

# Differential Antigen-presenting B Cell Phenotypes from Synovial Microenvironment of Patients with Rheumatoid and Psoriatic Arthritis

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**ABSTRACT. Objective.** To study the qualitative and quantitative phenotypic changes that occur in molecules involved in antigen presentation and costimulation in synovial B cells from rheumatoid arthritis (RA) and psoriatic arthritis (PsA).

**Methods.** The presence of HLA-DR, CD86, and CD40 in CD20+ cells was studied in RA synovium biopsies using immunohistochemistry and immunofluorescence. Expression was assessed by flow cytometry of the Class II molecules CD40, CD86, CD23, and CD27 on B cells from the synovial fluid (SF), with respect to peripheral blood, from 13 patients with RA and 15 patients with PsA. Expression of interferon-induced protein with tetratricopeptide repeats 4 (IFIT4) in immune-selected CD20+ cells from patients with RA was assessed by quantitative realtime PCR.

**Results.** Infiltrating synovial RA, B cells expressed HLA-DR, CD40, and CD86. Increased expression of CD86, HLA-DR, and HLA-DQ in B cells from SF was found in patients with RA and PsA. HLA-DP was also elevated in rheumatoid SF B cells; conversely, a significantly lower expression was observed in SF from patients with PsA. CD40 expression was increased in SF B cells from PsA, but not in patients with RA. Interestingly, CD20 surface expression level was significantly lower in SF B cells (CD19+, CD138-) from RA, but not in patients with PsA. CD27 upregulation and CD23 downregulation were observed in synovial B cells in both pathologies. Finally, a 4-fold increase in IFIT4 mRNA content was shown in B cells from SF in patients with RA.

**Conclusion.** Synovial B cells from patients with RA and patients with PsA express different antigen-presenting cell phenotypes, suggesting that this cell type plays a dissimilar role in the pathogenesis of each disease. (J Rheumatol First Release July 15 2015; doi:10.3899/jrheum.141577)

## Key Indexing Terms:

B LYMPHOCYTES  
PSORIATIC ARTHRITIS

RHEUMATOID ARTHRITIS  
ANTIGEN PRESENTATION

The depletion of B cells by rituximab (RTX), an anti-CD20 monoclonal antibody (mAb), has shown an unexpected positive clinical response in patients with rheumatoid arthritis (RA)<sup>1,2</sup>. This finding, in tandem with the arthritogenic

properties showed by antibodies against glucose-6-phosphate isomerase in an animal model of arthritis<sup>3</sup>, has revitalized interest in the contribution that B cells make to the pathogenesis of rheumatoid synovitis. Although it is tempting to

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think that the same biological therapy could also show benefits in psoriatic arthritis (PsA), RTX has achieved only modest improvements in musculoskeletal symptoms<sup>4,5</sup>. This, in addition to other evidence<sup>6,7,8,9</sup>, including the lack of detectable autoantibodies or the unusual presence of follicular aggregates in PsA, suggests that B cells do not play an equal role in the pathogenesis of RA and PsA.

Currently, the role that B cells play in the pathogenesis of arthritis is becoming better understood. Three potential mechanisms of action for B cells have been proposed<sup>10,11</sup>: (1) the formation of immune complexes<sup>12</sup>; (2) the local production of proinflammatory soluble factors, including tumor necrosis factor (TNF)- $\alpha$  or interleukin 6<sup>13</sup>; and (3) the regulation of T cell functions locally in the synovium<sup>14</sup> to thereby act as antigen-presenting cells (APC)<sup>15</sup>. Which one of these potential pathogenic B cell mechanisms is the most relevant? Or are there other mechanisms through which B cells participate in the pathogenesis of arthritis? These are the remaining unanswered questions.

T cell receptor recognizes antigenic peptides in the APC surface only when they are bound to appropriate Class II molecules of the MHC. However, to become effector cells, T cells must receive a second signal from the specific surface protein expressed by the APC, a process called costimulation. CD86 and CD40 are 2 surface receptors present on APC responsible for the costimulatory signals needed for T cell activation and survival. The role of B cells in presenting antigen to naive T cells has been explored in different experimental models<sup>16,17,18</sup>. In 2 murine models of arthritis (proteoglycan-induced arthritis and collagen-induced arthritis), data suggest that B cells are involved in the pathogenesis of arthritis by producing autoantibodies and by acting as APC<sup>19,20</sup>. In human RA, this has not been demonstrated.

The aim of this work was to study the phenotypic changes within a profile of molecules involved in the antigen presentation that occurs in B cells present in the synovial microenvironment of patients with RA and PsA. Our results show that synovial B cells of patients with RA develop phenotypic changes that allow them to act as APC. In patients with PsA, however, synovial B cells show a different phenotype of molecules involved in antigen presentation and costimulation with respect to RA. This suggests that B cells do not play the same role in the pathogenesis of both diseases, which might explain, at least in part, the dissimilar clinical responses shown by patients with RA and PsA to B cell-depletion therapy.

## MATERIALS AND METHODS

**Patients.** Samples of peripheral blood (PB) and synovial fluid (SF) were obtained from 13 patients with clinically active RA and 15 with PsA (28-joint Disease Activity Score–erythrocyte sedimentation rate, mean  $\pm$  SD 4.31  $\pm$  0.68 and 4.70  $\pm$  0.55, respectively). All patients met the American College of Rheumatology criteria for RA<sup>21</sup> and the CLASSification for Psoriatic ARthritis criteria for PsA<sup>22</sup>. Table 1 shows the clinical characteristics of patients included in our study. All patients gave informed consent, and the

study was approved by the Ethics Committee of the Hospital Universitario de Canarias (Tenerife, Spain).

