Synovial Infiltration with CD79a-positive B Cells, But Not Other B Cell Lineage Markers, Correlates with Joint Destruction in Rheumatoid Arthritis

YING-QIAN MO, LIE DAI, DONG-HUI ZHENG, LANG-JING ZHU, XIU-NING WEI, FRANK PESSLER, JUN SHEN, and BAI-YU ZHANG

ABSTRACT. ***Objective.*** The efficacy of B cell depletion in the treatment of patients with rheumatoid arthritis (RA) has revitalized interest in the pathogenic role(s) of B cells in RA. We evaluated the distribution of synovial B lineage cells and their correlation with histologic disease activity and joint destruction in RA.

Methods. Synovial tissue samples were obtained by closed-needle biopsy from 69 Chinese patients with active RA, from 14 patients with osteoarthritis (OA), and from 15 with orthopedic arthropathies (OrthA) as disease controls. Serial tissue sections were stained immunohistochemically for CD79a (pro-B cell to plasma cell), CD20 (B cells), CD38 (plasma cells), CD21 (follicular dendritic cells), CD68 (macrophages), CD3 (T cells), and CD34 (endothelial cells). Densities of positive-staining cells were determined and correlated with histologic disease activity (Krenn 3-component synovitis score) and radiographic joint destruction (Sharp score).

Results. Mean sublining CD79a-positive cell density was significantly higher in RA than in OA ($p < 0.001$) or OrthA ($p = 0.003$). Receiver operating characteristic curve analysis showed that CD79a-positive cell density differentiated RA well from OA [area under the curve (AUC) = 0.79] or OrthA (AUC = 0.75). Spearman's rank order correlation showed significant correlations between sublining CD79a-positive cell density and the synovitis score ($r = 0.714$, $p < 0.001$), total Sharp score ($r = 0.490$, $p < 0.001$), and the erosion subscore ($r = 0.545$, $p < 0.001$), as well as the joint space narrowing subscore ($r = 0.468$, $p = 0.001$) in RA.

Conclusion. Synovial CD79a-positive B cells may be a helpful biomarker for histologic disease activity in RA and may be involved in the pathogenesis of joint destruction in RA. (First Release Oct 15 2011; J Rheumatol 2011;38:2301–8; doi:10.3899/jrheum.110615)

Key Indexing Terms:
RHEUMATOID ARTHRITIS B CELL LINEAGE SYNOVITIS JOINT DESTRUCTION

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Rheumatoid arthritis (RA) is characterized by aggressive synovitis leading to joint destruction and disability. Rheumatoid synovitis reveals varying patterns involving all cells of chronic inflammation and increased proliferation of synovial lining cells, stromal fibroblasts, and blood vessels. There is some debate about which cell type contributes most to RA pathogenesis. The advent of targeted biological therapies has improved our understanding of the pivotal molecules/cells in disease pathogenesis. In particular, the potent efficacy of B cell depletion therapy [e.g., rituximab (RTX), a chimeric monoclonal antibody directed at the CD20 membrane protein present on B cells] in RA has revitalized interest in the pathogenic roles of B cells[1].

The role of B cells in RA clearly transcends the production of autoantibodies, as they also produce soluble factors (including cytokines and chemokines), present antigens to T cells, and form B cell aggregates in the synovium[2]. Svensson, *et al*[3] confirmed the pathologic relevance of B cells in arthritis in mice deficient of IgM transmembrane tail exons (μMT mice). These mice turned out to be resistant to colla-

gen-induced arthritis, presumably since B cell development was blocked at the pro-B stage. Further, severe combined immunodeficient mice were engrafted with synovium taken from patients with RA and treated with a 3-day course of anti-CD20 antibodies. The depletion of B cells led to dissociation of B cell follicular structures, and dramatically reduced interleukin 1ß (IL-1ß) and interferon-γ (IFN-γ) levels, indicating that B cells affect the ability of T cells and macrophages to generate these proinflammatory cytokines[4].

