Organism article

Association between -871C>T promoter polymorphism in the B-cell activating factor gene and the response to rituximab in rheumatoid arthritis patients

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Abstract

Objective. To determine whether a functional single-nucleotide polymorphism in the B-cell activating factor (BAFF) gene correlates with the response to treatment with rituximab (RTX) in RA.

Methods. SMART is a randomized open trial (NCT01126541) assessing two strategies of re-treatment in patients responding to 1-g infusion of RTX with MTX on days 1 and 15 after failure, intolerance or contraindication to TNF blockers. Among the 224 patients included, 115 provided informed consent, could be genotyped and were included in an ancillary study of SMART assessing European League Against Rheumatism (EULAR) response rate after the first course of RTX according to BAFF-871C>T polymorphism. Baseline clinical factors (patients and disease characteristics) and biologic factors (ESR, CRP, RF, anti-citrullinated peptide antibodies, serum immunoglobulins) were collected. Univariate analyses were performed to assess whether BAFF-871C>T polymorphism was associated with EULAR response at week 24. Results with \( P < 0.15 \) obtained in univariate analyses were then included in multivariate analysis adjusted on DAS28 level.

Results. Ninety-three patients (81%) were responders, of whom 31 (27%) were good responders. CC genotype was significantly associated with a higher response rate [92% of responders vs 64% for TT genotype, odds ratio (OR) = 6.9; 95% CI 1.6, 29.6; \( P = 0.03 \)]. These results were also confirmed in RF-positive patients (96% vs 58%, \( P = 0.006 \)). In multivariate analysis, C allele carriage was independently associated with response to RTX (OR = 4.1; 95% CI 1.3, 12.7; \( P = 0.017 \)).

Conclusion. The BAFF-871C>T polymorphism seems to influence the response to RTX in RA patients after failure or intolerance to TNF blockers.

Key words: rheumatoid arthritis, BAFF, genetic polymorphism, rituximab, clinical response.

Introduction

In RA, B cells play critical roles in inducing or maintaining autoimmune inflammation. The anti-CD20 monoclonal antibody that targets B cells, rituximab (RTX), is an effective treatment of RA that is refractory to TNF blockers [1].

B-cell activating factor (BAFF), also known as B lymphocyte stimulator, is a cytokine member of the TNF family secreted predominantly by myeloid cells and plays an important role in B-cell maturation, homeostasis and survival in RA [2].
Since the survival of some autoreactive B-cell clones may be dependent on levels of BAFF that are higher than those required by non-autoreactive cells [3], the up-regulation of BAFF at the mRNA and protein level following B-cell depletion therapy may profoundly affect the re-expansion of autoreactive B cells and the recrudescence of autoimmunity.

The BAFF-871C>T polymorphism (rs9514828) is reported to be correlated with serum BAFF level in haematological disorders [4], mixed cryoglobulinaemia associated with HCV [5] or primary SS [6, 7], but not in RA [8, 9].

Based on the hypothesis that leading changes in components of the BAFF/BAFF-R system could be associated with the clinical outcome after RTX treatment, and that BAFF-871C>T polymorphism could be implicated in the expression of BAFF, we hypothesized that the BAFF-871C>T polymorphism could be implicated in clinical outcome after RTX treatment. With this in mind, we assessed clinical response after a first course of RTX depending on the genotype carriage of the BAFF-871C>T polymorphism.

Patients and methods

Patients

The design of the study has already been described in detail elsewhere [10]. Briefly, a total of 224 patients who had had RA for at least 6 months and fulfilling the ACR 1987 criteria were included in the SMART study (eSSai MAAbthera sur la dose de Re-Traitement, NCT01126541). This study is a 2-year national multicentric randomized open label study evaluating the efficacy and tolerability of two doses of RTX for re-treatment after one course of RTX (1000 mg on days 1 and 15). Each patient received a stable dose of MTX (> 10 mg/week for at least 4 weeks) and had experienced an inadequate response or intolerance to TNF blockers or for whom TNF blockers were contraindicated. The present ancillary study of SMART focused on the first stage of the study where all patients received their first course of RTX, before the randomization into two groups assessing two different doses of re-treatment by RTX.