**Immunofluorescence and immunohistochemistry.** Synovial tissues were obtained by arthroscopic biopsy from active knee arthritis of patients with RA (4 women and 2 men). All patients were positive for rheumatoid factor (RF) and anticitrullinated peptide antibodies, with disease evolution ranging between 6 months and 3 years, and all biologics-naive. Sections were deparaffinized, rehydrated, and heated in 1 mM EDTA, pH 8 for antigen retrieval. The slides were incubated at 4°C overnight with mouse anti-human HLA-DR (clone TAL 1B5) or CD86 (clone D-6) mAb purchased from Santa Cruz Biotechnology Inc., followed by incubation with Alexa Fluor 594 goat anti-mouse IgG1 secondary antibodies for 1 h at room temperature (Molecular Probes, Invitrogen). Double-labeling of B cells was performed by sequential incubation with an anti-CD20-cy (L26 clone, Dako) mAb for 1 h at room temperature, followed by Alexa Fluor 488 goat anti-mouse IgG2a secondary antibodies (Molecular Probes).

Immunolabeling with mouse anti-human CD40 mAb (clone 2Q1331; Santa Cruz Biotechnology Inc.) was performed on frozen RA tissues, followed by labeling with avidin-biotin immunoperoxidase secondary reagents (Vector Laboratories). Color was developed with diaminobenzidine chromogen. Double-labeling of CD40 immunoperoxidase-labeled sections was performed by further anti-CD20-cy immunofluorescent labeling as indicated above.

Tissues were sequentially photographed on a Zeiss LSM 510 Meta confocal microscope (Zeiss).

**Cell isolation and culture.** Mononuclear cells were isolated from heparinized samples of PB and SF simultaneously obtained from patients with active RA or PsA by Biocoll density-gradient centrifugation (Biochrom AG). After washing in phosphate buffer, cells from the mononuclear band were analyzed by multicolor flow cytometry using CD20 or CD19 as B cell markers, as described below. In some samples of PB and SF from patients with RA, the B cell populations were isolated using negative immunoselection (StemCell Technologies), yielding a purity > 95%, ascertained by the positivity to CD19, in flow cytometry analysis.

**Flow cytometry analysis.** Mononuclear cells isolated from PB and SF from patients with RA and PsA were simultaneously labeled with 2 directly conjugated mAbs at 4°C for 30 min. One of these was phycoerythrin (PE)-conjugated anti-human CD20 mAb (clone LT20; Miltenyi Biotec GmbH), to select the B cell subset, and the other either a fluorescein isothiocyanate-conjugated anti-human mAb against CD40 (clone 5C3; Miltenyi Biotec), CD86 (clone FUN-1), CD27 (clone 9F4; Immunotools), HLA-DR (clone L243), HLA-DQ (clone SK10; all from BD Biosciences), HLA-DP (clone DP 11.1; Santa Cruz Biotechnology), or with the allophycocyanin-conjugated anti-human CD23 (Immunotools GmbH).

The surface expression of both CD20 and CD40 in B cells from SF and PB was assessed by flow cytometry analysis using triple-color staining. The surface expression of CD20 was analyzed in CD19-PE+ (clone LT19), and CD138-allophycocyanin(-), clone B-B4 (both from Miltenyi Biotec GmbH) mononuclear cells. Similarly, the surface expression of the costimulatory molecule CD40 was studied in CD20(+), CD138(-) mononuclear cells. The fluorescence levels of isotype-matching antibodies (Immunotools) were used as controls.

After washing in phosphate buffered saline, at least  $1 \times 10^4$  lymphocytes from each sample were analyzed using an Accuri C6 instrument (BD Biosciences), and the data were analyzed using BD Accuri C6 Software.

Because fluorescence conditions varied between experiments, data were normalized to express the relative mean fluorescence intensity (rMFI), according to the following equation:

$$\text{rMFI} = (\text{MFI}_{\text{SF}} - \text{MFI}_{\text{isotype control}}) \div (\text{MFI}_{\text{PB}} - \text{MFI}_{\text{isotype control}}) \times 100$$

**Quantitative real-time PCR (qRT-PCR).** For quantitative analysis of gene expression, the total RNA of B cells from PB and SF was isolated using Tripure Isolation reagent (Roche Diagnostics), followed by treatment with

Table 1. Characteristics of patients included.

Patient No.	Sex	Age	Diagnosis	RF	ACPA	Time of Evolution, Yrs	Treatment	CC Dose
1	F	67	RA	Pos	Pos	10	MTX	0
2	F	58	RA	Pos	Pos	8	MTX + PRED	15 mg/day
3	F	58	RA	Pos	Pos	13	NSAID	0
4	M	47	RA	Pos	Pos	10	MTX	0
5	M	47	RA	Pos	Pos	5	MTX	0
6	F	82	RA	Pos	ND	12	LEF + PRED	10 mg/day
7	F	43	RA	Neg	Neg	4	MTX	0
8	F	55	RA	Neg	Neg	2	NSAID	0
9	F	42	RA	Neg	ND	9	MTX + PRED	5 mg/day
10	F	42	RA	Neg	ND	12	MTX + PRED	7.5 mg/day
11	F	43	RA	Neg	Neg	3	Anti-TNF	0
12	F	78	RA	Neg	Neg	1	Anti-TNF	0
13	F	45	RA	Neg	Neg	8	MTX + LEF + PRED	7.5 mg/day
14	F	43	PsA	Neg	Neg	3	MTX	0
15	F	20	PsA	Neg	Neg	3	MTX	0
16	M	44	PsA	Neg	Neg	4	MTX + NSAID	0
17	M	54	PsA	Neg	Neg	3	Anti-TNF	0
18	M	48	PsA	Neg	Neg	< 1	None	0
19	M	70	PsA	Neg	Neg	6	MTX	0
20	F	44	PsA	Neg	Neg	1	MTX + NSAID	0
21	F	52	PsA	Neg	ND	4	None	0
22	M	41	PsA	Neg	ND	10	MTX	0
23	M	61	PsA	Neg	ND	13	MTX + NSAID	0
24	M	42	PsA	Neg	ND	6	NSAID	0
25	F	52	PsA	Neg	ND	4	NSAID	0
26	M	27	PsA	Neg	ND	1	MTX + NSAID	0
27	M	41	PsA	Neg	ND	1	NSAID	0
28	M	31	PsA	Neg	ND	7	Anti-TNF	0