Several clinical trials showed that RTX affected B cell levels in the synovium less than in the circulation. In an open-label and serial synovial biopsy study of RTX treatment (ARISE), all 13 patients with RA experienced nearly complete depletion of circulating B cells after therapy, but only a smaller although significant decrease in synovial B cells[5]. More recently, Teng, *et al*[6] performed an immunohistochemical analysis in 24 patients with RA treated with RTX, and showed that 12 weeks after treatment only CD79a-positive residual B cells were significantly lower in patients with Disease Activity Score 44 (DAS44) < 2.4 compared to patients with DAS44 > 2.4, but CD20cg staining was negative in nearly all patients. CD20–/CD79a+ B cells likely play a pivotal role in RA pathogenesis, but their depletion by RTX is hampered because they do not express CD20.

Thus, an understanding of which B cell subpopulation(s) are most relevant to RA pathogenesis is important in designing therapeutic strategies targeting B cells. Our study aimed to evaluate the distribution and densities of synovial B lineage cells in 69 Chinese patients with active RA and to determine which cells correlate most with disease activity and joint destruction.

MATERIALS AND METHODS

Patients. Sixty-nine Chinese patients with RA who fulfilled the 1987 revised criteria of the American College of Rheumatology (ACR) were recruited from the Department of Rheumatology of Sun Yat-sen Memorial Hospital. All patients had active disease, defined as a 28-joint DAS ≥ 3.2. Fourteen patients with osteoarthritis (OA) and 15 with noninflammatory orthopedic arthropathies [OrthA; consisting of plica syndrome (n = 5), meniscus injury (n = 3), ligament injury (n = 3), avascular necrosis of the femur (n = 2), and femur fracture (n = 2)] were recruited from the Department of Orthopedics as "less inflamed" disease controls, similar to OA[7,8]. This study was conducted in compliance with the Helsinki Declaration. The Medical Ethics Committee of Sun Yat-sen Memorial Hospital approved the protocol. All patients gave written informed consent.

Clinical assessments. Clinical data of all patients with RA were collected at baseline. Disease activity was assessed with the DAS28, the 28-joint tender and swollen joint count (TJC and SJC), duration of morning stiffness, hand grip strength, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and anticitrullinated peptide antibodies (ACPA). Disability status was evaluated with the Chinese language version of the Stanford Health Assessment Questionnaire (HAQ)[9].

Joint damage was scored according to hand/wrist radiographs (anteroposterior view) and the Sharp score[10]. Seventeen areas for erosion and 18 for joint space narrowing are assessed in each hand/wrist. The maximum score per single joint for erosions is 5, and for joint space narrowing 4, with the sum of the erosion and joint space narrowing subscores constituting the total Sharp score. Radiographs were scored by 1 experienced observer (SJ), who was not aware of the patients' clinical and histologic findings. In a subgroup of patients, radiographs were reevaluated by the same observer within 2–4 weeks. The correlation between these measurements was high (intraclass correlation coefficient 0.953–0.977).

Synovial tissues. A closed Parker-Pearson needle biopsy[11] was performed on 1 inflamed knee joint in all patients with RA. At least 6 pieces of synovial tissue were obtained per patient to minimize sampling error[12]. The OA and OrthA specimens were obtained by knee arthroplasty or arthroscopy. All samples were fixed in 10% neutral formalin and embedded in paraffin. Sections (5 μm) were cut serially and mounted on adhesive glass slides. Sealed slides were stored at –20°C until staining.

Immunohistochemistry. Serial sections of synovial tissues were stained with hematoxylin and eosin and a 3-step immunoperoxidase method. Sections were deparaffinized with xylol, ethanol, and demineralized water. Antigens were then retrieved by boiling in 1 mM EDTA (pH 8.0) for 15–20 min. After the sections had been washed in demineralized water and phosphate buffered saline (PBS), the primary antibody was added and incubated for 45 min at 37°C. After washing with PBS, the sections were incubated with EnVision Mouse or Rabbit conjugate for 15 min at 37°C. The color reaction was completed with the DAB-positive substrate. Sections were counterstained with hematoxylin.

Serial sections were stained with the following commercial antibody preparations (Invitrogen, Carlsbad, CA, USA): anti-CD20 (clone L26, B cells), anti-CD38 (clone SPC32, plasma cells), anti-CD79a (clone SP18, B lineage cells from pro-B cell to plasma cell stage), anti-CD3 (clone PS1, T cells), anti-CD68 (clone KP1, macrophage-like synoviocytes and macrophages), anti-CD34 (clone QBEnd/10, vascular endothelial cells), and anti-CD21 [clone 2G9, follicular dendritic cells (FDC)], according to standard staining protocols. All were mouse monoclonal antibodies except anti-CD79a, which was a rabbit monoclonal antibody. An automated immunostainer (PV-6000; Zymed Laboratories Inc., South San Francisco, CA, USA) was used. Parallel sections were incubated with irrelevant, isotype, and concentration-matched monoclonal antibodies as negative control. Absence of staining due to technical failure was excluded by including appropriate positive control tissues in each staining run.

