Abatacept Reduces Levels of Switched Memory B Cells, Autoantibodies, and Immunoglobulins in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Abatacept (ABA) is a chimeric molecule, able to block the CD28-mediated costimulatory pathway. To evaluate the hypothesis that, through this mechanism of action, ABA may down-modulate the immune responses of B lymphocytes in rheumatoid arthritis (RA), we investigated the serum levels of immunoglobulins (Ig), free light chains (FLC), anticitrullinated protein antibodies (ACPA), and rheumatoid factor (RF), as well as the number of B lymphocytes differentiated into post-switch memory cells in patients treated with ABA.

Methods. The serum levels of Ig, FLC, different ACPA, RF isotypes, and the B cell phenotype were longitudinally evaluated in 30 patients with RA treated with ABA.

Results. At baseline, the proportion of total and post-switch memory B cells was lower in RA than in healthy individuals. After 6 months of ABA treatment we observed significant reductions of serum levels of IgG, IgA, and IgM, as well as FLC, with a normalization in many patients who had initially abnormal values. A significant reduction of the titers of IgG- and IgA-ACPA, as well as of IgM-, IgA-, and IgG-RF was also observed. A decrease of autoantibodies below the upper limits of normal values was found in 2 of 26 patients (8%) initially seropositive for IgG-ACPA, 1 of 14 (7%) for IgA-ACPA, 5 of 22 (23%) for IgM-RF, 7 of 22 (30%) for IgA-RF, and 5 of 16 (31%) for IgG-RF. After treatment, the proportion of circulating post-switch memory B cells was also further significantly decreased.

Conclusion. ABA treatment in patients with RA can reduce signs of polyclonal B cell activation, inducing a trend toward normalization of serum levels of different classes of Ig and of FLC, decreasing titers of ACPA and RF, and percentages of post-switch memory B cells. (J Rheumatol First Release March 1 2014; doi:10.3899/jrheum.130905)

Key Indexing Terms:
ABATACEPT RHEUMATOID FACTOR FREE LIGHT CHAINS
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B cells play a central role in the pathophysiology of rheumatoid arthritis (RA)[1,2], and their hyperactivation is demonstrated by hypergammaglobulinemia and increased levels of serum free light chains (FLC)[3]. B cells can also produce rheumatoid factor (RF) and other autoantibodies, including anticitrullinated protein antibodies (ACPA), whose presence is associated with a more severe disease[1]. The ability to produce different classes of immunoglobulins (Ig), including autoantibodies, is acquired after antigen presentation in lymphoid tissue with the aid of T lymphocytes, by a fraction of B cells that have undergone somatic hypermutation. These cells can generally be identified phenotypically as post-switch memory cells (CD19+CD27+IgD–)[4], although a smaller CD27– population of memory B cells with mutated Ig genes has also been described[5]. Post-switch memory B cells have been shown to accumulate in the synovial compartments of patients with RA[6,7], and this underlines their relevance in the autoimmune/inflammatory process of RA.

B cells expressing the costimulatory molecules CD80 and CD86 can also act as antigen-presenting cells and therefore activate T cells providing signals to the CD28 receptor[1,2]. The relevance of this pathway as a therapeutic target in RA has been demonstrated by clinical results obtained with abatacept (ABA), a cytotoxic T lymphocyte-associated antigen 4 immunoglobulin (CTLA-4-Ig)

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fusion protein[8]. Through its CTLA4 portion, this agent can bind to CD80 and CD86 on B cells, thereby inhibiting CD28 costimulation[9]. CD28-mediated signals are relevant in the upregulation of CD154 (the ligand for CD40) on T cell surface, a key process in the acquisition of the T cell "helper" function[10]. The engagement of CD40 (constitutively present on B cell membrane) by CD154 plays a crucial role in the process of isotype switching and B cell maturation[10]. It can therefore be hypothesized that, blocking this pathway, ABA may downmodulate the immune responses of B lymphocytes and the production of autoantibodies[9]. However, not much information is currently available on the effect of ABA therapy on B cells and autoantibody levels in patients with RA[9].

The aim of our study was to evaluate whether the blockade of costimulation performed by ABA may reduce the ability of B lymphocytes to differentiate into post-switch memory B cells and to produce ACPA and RF. The levels of total serum Ig and of FLC were also evaluated.