RA: rheumatoid arthritis; ACPA: anticitrullinated protein antibodies; CC: corticosteroids; Pos: positive; Neg: negative; ND: not determined; MTX: methotrexate; NSAID: nonsteroidal antiinflammatory drug; PRED: prednisone; LEF: leflunomide; anti-TNF: anti-tumor necrosis factor; RF: rheumatoid factor; PsA: psoriatic arthritis.

chloroform and isopropanol before washing with ethanol 70% and absolute ethanol. cDNA was synthesized by a SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen), while fluorescence RT-PCR was performed by using SYBR Green (Bio-Rad Laboratories) in an MJ Research Opticon Engine (Bio-Rad). The following primers were used: interferon (IFN)-induced protein with tetratricopeptide repeats 4 (IFIT4) forward: 5'-AAC TAC GCC TGG GTC TAC TAT CAC TT-3', IFIT4 reverse: 5'-GCC CTT TCA TTT CTT CCA CAC-3', B2 microglobulin (B2MG2) forward: 5'-GCA GCA TCA TGG AGG TTT GAA-3', and B2MG2 reverse: 5'-CAT GGA GAC AGC ACT CAA AGT AGA A-3'. PCR was started with 1 cycle of 95°C for 10 min, followed by 40 cycles with denaturing at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s.

B2MG2 was co-amplified as an internal control to normalize the amount of IFIT4 mRNA. The RT-PCR data were quantified using the relative quantification ( $2^{-\Delta\Delta CT}$ ) method<sup>23</sup>. All data were analyzed using Opticon Monitor Analysis Software (Bio-Rad).

**Statistical analysis.** Differences between groups were analyzed for statistical significance using Wilcoxon signed-rank test for paired samples. P values < 0.05 were considered significant. Results are expressed as the arithmetic mean  $\pm$  SE of the mean.

## RESULTS

*Rheumatoid synovial membrane infiltrating CD20+ cells express HLA-DR and molecules involved in costimulation.* It has been suggested that B cells, in addition to their role as antibody-secreting cells, might act as APC and participate in

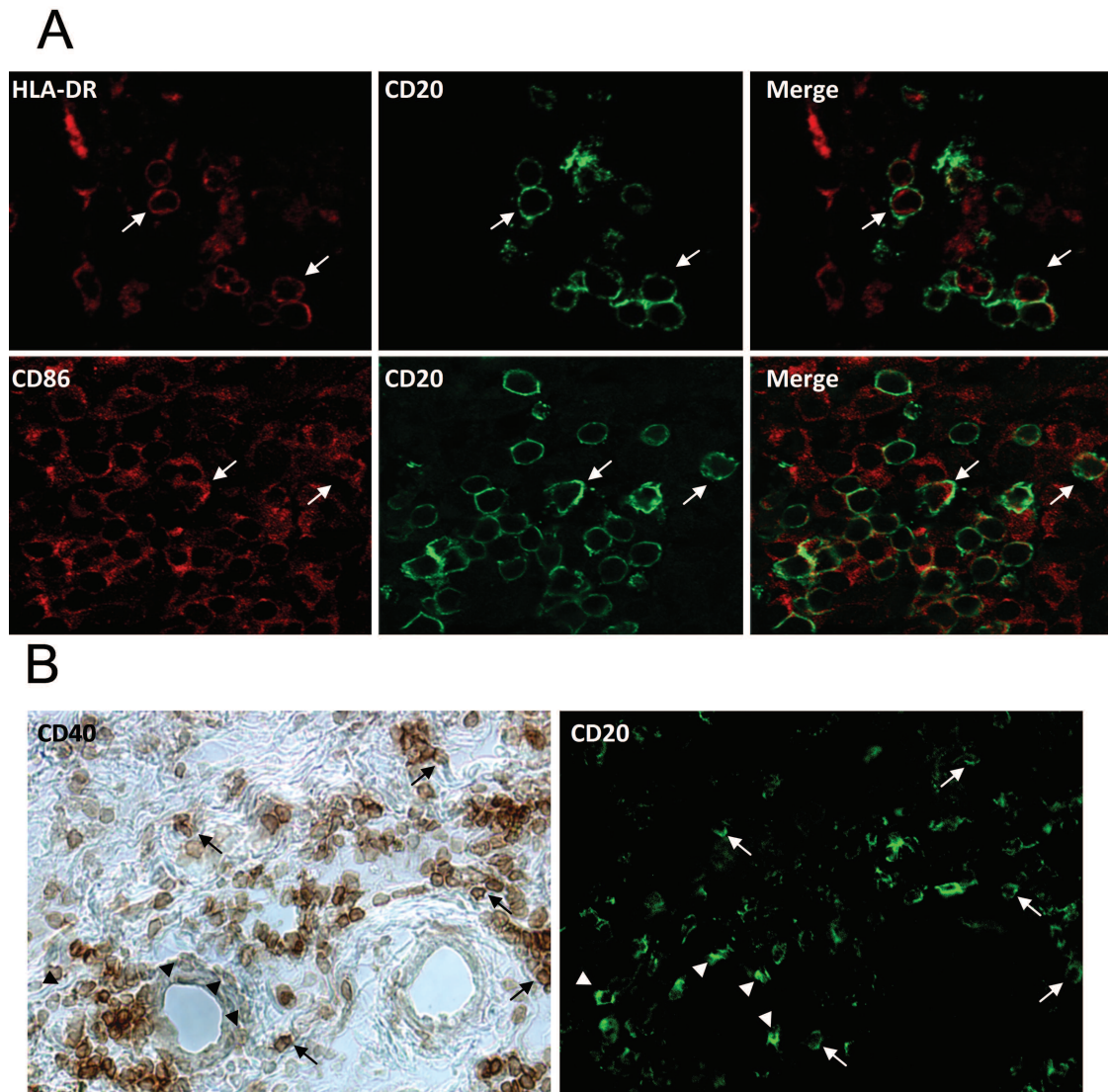
the pathogenesis of several autoimmune diseases<sup>15,24</sup>. However, the role of B cell as APC in human RA is still not fully clarified. To investigate this issue, we first studied the phenotype of B cells present in the synovial membrane infiltrate of patients with RA by immunofluorescence and immunohistochemistry techniques. We observed that CD20+ cells in synovial membrane samples from patients with RA showed a partial colocalization with both HLA-DR (Figure 1A) and the costimulatory molecules CD86 and CD40 (Figure 1A and 1B, respectively). Moreover, all of the molecules studied presented a relatively homogeneous surface-staining pattern (Figure 1).