Synovitis assessments. Only tissue pieces containing synovial lining and vascularized subintima were included in the analyses. At least 3 such pieces were evaluated in each specimen. Histologic changes in the hematoxylin-stained sections were graded with the synovitis score according to Krenn, *et al*[13,14] with the modification that the average of all fields containing synovial lining was recorded per specimen. Synovitis assessments were performed by 2 blinded observers (DL and MYQ) using an Olympus BX41-32PO2 microscope (Olympus Corp., Tokyo, Japan). Differences between the observers were resolved by mutual agreement.

The densities of CD79a, CD20, CD38, CD3, and CD68 positive-staining cells and vessels (confirmed by CD34 positive-staining) were determined by manual counting. A selection of 17 hpf (400×) in the superficial subintima were examined for each specimen[15]. A 1-mm graticule was used in each hpf with its edge placed just below the lining. The measured value per hpf was converted to the value per mm^2 by using $\times 0.0625^{-1}$ as the conversion factor[16]. The percentage of lining CD68-positive macrophage-like synoviocytes was scored semiquantitatively (0: absent, 1: 1%–25%, 2: 26%–50%, 3: 51%–75%, 4: 76%–100%).

Statistical analysis. Statistical analyses were performed with SPSS for Windows 13.0 statistical software (SPSS Inc., Chicago, IL, USA). Data were presented as frequencies and percentages for categorical variables and median and interquartile range (IQR) for continuous variables, unless stated otherwise. Because the densities of positive-staining cells and vessels were not distributed normally, nonparametric tests were used (Mann-Whitney rank-sum test between 2 groups, Kruskal-Wallis 1-way analysis of variance on ranks among 3 or more groups for continuous variables). Differences among cell-surface markers of B lineage cells were also assessed with receiver operating characteristic curve (ROC) analysis[17,18]. ROC analysis based on the logistic model was used to identify the differentiated ability of combination

of all 3 B-cell markers. Spearman's rank order correlation test was used to assess the relationship between synovial B lineage cells and the histologic and clinical measures.

RESULTS

Characteristics of the study patients. Demographic and clinical features of all patients with RA are shown in Table 1. Age, sex, and disease duration did not differ among the patients with RA, OA, and OrthA. Median DAS28 value was 5.7 (range 3.2–7.7), suggesting active disease. Median disease duration was 48 months (range 3 to 480 mo). The median synovitis score was 4, with a range of 1–7[19]. Of the patients with RA, 48% (33/69) had never taken corticosteroids or disease-modifying antirheumatic drugs (DMARD). A majority of these patients had taken only Chinese herbal medicine and/or painkillers to relieve arthralgia and did not seek conventional medical treatment until the onset of serious symptoms. At recruitment, 22% (15/69), all of whom were referred from other hospitals, had taken corticosteroids alone. The remaining 30% (21/69) were taking 1 or more DMARD, including methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine. None had been treated with biologic agents.

Synovial CD79a-positive cell density in the differentiation between RA and OA or OrthA. As shown in Figure 1, synovial cells expressing CD79a showed a typical morphology of lymphocytes or plasma cells. In the RA synovium, B lineage inflammatory cells infiltrated the sublining area in heterogeneous patterns: 19% (13/69) showed scarce B lineage inflammatory cells in the sublining area; 36% (25/69) showed diffuse infiltration of CD79a-positive and CD20-positive cells (diffuse synovitis, Figure 1A-1E), and 45% (31/69) showed aggregated CD20-positive B cells, generally combined with T cells and surrounded by CD38-positive plasma cells (aggregate or follicular synovitis, Figure 1F-1J). The aggregates varied in size ranging from small perivascular infiltrates to large aggregates. Germinal center formation, as confirmed by standard histologic criteria and immunohistochemical staining for CD21-positive FDC[2], was detected in 7 cases (10%).

