perivascular and diffuse	7/8	6/7	3/8	5/6
Surface lining cells	0	0	0	6/6

Table 2. Distribution of various cells in synovial tissue from 8 RA patients with synovitis. All numbers rounded to the nearest digit.

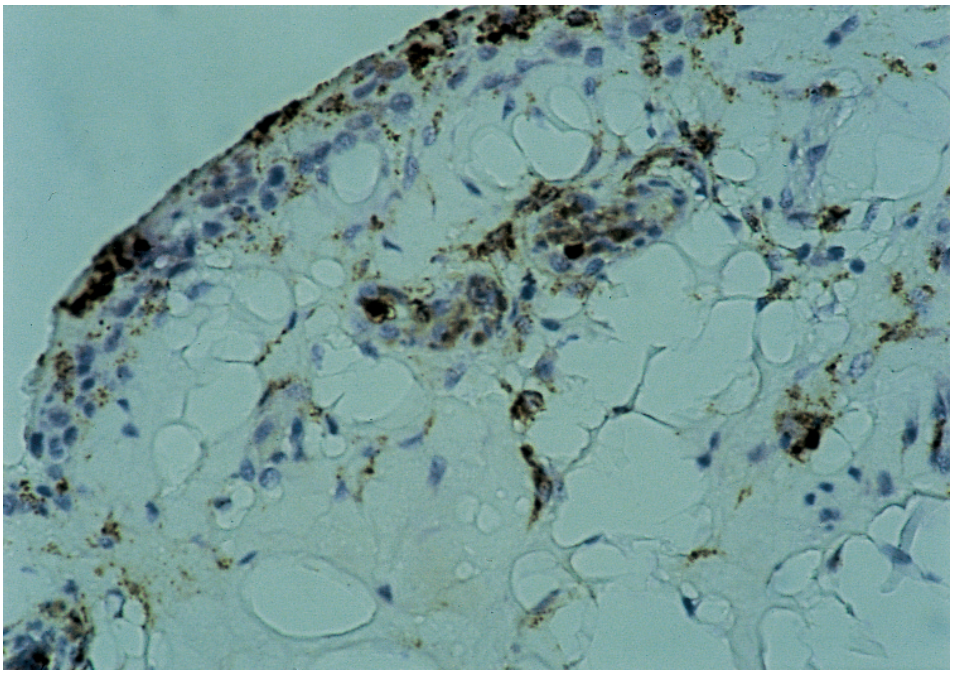
	Cells/mm^2, mean ± SD	Cells/mm^2, median; range
CD3+ T cells		
Perivascular	131 ± 148	91; 0–415
Diffuse	38 ± 66	15; 2–201
CD8+ T cells		
Perivascular	66 ± 73	47; 0–201
Diffuse	20 ± 18	13; 3–53
L26+ B cells		
Perivascular	10 ± 9	7; 2–23
Diffuse	7 ± 10	3; 0–21
CD68+ macrophages		
Perivascular	92 ± 117	37; 1–279
Diffuse	45 ± 59	19; 10–162
Surface lining cells	61 ± 40	48; 22–134

Blood vessel density varied among the patients, with an average of 67 ± 44 VWF+ blood vessels/mm^2 (median 53 vessels/mm^2, range 28–166). Six of 8 patients had fewer than 59 vessels/mm^2; Figure 1B shows typical synovial vascular density and VWF staining pattern in one such patient. In all cases, the inflammatory infiltrate of macrophages and T and B lymphocytes was limited predominantly to relatively superficial parts of the synovium, i.e., within 1 to 2 high power fields of the surface lining cells. No lymphoid aggregates were seen in any biopsy specimen.

DISCUSSION

Purely histologic studies in arthritis of less than 6 weeks' duration (very early synovitis) or less than 3 months' duration (early synovitis) have shown no lymphocytic nodules[2,3,5,6] and rare[3-5] or absent[2] plasma cells compared to synovitis of longer duration. Similarly, we noted an absence of lymphoid follicles and lesser numbers of L26+ B lymphocytes.

Our immunohistologic findings differ from previous studies of RA lasting more than 6 weeks and studies of chronic synovitis. Lymphocytic aggregates (defined as characteristic aggregation of at least 50 lymphocytes) were not seen in any of our patients, while they were observed in an earlier report in 44% of patients with RA of less than one