These results show that a fraction of the infiltrating B cells of the synovial membrane from patients with RA expressed both MHC Class II molecules and costimulatory molecules, both of which are crucial to the process of antigen presentation.

B cells from SF of patients with RA and PsA differentially express molecules involved in antigen presentation and costimulation.

To obtain quantitative data, we analyzed by multicolor flow cytometry the surface expression levels of MHC Class II, CD86, and CD40 in CD20+ cells from PB and SF of patients with RA and PsA. The percentages of CD20+ cells

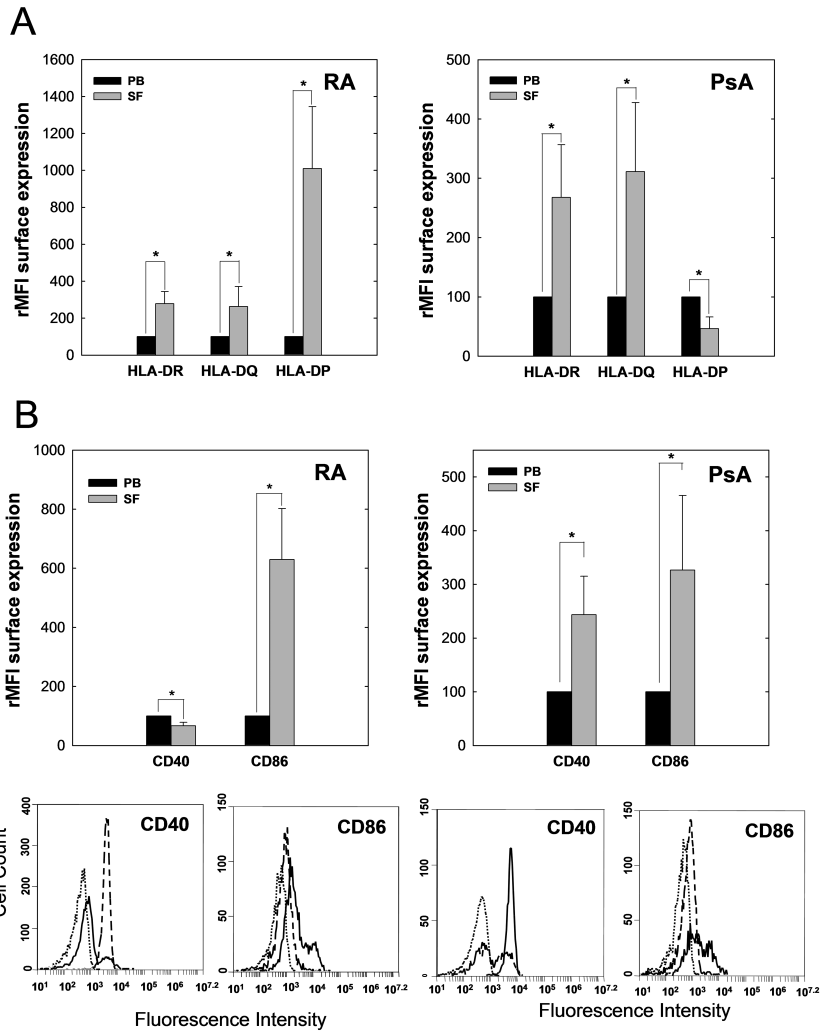




**Figure 1.** Synovium-infiltrated CD20+ B cells from patients with RA express HLA-DR and the costimulatory molecules, CD86 and CD40. **A.** RA synovial tissues were deparaffinized, rehydrated, and heated in EDTA for antigen retrieval and stained with HLA-DR or CD86 mAb, followed by incubation with Alexa Fluor 594 secondary antibodies (right panels). Double-labeling of B cells was performed by sequential incubation with an anti-CD20-cy, followed by Alexa Fluor 488 secondary antibodies (middle panels) and examination by confocal microscopy. **B.** Frozen RA tissues were immunolabeled with CD40 mAb, followed by avidin-biotin immunoperoxidase secondary reagents. Color was developed with diaminobenzidine chromogen (right panel). Double-labeling of CD40 immunoperoxidase-labeled sections was performed by further anti-CD20-cy immunofluorescence (left panel). Antigen colocalization is indicated by white arrows. Confocal microscopy images presented are representative examples of 6 independent synovial biopsies from patients with RA. Original magnification  $\times 400$ . RA: rheumatoid arthritis; mAb: monoclonal antibody.