Synovial B lineage cells, as well as CD3-positive T cells, CD68-positive macrophages, vascular density, and synovitis score, were significantly less in synovium of OA or OrthA than in the RA synovium (Table 2). The mean sublining CD79a-positive cell density was significantly higher in RA than in OA ($p < 0.001$) or OrthA ($p = 0.003$), with a similar trend for CD20-positive and CD38-positive cells. In the ROC analysis, CD79a differentiated RA from OA and OrthA better than CD20 or CD38 did (Table 3).

Correlation of synovial B lineage cell densities with the synovitis score. Synovial CD79a-positive cells, CD20-positive B cells, and CD38-positive plasma cells in RA correlated significantly with the synovitis score (Table 4; all $p < 0.001$). Additionally, synovial B lineage cell densities correlated with lining hyperplasia (all $p < 0.05$) and CD3-positive T cell density (all $p < 0.001$). However, the B lineage cell densities correlated neither with lining nor sublining CD68-positive cells nor with vascular density.

Correlation of synovial CD79a-positive cell density with disease activity. Slight correlations between synovial CD79a-positive cell density and CRP, CD20-positive B cell density and ESR, and CD38-positive plasma cell density and ESR/CRP were found (Table 4). There was no significant correlation between densities of any of the synovial B lineage cells and DAS28, RF, ACPA, and other measures such as TJC, SJC, duration of morning stiffness, hand grip strength, or HAQ score (Table 4). About 80% of patients with RA in our study were positive for RF and/or ACPA, and synovial B lineage cell densities were similar in seropositive and seronegative patients (data not shown).

Correlation of synovial CD79a-positive cell density with joint destruction. Spearman's rank correlation analysis showed that synovial CD79a-positive cell density correlated positively with the total Sharp score ($r = 0.490$, $p < 0.001$), the erosion subscore ($r = 0.545$, $p < 0.001$), and the joint space narrowing subscore ($r = 0.468$, $p = 0.001$; Table 4 and Figure 2). There was no significant correlation between synovial CD20-positive B cell or CD38-positive plasma cell densities and the Sharp score. Additional correlation analysis showed that synovial T cells, lining or sublining CD68-positive cells, and capillary angiogenesis did not correlate with the Sharp score either.

Synovial CD79a-positive cell density was higher in patients with longstanding RA (disease duration > 2 years, n =

Table 1. Baseline characteristics of the 69 patients with rheumatoid arthritis (RA).

Characteristics	
Demographic	
Age, yrs, median (range)	55 (21–75)
Women, n (%)	56 (81)
Disease status	
Disease duration, mo, median (range)	48 (3–480)
Rheumatoid factor-positive, n (%)	55 (80)
ACPA-positive, n (%)	55 (80)
DAS28, median (range)	5.7 (3.2–7.7)
Synovitis score*, median (range)	4 (1–7)
Medications, n (%)	
Corticosteroids	31 (45)
Methotrexate	16 (23)
Leflunomide	7 (10)
Sulfasalazine	3 (4)
Hydroxychloroquine	3 (4)

* Synovitis score according to Krenn, *et al*[13,14], based on the following basic morphological measures of synovitis: intimal hyperplasia, inflammatory infiltration, and activation of resident cells/synovial stroma, including fibroblasts, endothelial cells, histiocytes, macrophages, and multinucleated giant cells. All measures are graded: 0 (absent), 1 (slight), 2 (moderate), or 3 (strongly positive). The values of all measures are summarized, resulting in a final score between 0 and 9. Median reference value for RA = 5.0[19]. ACPA: anticitrullinated protein antibody; DAS28: 28-joint Disease Activity Score.