MATERIAL AND METHODS

Patients. Thirty consecutive patients with RA treated for at least 6 consecutive months with ABA were enrolled in our study. Their main clinical and demographic characteristics are shown in Table 1. Four patients had been previously treated with the anti-B cell agent rituximab (RTX). Median time from the last RTX infusion at the moment in which ABA was started was 14.5 months (range 8–68). No patients had renal failure or monoclonal gammopathies. The clinical disease activity and the response to the treatment were evaluated respectively with the DAS28 (based on CRP) and the European League Against Rheumatism (EULAR) criteria of response to the treatment[11].

The local ethics committee approved our study, and all patients provided informed consent.

Twenty-four blood donors [18 women (75%); median age 39 yrs (25th–75th percentile: 34–46)] served as healthy controls (HC).

Serum analysis. Serum samples were collected and stored at –80°C immediately before the first administration of ABA (T0) and then after 6 months (T6). In 16 patients, a further sample was collected after 12 months (T12). Testing for the different assays was carried out simultaneously on all serum samples at the end of the study.

Table 1. Demographic features of patients with rheumatoid arthritis (n = 30). Data are expressed as the median (25th–75th percentile) and range unless otherwise indicated.

Sex (male/female), n	4/26
Age, yrs	53 (44–60)
Disease duration, yrs	6.5 (2.25–11.75)
Smokers, n (%)	13 (43)
No. previous DMARD	3 (1–5)
No. previous biological agents	2 (0–3)
TNF-α blocking agents	24
Rituximab, no. patients	4
Tocilizumab, no. patients	4
Anakinra, no. patients	6
ABA as firstline biological treatment	5
Concomitant use of methotrexate, n (%)	24 (80)
Median dosage of methotrexate at baseline	12.5 (5.62–15)
DAS28-CRP at baseline	5.12 (4.71–5.93)
Serum creatinine (mg/dl)	0.70 (0.64–0.72)

DMARD: disease-modifying antirheumatic drug; TNF: tumor necrosis factor; ABA: abatacept; DAS28-CRP: 28-joint Disease Activity Score based on C-reactive protein.

Serum IgG, IgA, and IgM levels were measured by a nephelometric immunoassay method (Siemens Healthcare Diagnostics Products GmbH) using a Dimension Vista 500 (Siemens). Reference ranges were provided by the package insert of the commercial kit and were derived by a consensus of a group of professional societies and diagnostic companies based on the standardization against the calibrated reference material 470[12].

Serum FLC levels were measured by a latex-enhanced immunoassay (Freelite, The Binding Site) with use of the turbidimetric platform SPA-PLUS analyzer (The Binding Site). The diagnostic ranges had been previously established by the manufacturer to include 100% of a reference population of 282 serum samples[13].

IgG-ACPA and IgA-ACPA were tested using a commercially available third-generation indirect solid-phase ELISA kit (Quanta-Lite CCP 3.1; Inova Diagnostics). The upper limit of normal (ULN; 20 U/ml) was set in accordance with the manufacturer's recommendations. Serum samples showing high concentration (> 250 U/ml) were evaluated after further dilutions (1/4 and, when necessary, 1/16) and then corrected for these additional dilution factors. The different RF isotypes (IgM, IgA, and IgG) were assessed using ELISA kits (Quanta-Lite RF; Inova Diagnostics). According to the manufacturer's recommendations, the test ULN was 6 U/ml. Only high titer IgM-RF (> 100 U/ml) were evaluated after further 1/20 dilution.

Flow cytometry. B cell counts were determined by flow cytometry (Cytomics FC-500, Beckman Coulter Inc.). Briefly, 100 μl of fresh whole blood were stained for 20 min at 4°C with a mixture of PC5-CD19, PE-CD27, and FITC-IgD (from Beckman Coulter, or R&D Systems Inc.), to identify naive (CD19+CD27–IgD+), memory (CD19+CD27+), or post-switch memory (CD19+CD27+IgD–) populations[4]. Absolute cell count was determined by single-platform analysis using Flow-Count beads (Beckman Coulter).

Statistical analysis. Data are expressed as the median (25th–75th percentile). The comparison between quantitative variables among different groups was performed by Mann-Whitney U test, while Wilcoxon signed-rank test was applied to assess variation within paired quantitative variables. The association between nominal variables was assessed with chi-square test with Yates' correction or Fisher's exact test. The correlation between quantitative variable was evaluated with the linear simple regression.