in both compartments were similar in range and tendency in both pathologies (RA  $8.53 \pm 1.75$  vs  $1.60 \pm 0.41$   $p < 0.01$  and PsA  $8.66 \pm 1.92$  vs  $2.87 \pm 0.57$ ,  $p < 0.01$ ). Using double-staining flow cytometry analysis, SF CD20+ cells showed a significantly higher expression of HLA-DR and HLA-DQ receptors compared with PB in both diseases (Figure 2A). HLA-DP expression was also increased in SF B cells from patients with RA (independently of the presence or absence of RF; Appendix 1), but in patients with PsA, a signif-

icantly lower expression of this MHC Class II molecule was observed in SF compared with PB (Figure 2A). The surface-expression level of the costimulatory molecule CD86 was significantly higher in CD20+ cells from SF compared with those from PB in both the RA and PsA groups (Figure 2B). With regard to CD40 surface expression, in patients with RA, CD20+ cells from SF showed a lower expression compared with PB. However, in B cells from patients with PsA, CD40 expression levels were higher in SF than in PB (Figure 2B).



**Figure 2.** CD20+ B cells from PB and SF of patients with RA and PsA show a differential surface expression of HLA Class II and CD40. Mononuclear cells from PB and SF of patients with RA and PsA were isolated, double-stained, and analyzed by flow cytometry. **A.** Surface expression levels of HLA-DR, HLA-DQ, and HLA-DP in CD20+ cells from PB and SF in patients with RA and PsA. Data represent the mean  $\pm$  SD of rMFI in SF compared to PB cells (considered 100%), from 5 independent experiments (patients with RA and PsA). **B.** Surface expression of CD40 and CD86 in CD20+ cells from PB and SF in patients with RA and PsA. Data represent the mean  $\pm$  SD from 5 independent experiments (patients with RA and PsA) of rMFI compared with cells from PB (considered 100%). Representative flow cytometry histograms of surface expression of CD40 and CD86 in CD20+ cells from RA (left) and PsA (right) are shown. Dotted histogram represents the isotype-matching control, and dashed and closed histograms represent the expression in B cells from PB and SF, respectively. \*  $p < 0.05$  by Wilcoxon signed-rank test. PB: peripheral blood; SF: synovial fluid; RA: rheumatoid arthritis; PsA: psoriatic arthritis; rMFI: relative mean fluorescence intensity.

Taken together, the data show that B cells present in the synovial microenvironment of patients with RA and PsA undergo differential changes in the phenotype of the surface proteins involved in antigen presentation.

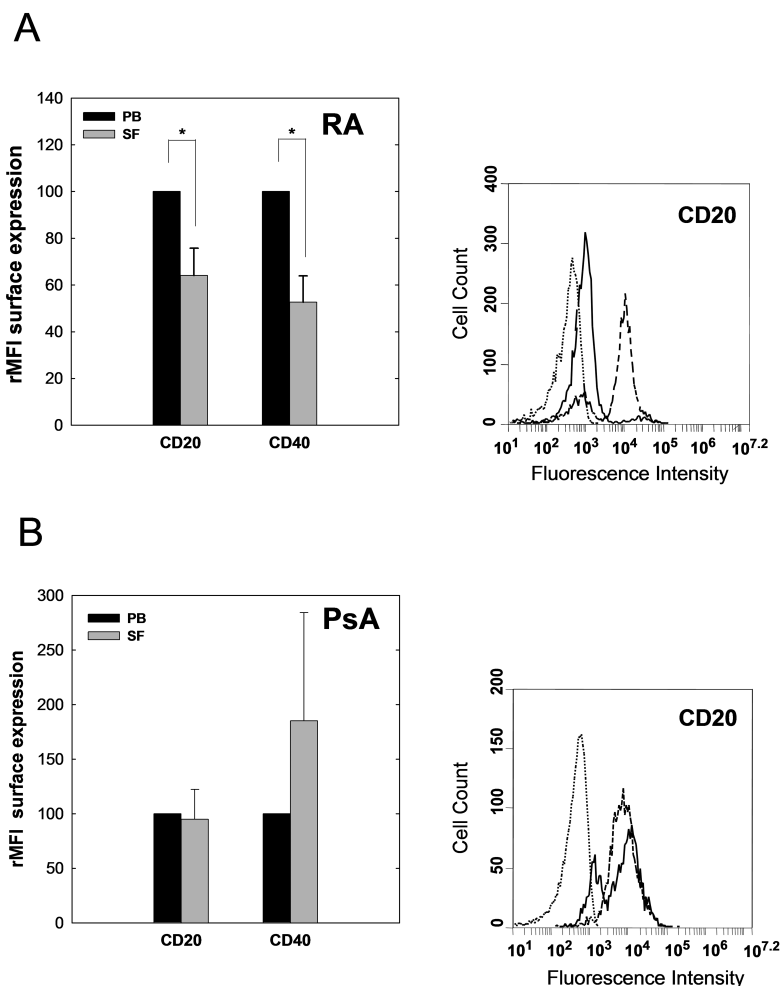
*B cells present in the synovial microenvironment of patients with RA downregulate CD20 and CD40 expression.* The foregoing results showed that B cells from SF of patients with RA had a reduced surface expression of CD40, a molecule physically and functionally related to CD20<sup>25</sup>.