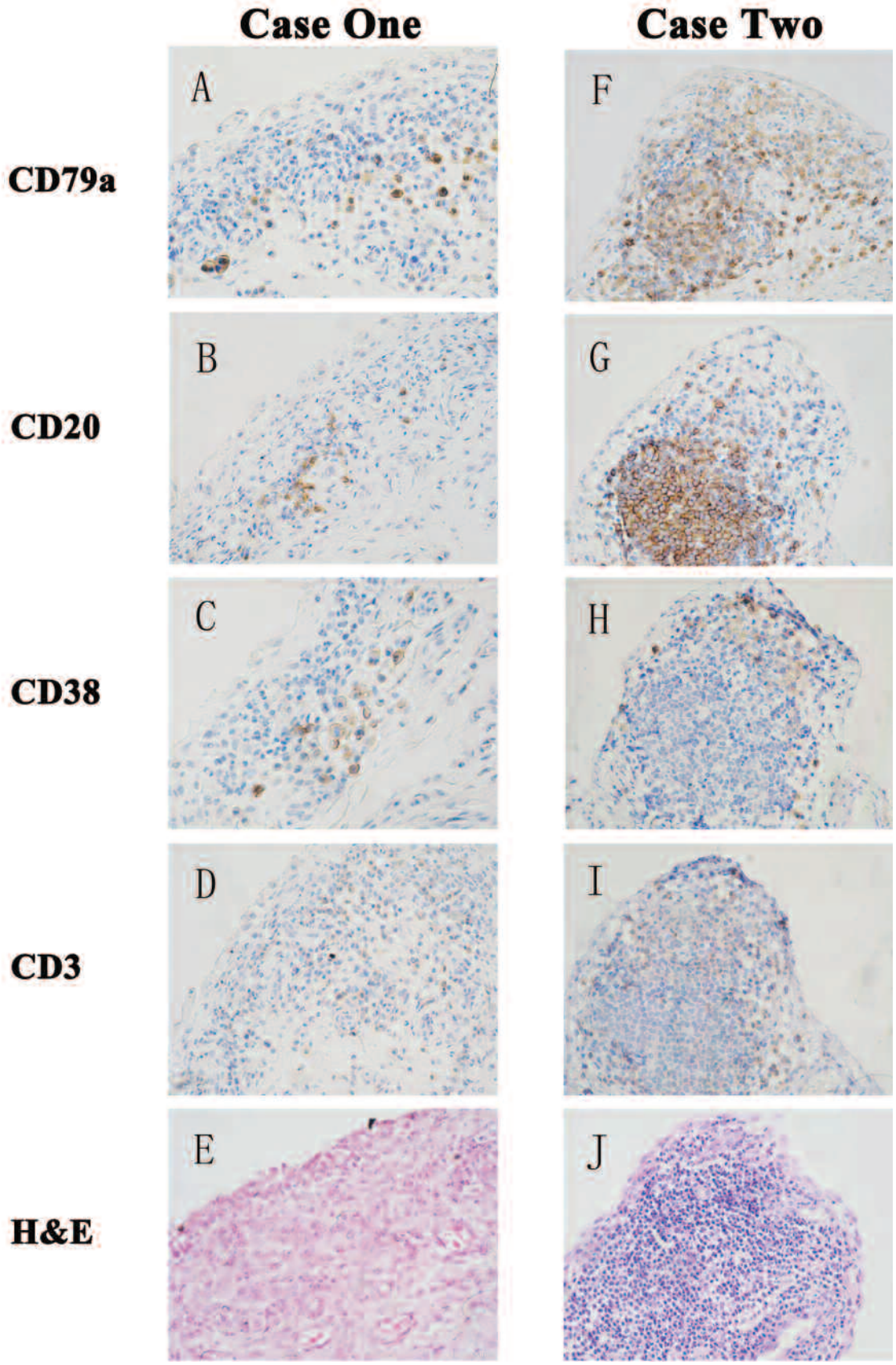

Figure 1. Different distributions of B lineage infiltrated cells in representative synovia from 2 different patients with RA. Synovial cells expressing CD79a (A, F) showed a typical morphology of lymphocytes or plasma cells. Case 1 showed diffuse infiltration of CD79a-positive cells (A), CD20-positive B lymphocytes (B), and CD38-positive plasma cells (C), together with a small number of CD3-positive T cells (D) and low-grade synovitis (Krenn synovitis score 3[13,14]; panel E). Case 2 showed aggregated CD79a-positive cells (F), CD20-positive B lymphocytes (G) generally combined with CD3-positive T cells (I) with a surrounding of CD38-positive plasma cells (H), and high-grade synovitis (synovitis score 6; panel J). In panels A to D and F to I, immunohistochemical stains with DAB as chromogen (brown); in panels E and J, hematoxylin-eosin stains; original magnification ×400.

Table 2. Synovial infiltration of B lineage cells in patients with RA and disease controls. Data are presented as median (interquartile range) and analyzed with the Mann-Whitney U test. Cell data are cells/mm^2.