RESULTS

Evidence of B cell hyperactivation before ABA treatment. Before starting treatment with ABA (T0), patients with RA had higher serum levels of IgM, IgA, and FLC than those observed in HC (Table 2). In comparison with reference ranges, raised levels of serum IgG, IgA, or IgM were observed in 17%, 37%, and 20% of patients, respectively, whereas no one had hypogammaglobulinemia. Measurement of FLC demonstrated raised levels of κ chains in 18 out of 29 evaluated patients (68%) and of λ chains in 5/29 (17%). The κ:λ ratio was above normal levels in 8 of 29 patients (27%), and normal in 21/29.

As far as autoantibodies (Table 3), at T0, 87% and 47% of patients tested positive (> 20 IU/ml) for IgG- and IgA-ACPA, respectively, whereas 73%, 73%, and 53% showed the presence (> 6 U/ml) of IgM-, IgA-, and IgG-RF.

Table 2. Variations of Ig and FLC after therapy with ABA and comparison with healthy controls. Data are expressed as the median (25th–75th percentile).

	Reference Values	Healthy Controls, n = 24	RA, n = 30, T0	RA, n = 30, T6	p, T0 vs T6	RA, n = 16, T6	RA, n = 16, T12	p, T6 vs T12
Serum IgG, mg/dl	700–1600	1140 (1065–1410)	1330 (1085–1385)	1070* (901–1210)	0.0002	990 (880–1090)	958** (904–1044)	NS
Serum IgA, mg/dl	70–400	199 (161–284)	361*** (260–513)	287** (206–420)	0.0002	207 (172–328)	201 (176–282)	0.047
Serum IgM, mg/dl	40–240	127 (103–160)	181* (124–208)	145 (102–170)	0.002	127 (102–169)	102 (88–159)	NS
κ chain, mg/l	3.3–19.4	12.8 (11.1–15.6)	24.3*** (18.0–33.6)	19.6*** (13.9–21.9)	0.003	15.9 (13.9–19.9)	16.1* (13.3–19.6)	NS
λ chain, mg/l	5.7–26.3	12.2 (9.9–15.1)	16.5** (12.5–20.3)	13.0 (10.7–17.1)	0.01	12.9 (11.1–14.4)	12.2 (9.9–14.5)	NS
κ:λ ratio	0.26–1.65	1.07 (0.95–1.24)	1.37** (1.19–1.73)	1.28** (1.09–1.53)	0.02	1.28 (1.15–1.49)	1.25* (1.14–1.59)	NS

* $p < 0.05$ of controls; ** $p < 0.01$ of controls; *** $p < 0.001$ of controls. NS: not significant; Ig: immunoglobulin; FLC: free light chains; ABA: abatacept; RA: rheumatoid arthritis; T0: first administration of ABA; T6: after 6 months of ABA treatment; T12: after 12 months of ABA treatment.

Table 3. Variations of ACPA and RF after therapy with ABA. Data are expressed as the median (25th–75th percentile), except for reference values and p values.

Ig (IU/ml)	Reference Values	RA, n = 30, T0	RA, n = 30, T6	p, T0 vs T6	RA, n = 16, T6	RA, n = 16, T12	p, T6 vs T12
IgG-ACPA	< 20	283 (165–1497)	184 (68–1178)	0.05	140 (63–235)	130 (49–229)	NS
IgA-ACPA	< 20	20 (6–258)	13 (4–224)	0.01	7 (3–39)	10 (3–35)	NS
IgM-RF	< 6	87 (9–150)	31 (3–101)	0.03	31 (2–99)	24 (2–81)	< 0.01
IgA-RF	< 6	23 (10– > 100)	16 (4–95)	0.01	14 (2–29)	8 (3–27)	0.02
IgG-RF	< 6	7 (2–13)	3 (0.3–14)	0.03	3 (2–10)	3 (0.2–8)	NS

NS: not significant; ACPA: anticitrullinated protein antibodies; Ig: immunoglobulin; ABA: abatacept; RA: rheumatoid arthritis; T0: first administration of ABA; T6: after 6 months of ABA treatment; T12: after 12 months of ABA treatment; RF: rheumatoid factor.