Interestingly, it has been shown that CD40 engagement by its counter-receptor, CD40L in normal B lymphocytes, a process that happens during antigen presentation, causes the downregulation by internalization of both CD40 and CD20<sup>26</sup>. To determine whether CD40 downregulation in B cells was associated with changes in CD20 expression, the surface expression of both molecules was assessed in mononuclear cells isolated from PB and SF of patients with RA using triple-staining flow cytometry analysis. Interestingly, CD20

expression was significantly lower in SF than in PB B cells (CD19+, CD138-) from patients with RA (Figure 3A). When CD40 surface expression was assessed in CD20+ and CD138- cells, B cells from SF again showed a lower surface expression of this costimulatory molecule than those from PB. When RF-positive (n = 6) and -negative (n = 7) patients with RA were analyzed separately, the SF B cells from both subgroups showed a lower expression of CD20 and CD40 with respect to PB (Appendix 1). Remarkably, in patients with PsA, the expression levels of CD20 between B cells isolated from PB and SF were not statistically different (Figure 3B).

These data show that B cells present in the synovial microenvironment of patients with RA reduce the surface expression of CD20 and CD40, which resembles what occurs *in vitro* upon CD40 activation.

*CD23 and CD27 exhibit divergent expression levels in B cells from PB and SF in patients with RA and PsA.* CD27 is a member of the TNF receptor family expressed on T and B lymphocytes. In B cells, CD27 increases its expression upon antigen contact, indicating the transition from naive to memory B cells<sup>27</sup>. CD23, a low affinity receptor for IgE, has been described as an acquired marker of activated B cells,



**Figure 3.** Downregulation of CD20 and CD40 in B cells from SF compared to PB in patients with RA. Mononuclear cells from PB and SF of patients with RA and PsA were isolated, triple-stained, and analyzed by flow cytometry. A. Graph bar showing the surface expression of CD20 and CD40 in nonplasmatic (CD138-) B cells isolated from PB and SF of patients with RA. Data represent the mean  $\pm$  SD of rMFI in SF with respect to PB cells (considered 100%) from 5 independent experiments. B. Results from the same experiments as panel A, but for patients with PsA. Data are expressed as the mean  $\pm$  SD from 4 independent experiments. Representative flow cytometry histograms of surface expression of CD20+ in CD19+, CD138- cells from RA (up) and PsA (down) are shown. Dotted histogram represents the isotype-matching control, and dashed and closed histograms the expression in B cells from PB and SF, respectively. \*  $p < 0.05$  by Wilcoxon signed-rank test. SF: synovial fluid; PB: peripheral blood; RA: rheumatoid arthritis; PsA: psoriatic arthritis; rMFI: relative mean fluorescence intensity.

and it is cleaved after antigen interactions<sup>28</sup>. Double-staining flow cytometry analysis revealed a significant decrease in CD23 surface expression levels in CD20+ B cells from SF compared with PB, while an increase of CD27 surface expression was detected in SF compared with PB in both pathologies (Figure 4).

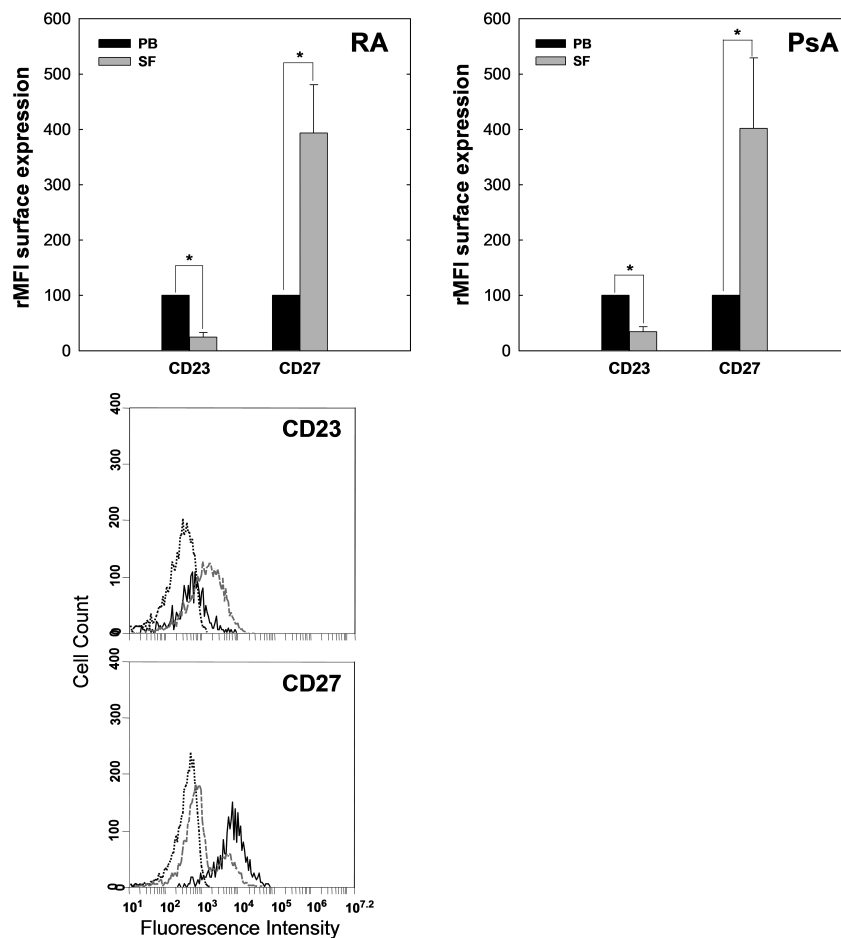
These data suggest that B cells in the SF of patients with chronic arthritis are activated and have been in contact with the antigen, 2 conditions necessary for acting as effective APC. *IFIT4* mRNA is increased in B cells from SF in patients with RA. *IFIT4* is a novel gene whose function is not fully understood. Evidence suggests that *IFIT4* might play a role in the cell differentiation into APC<sup>29</sup>. Therefore, we studied the