Cell Lineage	OA, n = 14	p, RA vs OA	RA, n = 69	p, OrthA vs RA	OrthA, n = 15
CD79a+ cells	17.1 (13.2–209.2)	< 0.001	1092.1 (309.2–2098.7)	0.003	197.4 (13.2–686.8)
CD20+ cells	18.4 (13.2–211.2)	0.006	384.2 (52.6–797.4)	0.004	15.8 (13.2–67.1)
CD38+ cells	21.1 (21.1–44.7)	0.016	600.0 (13.2–817.1)	0.023	21.1 (21.1–50.0)
CD3+ T cells	113.2 (50.0–330.0)	0.015	578.9 (155.3–1117.1)	< 0.001	13.2 (13.2–201.3)
SD68+ macrophages	30.0 (15.3–87.5)	0.006	530.3 (103.8–855.3)	0.006	48.0 (30.0–86.0)
Vascular density (/mm^2)	107.5 (96.5–129.6)	0.03	151.3 (134.9–177.0)	0.015	86.0 (64.8–103.5)
Synovitis score (range 0–9)	3 (2–3)	< 0.001	4 (3–4.5)	< 0.001	2 (1–3)

OA: osteoarthritis; OrthA: orthopedic arthropathies.

Table 3. Areas under the curve (AUC) and p values of synovial CD79a, CD20, and CD38 expression as diagnostic biomarkers.

	AUC	p	95% CI	Tradeoff Value	Youden Index	Sensitivity, %	Specificity, %
RA vs OA							
CD79a	0.790	0.001	0.661–0.919	316.0	0.552	69.5	85.7
CD20	0.688	0.027	0.533–0.844	63.2	0.439	72.5	71.4
CD38	0.698	0.021	0.587–0.808	157.6	0.555	62.7	92.9
Combination of CD79a, CD20, and CD38	0.838	< 0.001	0.747–0.929	0.656*	0.657	72.8	92.9
RA vs OrthA							
CD79a	0.753	0.003	0.633–0.872	522.4	0.527	59.3	93.3
CD20	0.734	0.005	0.597–0.870	67.2	0.510	71.0	80.0
CD38	0.687	0.024	0.578–0.796	326.4	0.522	52.2	100
Combination of CD79a, CD20, and CD38	0.765	0.002	0.661–0.869	0.923**	0.559	56	100

* Predictive probability was calculated through the logistic regression formula $p = 1/[1+e^{-(0.412-0.003*CD20+0.003*CD38+0.002*CD79a)}]$. If the predictive probability was > 0.656, the sensitivity of diagnosing RA was 72.8% and the specificity was 92.9%. ** Predictive probability was calculated through the logistic regression formula $p = 1/[1+e^{-(0.265-0.0001*CD20+0.03*CD38+0.0001*CD79a)}]$. If the predictive probability was > 0.923, the sensitivity of diagnosing RA was 56% and the specificity was 100%. OrthA: orthopedic arthropathies.

46; median 1315.8 cells/mm^2, IQR 586.8 to 2313.2 cells/mm^2) than in those with early RA (disease duration ≤ 2 years, n = 23; median 1028.9 cells/mm^2, IQR 13.2 to 1192.1 cells/mm^2; p = 0.045). Expression of CD20-positive or CD38-positive cells did not differ between these 2 groups. In patients with longstanding RA, synovial CD79a-positive cell density correlated positively with the total Sharp score (r = 0.544, p = 0.003), the erosion subscore (r = 0.550, p = 0.003), and the joint space narrowing subscore (r = 0.516, p = 0.006).

DISCUSSION

Using immunohistochemical analysis of the B cell markers CD79a, CD20, and CD38 in synovial tissue samples and quantitative analysis of positive-staining cells, we found a higher synovial CD79a-positive cell density in RA than in OA or OrthA and its significant positive correlation with synovitis score, T cell density, and radiographic erosion as well as joint space narrowing subscores in RA.

Transcription of CD79 protein can be identified in the majority of B cells, which begins at the earliest pro-B stage of development and continues throughout B cell differentiation to the plasma cell stage[20]. CD79 encompasses 2 transmembrane components: CD79a and CD79b. Although the cellular distribution of human CD79b in normal lymphoid tissue is

Table 4. Correlations between synovial B lineage cells and measures of synovial or systemic inflammation and joint destruction in 69 patients with RA. Data expressed as r values (correlation coefficient0 according to Spearman's rank order correlation test.