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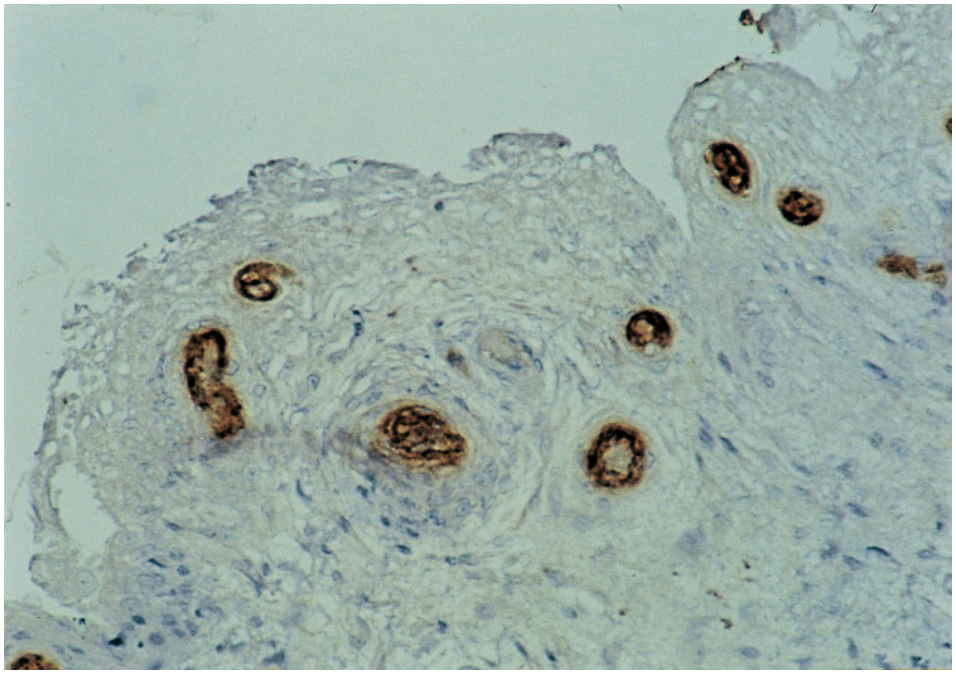

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Figure 1. A. Synovial tissue from a patient showing CD68 positive cells in all distributions, particularly perivascularly (original magnification 400×). B. Synovial tissue showing blood vessels staining positive for von Willebrand factor (original magnification 400×).

year (no definition of aggregates given)[7]. Lymphocyte aggregates have been reported in the synovium of patients with disease duration of 2 to 15 years[11,12]. We believe that the presence of lymphocyte aggregates may be related to longer disease duration (> 4–6 weeks), more severe disease, or both. Further, we detected a mean of 67 vessels/mm^2 in our patients. In contrast to the average of historic controls of 242–270 vessels/mm^2 [13-15], we noted no evidence of increased synovial vascularity at this stage. Previous studies of rheumatoid synovitis in patients with longer disease duration (2.7–15 yrs) showed varied results: in some, the number of vessels was higher than the above controls[13,14], in others it was lower[15,16].

Moreover, the numbers of various cell populations in our

patients were lower than in 2 reports of synovitis of longer duration in DMARD- and steroid-naïve RA patients[16,17]. The earlier studies found much higher numbers of macrophages[16,17], B cells[16,17], and CD8+ T cells[16]. This difference may be due either to the shorter duration of disease in our patients or to differences in treatment regimens or methodology, i.e., patient selection criteria, biopsy processing, and scoring techniques. Since quantitative analysis was not performed in the earlier reports of very early synovitis and synovitis up to 6 months to one year[2-8], the number of various cell subpopulations could not be compared. Lastly, we found most of the T and B lymphocytic infiltrate in the superficial layer of synovium, i.e., within 1 to 2 high power fields of the synovial lining cells. The exact significance of this finding is unclear, since most studies of early synovitis and synovitis of greater than 6 weeks' duration provide no details regarding this. It is, however, possible that, at least initially, the inflammatory infiltrate is limited to a superficial site, and involvement of deeper tissue occurs with increase in duration and/or severity of disease.

In an earlier study comparing 3 groups of patients with different duration of disease and synovitis, Konttinen, *et al* concluded from cellular immunohistopathology of RA synovium that the target organ undergoes sequential changes during the course of the disease[6]. Despite the differences noted above, we acknowledge that the distinction between very early and early arthritis is somewhat arbitrary at this point and needs further study.

Some findings noted in previous studies of chronic synovitis were also seen in our patients, including relatively lower frequency and numbers of B lymphocytes versus T lymphocytes[11,12], marked variation of various cell subpopulations between patients, and presence of a mixture of CD4+ and CD8+ lymphocytes[7] (since only half of CD3 positive cells were CD8 positive, we presume that most of the remaining CD3 positive cells were CD4 positive). Interestingly, synovial changes have been noted in asymptomatic joints in patients with RA that are similar to our findings with respect to the predominant perivascular distribution of T lymphocytes, the paucity of B lymphocytes[18,19], and no evidence of lymphocyte aggregates or increased vascularity[18].

The limitations of our study include an absence of a control group of patients with RA with longer disease duration studied in parallel, and use of nonsteroidal antiinflammatory drugs by some of our patients at the time of biopsy, which may have affected synovial histology[20,21]. Nevertheless we were able to exclude patients with DMARD and corticosteroid use, which could have caused more significant alterations[22-24]. A larger number of patients and longer followup could have added useful information to our study.

Our study is the first detailed quantitative immunohistological analysis of patients with RA having disease duration of 6 weeks or less. All our patients were DMARD- and corticosteroid-naïve, which decreases the problem of sorting out disease-mediated changes from those due to medication or to medication in combination with disease. We also provide a quantitative analysis, allowing easy comparison for future more extensive studies of very early, early, and late RA.

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