presence of transcripts of this molecule in negative immuno-selected CD19+ B cells isolated from PB and SF of patients with RA. Results from qRT-PCR assays, expressed as  $2^{-\Delta\Delta Ct}$ , showed a 4-fold increase in *IFIT4* mRNA content in B cells from SF compared with PB in samples from 3 patients with RA ( $p < 0.05$  by Wilcoxon signed-rank test).

This increase in *IFIT4* transcript expression in the synovial compartment strengthens the idea that B cells might act as APC in the inflammatory focus of patients with RA, thereby contributing to the pathogenesis of this complex disease.

## DISCUSSION

The most important findings of this work can be summarized as follows: (1) B cells present in the infiltrate of rheumatoid



**Figure 4.** B cells from PB and SF of patients with RA and PsA show opposite expression levels for CD23 and CD27. Graph bars show the surface expression of CD23 and CD27 in CD20+ cells isolated from PB and SF of patients with RA (left) and PsA (right). Data represent the mean  $\pm$  SD of rMFI in SF with respect to PB cells (considered 100%) from 7 independent experiments. Representative flow cytometry histograms of the surface expression of CD23 and CD27 in CD20+ cells from RA are shown. Dotted histogram represents the isotype-matching control, and dashed and closed histograms the expression in B cells from PB and SF, respectively. \* $p < 0.05$  by Wilcoxon signed-rank test. PB: peripheral blood; SF: synovial fluid; RA: rheumatoid arthritis; PsA: psoriatic arthritis; rMFI: relative mean fluorescence intensity.



synovial membrane express molecules involved in antigen presentation and costimulation; (2) B cells from the synovial microenvironment of patients with RA show phenotypic changes, suggesting that they can act as APC; and (3) in patients with PsA, synovial B cells also exhibit changes, both in MHC Class II and costimulatory molecules, but involving fewer molecules than those observed in B cells from patients with RA. These findings suggest that B cells might act as APC locally in the rheumatoid synovium, a role that apparently is less evident in PsA.

B cells show effector and regulatory functions in autoimmunity<sup>30</sup>. Results obtained by implanting samples of synovial membrane from patients with RA into immunodeficient mice have demonstrated that the presence of B cells in the synovium is necessary for T cell activation in the rheumatoid synovium<sup>14</sup>. This strongly suggests that B cells exert their pathogenic role locally into the rheumatoid synovial membrane, where, in addition to other actions, they can act as APC<sup>10,11</sup>. In our study, we have investigated the changes that occur in the surface-expression profile of molecules involved in the antigen-presentation process in synovial B cells from patients with RA and PsA. Our results revealed that B lymphocytes infiltrating the synovial membrane of patients with RA express HLA-DR, CD86, and CD40 (colocalizing partially with CD20). This is in agreement with reports contending that CD20 may exist as a supramolecular complex with CD40 and MHC Class II molecules on the surface of B cells<sup>25</sup>. However, the functional consequences of these molecular interactions remain to be elucidated.

When we analyzed comparatively the surface expression level of MHC Class II molecules in CD20+ cells from PB and SF of patients with RA by flow cytometry, SF B cells from the joints of rheumatoid patients showed a strong upregulation of HLA-DR, HLA-DQ, and HLA-DP compared with PB. This is similar to what occurs in dendritic cells, the professional APC, in which inflammatory stimuli induce a rapid accumulation of a large number of long-lived peptide-loaded MHC Class II molecules capable of stimulating T cells<sup>31</sup>. In patients with PsA, a similar result was observed, with the exception of HLA-DP, a molecule that displayed a significant reduction in SF B cell surface expression compared with PB. This might indicate that, in contrast to HLA-DR and HLA-DQ, HLA-DP isotype might not be implicated in antigen presentation by B cells in PsA. In this regard, several studies have suggested that in PsA, there is a more significant association with MHC Class I than with Class II molecules<sup>32,33</sup>. Confirmation of these findings could lead to the use of the relative expression of HLA-DP between B cells from PB and SF as a laboratory test to differentiate between patients with RA and patients with PsA during early stages of the diseases.

It has been shown that CD80 and CD86 are important in strengthening the interaction and amplification of T cell