Measure	CD79a+ Cell	CD20+ Cell	Cd38+ Cell
Synovial inflammation			
Synovitis score	0.714***	0.701***	0.524***
Lining layer			
Hyperplasia	0.334*	0.301*	0.278*
Lining CD68+ cells	0.054	0.230	0.280*
Sublining area			
CD3+ T cell	0.517***	0.588***	0.705***
CD68+ macrophage	0.239	0.355*	0.387*
Vascular density	0.344	0.197	0.311
Disease activity			
DAS28	–0.024	0.030	0.129
28-joint tender joint count	–0.180	–0.105	–0.011
28-joint swollen joint count	–0.139	–0.109	–0.046
Duration morning stiffness	–0.142	–0.029	–0.121
Hand grip strength	–0.230	–0.040	–0.051
HAQ score	0.079	0.005	0.009
ESR	0.251	0.268*	0.365**
CRP	0.281*	0.203	0.392**
Rheumatoid factor	0.006	0.106	0.155
ACPA	–0.118	0.162	0.274
Joint destruction			
Total Sharp score	0.490***	0.205	0.107
Erosion subscore	0.545***	0.257	0.101
Joint space narrowing subscore	0.468**	0.220	0.159

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. DAS28: Disease Activity Score 28-joint assessment; HAQ: Health Assessment Questionnaire; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ACPA: anticitrullinated protein antibody.

similar to that of CD79a, the latter is expressed on CD79b-negative plasma cells and appears before CD79b in B cell development[20]. Based on extensive data, CD79a is a B cell-specific antigen that is expressed in all stages of B cell maturation, making it one of the most diagnostically sensitive and specific pan-B cell markers for routine immunophenotypic analysis. A previous ROC analysis showed that synovial CD20-positive and CD38-positive cells differentiated RA from OA with a high area under the curve[17]. The present ROC analysis supports those results, and shows that these markers also differentiate RA from OrthA. Further, our study showed that CD79a distinguished RA from OA or OrthA better than CD20 and CD38 did, likely due to its higher accuracy for the detection of B lineage cells in synovium.

Consistent with previous studies, which showed frequencies of 44%–90%[2], 45% of the aggregate or follicular synovitis specimens in our study possessed considerable B cell infiltration. As T/B cell aggregates enlarge progressively, they may acquire features similar to secondary lymphoid organs, a process known as lymphoid neogenesis[2]. In our study, 10% of the follicular synovitis specimens displayed histologic features of germinal centers (GC) with CD21-positive follicular dendritic cells. We also found that the DAS28 was significantly higher in patients with GC-positive synovitis, and that the rates of disease remission in patients with diffuse and aggregate synovitis were 85.7% and 40%, respectively, while none of patients with GC-positive synovitis achieved disease remission after 3 months' therapy (data not shown). B cell depletion results in the disruption of lymphocyte aggregates[4,5], supporting the notion that B cells play a key role in

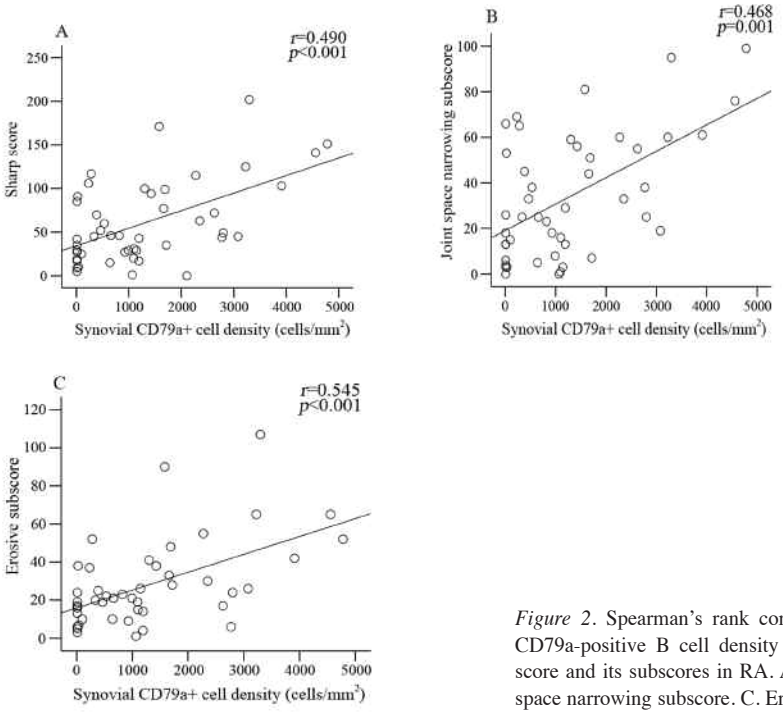

Figure 2. Spearman's rank correlation analysis of synovial CD79a-positive B cell density with the radiographic Sharp score and its subscores in RA. A. Total Sharp score. B. Joint space narrowing subscore. C. Erosive subscore.