Patients previously treated with RTX (minimum interval was 8 mos) did not differ from the other patients at T0 in Ig and FLC serum levels, but in 2 cases ABA was started before complete B cell reconstitution. These patients were therefore excluded from B cell analysis.

At T0, the proportions of circulating total memory B cells (CD19+CD27+) and of not-switched memory B cells (CD19+CD27+IgD+) were lower in patients with RA than in HC ($p = 0.019$ and $p = 0.05$, respectively; Figure 1; Table 4), whereas there was no difference in post-switch memory B cells (CD19+CD27+IgD–). Moreover, setting a cutoff at the 25th percentile of HC range, 28% and 71% of patients with RA at baseline had a low absolute number of circulating naive or memory B cells, respectively.

ABA treatment modulates the B cell compartment. After 6 months of ABA treatment (T6), 70% and 53% of patients achieved the EULAR good clinical response and clinical remission, respectively.

At this time, we observed a significant reduction of serum levels of IgG, IgA, and IgM (Table 2). Baseline abnormal values of IgG, IgA, and IgM normalized at 6 months in 2 of 5 (40%), 3 of 11 (27%), and 4 of 6 (67%) patients, respectively. Analogously, both serum free κ and λ chains decreased significantly after 6 months of therapy (Table 2), normalizing respectively in 6 of 18 (33%) and 4 of 5 (80%) patients with raised levels at T0. Despite this decrease in both types of light chains, the κ:λ ratio was also significantly reduced after therapy (Table 2), normalizing in 4 of 8 patients (50%) with raised ratio at T0.

We observed also a significant reduction of the titers of IgG- and IgA-ACPA, as well as of IgM-, IgA-, and IgG-RF (Table 3). A decrease of autoantibodies below the ULN values was observed in 2 of 26 patients (8%) initially seropositive for IgG-ACPA, 1 of 14 (7%) for IgA-ACPA, 5 of 22 (23%) for IgM-RF, 7 of 22 (30%) for IgA-RF, and 5 of 16 (31%) IgG-RF, whereas only 1 patient initially seronegative showed a weak (8.3 IU/ml) new positivity for IgG-RF at T6.

Finally, the proportion of post-switch memory B cells were found to be significantly decreased following ABA treatment ($p = 0.03$; Figure 1; Table 4).

In 16 patients, serum samples were obtained after 12 months of therapy (T12). There was a further significant decrease of total serum IgA, and of IgA- and IgM-RF, compared to values observed at T6 in these individuals (Tables 2 and 3). One patient who still had raised levels of serum IgA and 1 who still had positive IgA-RF at T6 normalized at T12.

All the variations here described were statistically significant even if patients who had received RTX prior to ABA therapy (n = 4) were excluded from the analysis. The only exception was the reduction of the titer of IgG-RF, which after exclusion of these patients, was significant only at T12 and not at T6.

Analysis of patients with low numbers of circulating B memory cells. As described in RA[6,14], a large proportion of our patients had low numbers of circulating memory B cells. Comparing these patients with the others, no difference was