activation, as well as in influencing B cells through bidirectional signaling<sup>34</sup>, and in collaboration with CD40, assisting the activation, proliferation, differentiation, survival, and generation of memory B cells<sup>35</sup>. Our results showed a general increase in CD86 expression in B cells from the synovial microenvironment in RA and PsA that was consistent with previous reports in juvenile idiopathic arthritis (JIA) and systemic lupus erythematosus (SLE)<sup>36,37,38</sup>. In patients with PsA, B cells from SF showed an upregulation of CD40 compared with PB that is consistent with published data showing an increase of CD40 and CD80 in B cells from patients with active SLE<sup>38</sup>. However, we observed the opposite result in patients with RA: a loss of CD40 surface expression in B cells from SF versus PB. Interestingly, despite the fact that CD20 antigen has been described as a molecule resistant to modulation<sup>39,40,41</sup> and therefore considered an ideal therapeutic target, our results revealed a decrease of CD20 expression, in tandem with CD40, on nonplasmatic (CD138-), CD19+ B cells from rheumatoid joints compared with cells from PB. However, in SF B cells from patients with PsA, this downregulation of CD20 was not observed. In the patients with RA included in our study, B cells from SF showed a downregulation in CD20 and CD40 expression with respect to PB, independently of the presence of RF. Although the number of patients was small, these data suggest that the phenotypic differences between synovial B cells from patients with RA and PsA cannot be explained by the presence or absence of RF. It is well known that CD20 expression is lost during cell differentiation into plasma cells (CD138+). In addition, it has been reported that downregulation of CD20 on B cells occurs upon CD40 activation by a mechanism involving the internalization of both molecules<sup>26</sup>. Signaling through CD40 has been shown to be relevant because studies on CD40-/- mice established that B cells fail to proliferate and undergo isotype switching<sup>42,43</sup>. Moreover, it has been shown that the signaling pathway that occurs after CD40 ligation activates genes involved in both cytokine production and upregulation of costimulatory molecules, such as CD80/CD86, MHC Class II, and other activation markers in B cells and in dendritic cells<sup>11,44</sup>. Taking all evidence into account, decrease in the global expression of CD40 and CD20, together with the presence of partial CD20/CD40 colocalization in B cells from RA joints, suggests that in the rheumatoid synovial microenvironment, the CD40 from B cells is very likely engaged by its counter-receptor, a process that happens during antigen presentation.

The expression of activation antigen CD23 is induced by the direct T cell contact *in vitro*<sup>45</sup>. Upon activation, CD23 is shed from the cell surface of B cells by members of a disintegrin and metalloproteinase domain family<sup>46</sup>. Our results showed that B cells from SF of patients with RA diminished the CD23 expression compared to PB, a finding consistent with the high concentration of the soluble form of CD23



described in SF from patients with RA<sup>47,48</sup>. We obtained a similar result when the surface expression of CD23 was assessed in SF B cells from patients with PsA. With respect to CD27, a molecule involved both in the transition from naive to memory B cells and in naive T cell activation and costimulation<sup>49</sup> showed an increased expression in SF B cells compared with PB in both patients with RA and PsA, a finding consistent with those previously described in JIA and patients with RA<sup>36,37</sup>. These data suggest that B cells in the SF of patients with chronic arthritis have been in contact with the antigen (upregulate CD27) and show an activated phenotype (downregulate CD23), 2 conditions necessary for acting as effective APC.

IFIT4 is an IFN-inducible protein expressed in immune tissues and cells. IFIT4, as the immunoglobulin-like transcript-4<sup>50</sup>, has been involved in the phenotypic changes in monocytes associated with the ability to present antigens. Previous studies have suggested that IFIT4 is involved in monocyte differentiation into dendritic cells. In fact, it has been demonstrated that IFIT4 primed DC-like cells exhibited higher expression levels of CD40, CD80, CD86, and HLA-DR, as well as an increased ability to stimulate T cell proliferation<sup>29</sup>. Our results showed that IFIT4 expression, as quantified by RT-PCR, was higher in negatively immunoselected CD19+ B cells from SF compared with those from PB in patients with RA, an indirect finding that supports the potential capacity of B cells to behave as dendritic cells in the rheumatoid synovium. However, the putative role of this IFN-induced gene in B cells remains unclear.

Because of the design of our study, an initial estimation of sample size was not obtained. Consequently, the main limitation of our study is the small sample size that precludes proper analysis of the potential effect that, for instance, medications including corticoids or time of disease evolution might have on our conclusions.

Taken together, these results support the contention that B cells present at the synovial membrane in patients with RA potentially contribute to the pathogenesis of this disease by acting as APC. In patients with PsA, synovial B cells exhibit a phenotype of the molecules involved in antigen presentation dissimilar to RA. This finding suggests that B cells play different pathogenic roles in RA and PsA. It might also shed light on the reasons for the different clinical responses of these 2 pathologies to B cell-depletion therapy.

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**APPENDIX 1.** Surface expression of molecules on B cells from SF and PB in patients with RA and PsA. Values are mean  $\pm$  SD of rMFI in SF regarding PB cells (considered 100%) from all experiments.

Surface Molecules		Patients with RA		Patients with PsA, n = 15
		RF+, n = 6	RF-, n = 7	
CD20	PB	100	100	100
	SF	60 $\pm$ 11	70 $\pm$ 5	95 $\pm$ 27
CD40	PB	100	100	100
	SF	50 $\pm$ 6	54 $\pm$ 8	172 $\pm$ 99
CD86	PB	100	100	100
	SF	569 $\pm$ 112	676 $\pm$ 117	326 $\pm$ 138
DR	PB	100	100	100
	SF	180 $\pm$ 51	307 $\pm$ 213	268 $\pm$ 88
DQ	PB	100	100	100
	SF	154 $\pm$ 19	334 $\pm$ 314	311 $\pm$ 116
DP	PB	100	100	100
	SF	824 $\pm$ 453	1447 $\pm$ 340	46 $\pm$ 19
CD23	PB	100	100	100
	SF	22 $\pm$ 10	31 $\pm$ 12	34 $\pm$ 9
CD27	PB	100	100	100
	SF	200 $\pm$ 58	529 $\pm$ 72	401 $\pm$ 127

SF: synovial fluid; PB: peripheral blood; RA: rheumatoid arthritis; PsA: psoriatic arthritis; rMFI: relative mean fluorescence intensity; RF: rheumatoid factor.