the formation of lymphoid neogenesis, an ideal milieu for B cell-T cell contacts and local interactions. The synovitis score correlated positively with synovial B lineage cell densities including CD79a, suggesting that synovial B lineage cells may be indicators of histologic disease activity in RA.

Joint destruction is the most important pathogenic process leading to disability in RA, underscoring the need to identify synovial factors that mediate this process. For technical reasons, studies always use serial synovial samples from knee joints, although the available scores of radiographic joint destruction evaluate the small joints. It has been proved that features of synovial inflammation in paired synovial biopsy samples from inflamed knee joints and inflamed wrist or metacarpophalangeal joints in the same patient with RA were comparable, particularly with regard to sublining cellularity[21]. An early synovial biopsy study of 12 patients with RA suggested that the intensity of synovial CD68-positive macrophage infiltration at baseline was associated with a progressive Larsen radiological score[22]. Subsequent results demonstrated an association between the number of both sublining T cells and fibroblast-like synoviocytes and deterioration in the Larsen score in 36 patients with early RA[23]. Differences in patient characteristics and the methods of determining joint destruction may explain this discrepancy. Compared to the Larsen score, the Sharp score provides a higher level of detail and appears to be more sensitive in early disease, possibly because it provides separate scores for joint erosion and joint space narrowing[24]. Our study is, to our knowledge, the first to attempt to identify the relationship between synovial indices and radiographic joint destruction in a relatively large cohort of patients. We demonstrated a significantly positive correlation of synovial CD79a-positive B cells with erosion and joint space narrowing Sharp subscores. Additionally, we found that synovial CD79a-positive cell density was higher in patients with longstanding RA than in those with early RA (disease duration ≤ 2 yrs), suggesting that synovial CD79a-positive cells increased with disease duration. Since joint destruction also increases with disease duration, this subgroup analysis may indicate that synovial CD79a-positive cells are predictors of joint destruction. Interestingly, we also found that the correlation coefficient (r value) between synovial CD79a-positive B cells and erosion or joint space narrowing Sharp subscores seemed slightly higher in patients with longstanding RA than in all patients with RA. However, radiographic progression at followup should be determined to further identify whether synovial CD79a-positive B cell infiltration predicts joint destruction in RA.

CD79a protein is the signal transduction portion of B cell receptor (BCR), which has important signaling functions to influence antigen internalization and increase the efficiency of antigen presentation[25]. Expression of the BCR on developing B lymphocytes could not take place without the CD79a components. Ablations or mutations of the genes encoding CD79a result in developmental arrest at the pre-B cell stage and agammaglobulinemia[26]. CD79a+, CD20–, and CD138– plasma cells formed a distinct subset of plasma cells; as previously observed, these plasma cells could play a more pivotal role in disease activity than CD138-positive plasma cells in patients with RA, before and after RTX treatment[6]. Another histologic observation showed that synovial inflammatory tissue could break the adjacent cortical bone barrier in joints from patients with RA and result in formation of CD79a-positive B cell-rich aggregates in subcortical bone marrow, which mediated the process of cartilage resorption and local bone erosions[27]. Our study showed a significantly positive correlation of synovial CD79a-positive cells, not CD20-positive mature B cells or CD38-positive plasma cells, with the Sharp score, which indicated the synovial load with CD79a-positive cells may mediate the process of joint destruction in RA.

Our results indicate that synovial CD79a-positive B cells may be helpful biomarkers for histologic disease activity in RA and may be involved in the pathogenesis of joint destruction in RA. Anti-CD79 antibodies have been proven to be effective as immunosuppressive agents in an animal model of autoimmune disease[28]. It is worthy of further study using anti-CD79 antibodies or siRNA silencing to investigate the exact mechanism of how CD79a-positive cells affect local inflammation and joint destruction in RA.

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