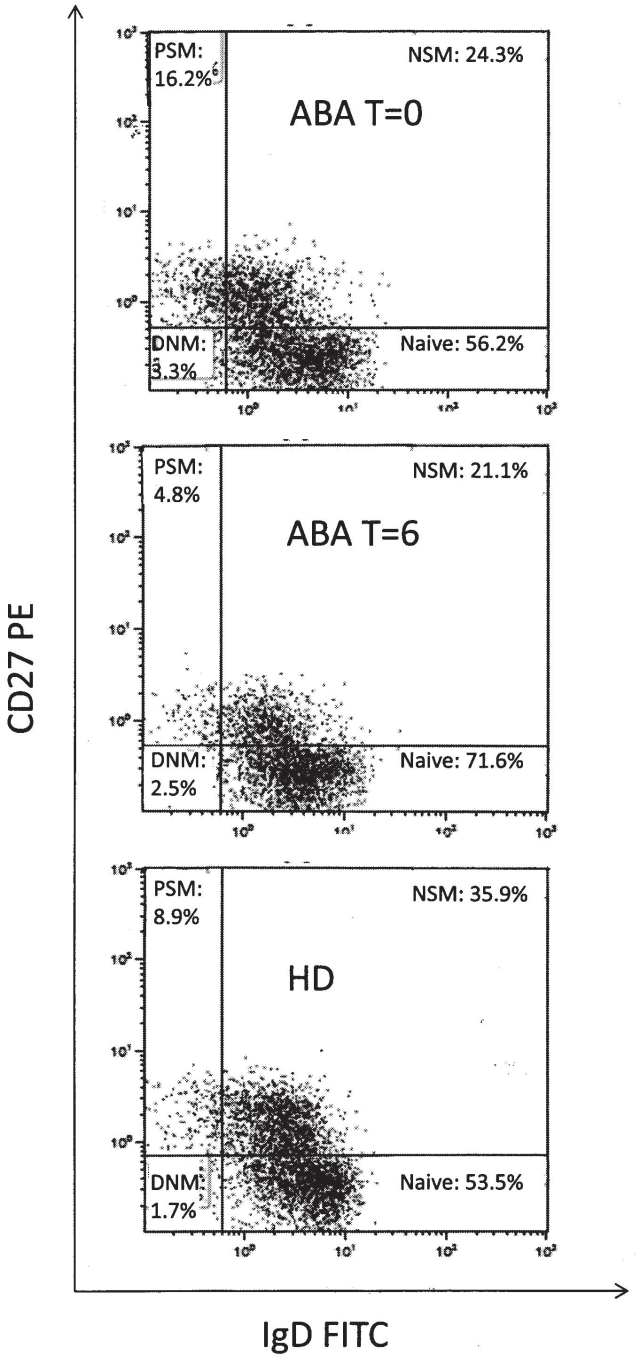

Figure 1. Flow cytometry evaluation of B cell subsets in a representative patient with rheumatoid arthritis before therapy with abatacept (ABA; T=0) and after 6 months (T=6), and in a representative healthy donor (HD). Dot-plot analysis is of CD19+ gated lymphoid cells stained with CD27 and antiimmunoglobulin D (IgD). PSM: post-switch memory cells; NSM: not-switched memory cells; DNM: double-negative memory cells.

observed in Ig, FLC, and autoantibody levels. The absolute number of circulating memory B cells at T0 was inversely correlated with the level of disease activity by Disease Activity Score 28 (DAS28) at T0 (r –0.47, p = 0.02), but treatment-induced change in levels of circulating memory B cells did not correlate with changes in DAS28 score.

However, the reduction of Ig, FLC, and autoantibodies after ABA therapy was significant only in patients with low numbers of circulating memory B cells and not in the others (with the exception of the decrease of IgM-RF (data not shown).

Comparison between patients who achieved clinical remission and those who did not. The decrease of FLC, ACPA, and RF was significant only in patients with clinical remission at T6, but not in those without it, whereas serum IgG and IgA levels decreased in both groups (Table 5). Moreover, the reductions of free λ chains and IgM-RF were significantly correlated with the reduction of DAS28-C-reactive protein (CRP) (r 0.47, p = 0.012; and r 0.46, p = 0.03, respectively).

Evaluating B cell markers as predictors of response to ABA, baseline free λ chain serum levels were lower in patients achieving clinical remission at T6 [14.7 mg/l (12.1–17.0) vs 18.5 (14.1–27.2); p = 0.045]. The differences of the other measurements did not reach significance, although they often approached it [in particular, IgA-ACPA titers in patients achieving remission were 6.7 IU/ml (4.8–55.1) vs 197.4 IU/ml (9.6–370.1); p = 0.058].

DISCUSSION

Our current study shows that ABA costimulation blockade in patients with RA induces a trend toward normalization of serum levels of total Ig of different classes and of FLC, variables that were above normal values in 17–68% of our patients before starting ABA. FLC are produced by B cells, plasma blasts, and plasma cells[15] and raised levels have been shown to correlate with disease activity in RA[3,15,16]. Interestingly, the reduction of FLC was significant in patients achieving clinical remission after ABA therapy, but not in those who did not. Similar results were observed in patients with RA who were treated with rituximab, but not with tumor necrosis factor (TNF)-blocking agents[15]. Moreover, in the present series, the decrease of λ serum free chains was correlated with clinical improvement, and their baseline levels appeared to be the best B cell marker associated with clinical response to ABA. This is at variance with what was observed in patients treated with RTX, in which the presence of autoantibodies and the levels of serum IgG were described as the better predictors of response[17]. However, our results derive from the observation of a small cohort, and further studies are needed to clarify whether they really reflect drug-specific differences (possibly related to different mechanisms of action). In the French nationwide registry, response to ABA was associated with ACPA positivity, but FLC were not evaluated[18].

We have observed a significant decrease of the serum titers of IgG- and IgA-ACPA, as well as of IgM-, IgA-, and IgG-RF. The probability of a seroreversion within normal

Table 4. Variations of B cell subset proportions after therapy with abatacept (ABA), and comparison with healthy controls. Except for p values, data are expressed as median (25th–75th percentile).

	Healthy Controls, n = 24	RA, n = 28,T0	RA, n = 28,T6	p, T0 vs T6	RA, n = 14, T6	RA, n = 14, T12	p, T6 vs T12
Naive B cells (% among CD19+ cells)	61.6 (53.6–69.8)	71.7 (64.4–83.5)	80.5** (70.9–85.4)	NS	82.1 (71.7–86.8)	77.1** (75.1–82.4)	NS
Memory B cells (% among CD19+ cells)	38.4 (30.2–46.4)	28.3* (16.5–35.6)	16.1*** (13.1–26.2)	NS	20.3 (14.1–30.5)	18.4*** (17.0–23.3)	NS
Not switched memory B cells (% among CD19+ cells)	26.6 (19.4–33.5)	18.2* (10.2–20.7)	13.4*** (9.4–18.5)	NS	13.5 (11.2–18.6)	12.3*** (7.0–14.7)	0.05
Post-switch memory B cells (% among CD19+ cells)	10.1 (7.1–12.2)	9.7 (5.7–12.9)	6.5* (5.5–6.7)	0.03	6.6 (5.8–6.7)	5.7 (2.8–8.3)	NS

* $p < 0.05$ of controls; ** $p < 0.01$ of controls; *** $p < 0.001$ of controls. NS: not significant; RA: rheumatoid arthritis; T0: first administration of ABA; T6: after 6 months of ABA treatment; T12: after 12 months of ABA treatment.

Table 5. Comparisons of variations of Ig, FLC, ACPA, and RF in patients with and without disease remission after 6 months of abatacept therapy. Data are expressed as the median (25th–75th percentile).

	Remission, n = 16			Not Remission, n = 14		
	T0	T6	p	T0	T6	p
Serum IgA, mg/dl	322 (248–363)	242 (182–343)	0.016	463 (360–694)	353 (269–474)	0.007
Serum IgM, mg/dl	175 (149–210)	163 (112–185)	NS	189 (108–195)	127 (74–152)	0.013
Serum IgG, mg/dl	1295 (1014–1370)	1075 (885–1112)	0.006	1340 (1140–1390)	1070 (929–1230)	0.009
κ chain, mg/l	23.0 (16.4–28.9)	17.3 (13.6–20.3)	0.019	35.5 (19.1–36.1)	20.3 (14.7–26.2)	NS
λ chain, mg/l	14.7 (12.1–17.0)	11.7 (10.7–14.5)	0.016	18.9 (15.4–27.8)	14.1 (12.9–23.1)	NS
IgA ACPA, IU/ml	7.5 (5.2–80.9)	7.4 (3.1–39.5)	0.011	203.2 (13.3–378.4)	159.8 (10–268.4)	NS
IgG ACPA, IU/ml	242 (174–425)	140 (83–273)	0.022	1004.8 (31.4–1884.8)	606.4 (18–1272)	NS
IgA RF, IU/ml	15.3 (10.4–41.5)	10.2 (3–23.4)	0.014	35.4 (12.6– > 100)	23.4 (5–> 100)	NS
IgM RF, IU/ml	101 (34.3–129.4)	40.5 (4.5–97.7)	0.008	31 (4.3–455)	4.15 (1.0–289)	NS
IgG RF, IU/ml	7.3 (3.75–10.8)	3 (2.1–9.9)	NS	4.2 (1.6–12.8)	1.5 (0.2–15.4)	NS

NS: not significant; T0: first administration of ABA; T6: after 6 months of ABA treatment; ACPA: anticitrullinated protein antibodies; RF: rheumatoid factor; FLC: free light chains; Ig: immunoglobulin.

values after 6 months of ABA therapy appeared to be more frequent for RF (23–31% of patients initially seropositive, according to the different isotypes) than for ACPA (7–8%). These results are in agreement with those of several studies in patients with RA receiving treatments other than ABA. Data from a cohort of 143 seropositive patients with RA demonstrated that, after 6 months of therapy, RF and ACPA titers decrease significantly, but the median changes were –36% of the baseline value and –15%, respectively[19]. Conflicting data have been reported on the effect of TNF-blocking agents: most studies describe a decrease in RF, whereas ACPA are generally reported to decrease significantly only in responders[20,21,22,23,24]. The effect on autoantibodies is more evident in patients treated with RTX[25], but even then the reduction of RF titer is more rapid and pronounced than in ACPA[26]. These data prompted the suggestion that RF is preferentially produced by short-lived plasma cells, whereas ACPA is predominantly produced by rather longer-lived plasma cells. We suggest therefore that the reductions of IgM-RF and of FLC λ serum levels after ABA therapy were directly correlated with clinical improvement, whereas the modifications of ACPA were not, because FLC and RF levels may better reflect the activation state of the B cells, while ACPA may be more associated to the immunological memory.

At T0, the proportions of circulating total memory B cells and of not-switched memory B cells were lower in patients with RA than in HC. This is in accordance with results that have shown that this effect is present from the early phase of RA[14]. It has been hypothesized that in patients with RA, circulating not-switched memory B cells are recruited to the synovial membrane or the secondary lymphoid organs[6,14]. The observation that reductions of Ig, FLC, and autoantibody levels after ABA therapy were more evident in patients with low absolute numbers of memory B cells might suggest that in these patients (in which more B cells might be present in the synovial and lymphoid tissue), ABA might better control signs of B cell hyperactivation.

Finally, we observed that ABA therapy can limit the differentiation of B lymphocytes to the effector population of post-switch memory cells. Post-switch memory cells are also reported to accumulate in the synovium of patients with

established RA[6,7]. Indeed, depletion of this B cell population can be induced also by therapy with RTX and was associated with good clinical response[27]. Taken together, all these data suggest that the blockade of costimulation can reduce B cell ability to differentiate into post-switch memory B cells and produce autoantibodies. Accordingly with our hypothesis, data available from immunohistological analysis of synovial tissue from patients with RA treated with ABA also provide evidence for a modest but significant reduction observed in mature B cells[28,29].

The effect of ABA therapy on the B cell compartment of patients with RA shown in our results was not previously described, but was observed in patients receiving other drugs. This kind of observation holds true for many effects of the biological and nonbiological therapies on other cell targets, and has led to the hypothesis of a possible common final pathogenic pathway leading to RA, which may be targeted by various and differently acting therapeutic agents[30]. Nevertheless, the specific effect of ABA on the costimulation blockade provides a clear possible mechanism of action accounting for the here-described effects of the drug on B cells[9]. In murine models, ABA administration blocked antigen-specific T cells in the lymph nodes to acquire a phenotype associated with migration to B cell follicles. This led to reduced specific antibody responses, despite normal B cell clonal expansion[31]. In accordance with the hypothesis of a direct effect of ABA in lymphoid organs, data from a human RA synovium/severe combined immunodeficiency mouse model suggested that ABA does not act directly on synovial T cells, but more likely prevents T cell activation at a systemic level of the immune system[32]. The data here presented, and the results of previous studies on T cells[33,34], are in agreement with a model in which ABA plays a "central effect," modulating T and B cell differentiation after antigenic presentation and their trafficking (also in human settings), thereby modifying the pathophysiology of RA.

The effects of ABA on B cells may have clinical implications, in particular for the response to vaccination and longterm humoral memory. In fact, it has been shown that ABA (in combination with methotrexate) significantly reduced the humoral response to the 2009 pandemic influenza A/H1N1 vaccine in patients with RA compared to patients with RA treated with methotrexate only[35]. Because others have observed similar findings[36], even though they need to be confirmed, clinicians may consider vaccinating patients against pathogens before starting ABA therapy. Despite these data, which suggest a possible impairment of the adaptive immune response, longterm safety of therapy with ABA in the clinical setting is confirmed by reassuring results showing no unexpected events and low incidence rates of serious infections and malignancies[37].

These data provide new insight on the effects of CD28 costimulation blockade in patients with RA, demonstrating a reduction of polyclonal B cell activation.

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