Study protocol

All patients received their first course of RTX (1000 mg infusions on days 1 and 15). Treatment efficacy was evaluated 24 weeks after the first RTX infusion according to the European League Against Rheumatism (EULAR) response [11]. This response was used to classify patients as responders (good or moderate) or non-responders and is based on the individual amount of change in DAS28 and the level (low, moderate or high) reached. Briefly, the DAS28 is a composite criterion used to assess disease activity and includes the number of tender joints of 28 joints, the number of swollen joints of 28 joints, global assessment by the patient on a visual analogue scale (0–100 mm) and the CRP level (mg/l). A patient is considered in remission if DAS28 is < 2.6, as low disease activity if DAS28 is between 2.6 and 3.2, as moderate activity if DAS28 is between 3.2 and 5.1 and as high activity if DAS28 is > 5.1. The change in DAS28 and the score reached after treatment is used to classify the patients as good, moderate or non-responders.

This study was approved by the local ethics committee (Groupe Hospitalier Pitié-Salpêtrière, Paris) and all patients gave their informed consent according to the Declaration of Helsinki.

In this ancillary study we investigated the influence of the BAFF-871C>T polymorphism on the therapeutic response to the first course of RTX at 24 weeks, before re-treatment, in patients who gave specific additional consent for this pharmacogenetic study.

Serum B-cell markers

Serum samples taken before the first RTX infusion were used to measure RF by nephelometry (BN Prospec, Dade Behring, Paris, France) and anti-citrullinated peptide antibodies (ACPAs) by ELISA (DiaSorin, Saluggia, Vercelli, Italy). The cut-off was 15 IU/l for RF and 25 IU/l for anti-CCP antibodies. The serum concentrations of IgG, IgA and IgM were assessed by nephelometry (BN Prospec, Dade Behring). The upper limits of normal were 12.66 g/l for IgG, 2.69 g/l for IgA and 2.09 g/l for IgM. The cut-offs for immunoglobulin levels are based on testing performed previously in 3500 healthy French donors. BAFF was measured at baseline, before the RTX course, by Quantikine ELISA (R&D systems, Lille, France).

BAFF-871C>T genotyping

Whole blood was collected before the first RTX infusion in EDTA, and DNA was extracted by the salting-out method according to standard protocol. Genotyping of rs9514828 was performed using allele-specific kinetic PCR (KBiosciences, Herts, UK) using the KASPar method (accuracy generally > 99%, error rate < 0.3%).

Statistical analysis

Statistical analysis was performed with SAS 9.1 software (SAS Institute, Cary, NC, USA). Tests were two-sided and type I error was set at 0.05. Missing data were not replaced. Continuous data are described as mean (s.d.) or median [interquartile range (IQR)]. Baseline characteristics were compared across BAFF-871C>T genotypes using parametric (Student’s t-test or analysis of variance) or non-parametric test (Wilcoxon or Kruskal-Wallis test) for continuous data and χ² test or Fisher’s exact test for qualitative variables. Response rates were compared across BAFF-871C>T genotypes using the Cochran-Armitage trend test and then the χ² test or Fisher’s exact test when appropriate. The relationship between EULAR response at 24 weeks and explanatory variables was analysed by logistic regressions. These variables were selected by univariate logistic regressions among BAFF-871C>T polymorphism carriage, patients and disease characteristics and B-cell activation markers at baseline. Significant variables after univariate regressions (P < 0.15) were then entered in a step-wise multivariate
model adjusted to the DAS28-CRP. Results are expressed as the odds ratio (OR) with 95% CI.

The association between BAFF-871C>T genotypes and serum BAFF level was assessed with variance analysis. The association between BAFF-871C>T polymorphism and the risk of serious adverse events was investigated with Fisher’s exact test. All the analyses were performed on patients who received one course of RTX and who gave their informed consent to the genetic testing.

Results

Characteristics of the study population

Of the 224 RA patients [age 56 (s.d. 11) years, disease duration 13 (s.d. 9) months, 84% of women] who received one course of RTX, 209 had an inadequate response to anti-TNF and 16 had a contraindication to TNF blockers. Among them, 109 did not give their consent for the genetic search. Thus 115 patients could be genotyped and gave their informed consent to the genetic testing.

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Demographic characteristics did not differ across the different genotypes. Although the proportion of ACPA-positive patients was similar between the different genotypes, patients with the TT genotype were less often RF positive and the duration of TNF blockers before inclusion in SMART was shorter in these patients, but these differences were not statistically significant. The patients included in the study had longer disease duration, more active disease and more often erosions (P = 0.02, P = 0.03 and P = 0.04, respectively) in comparison with the patients who did not give consent for this ancillary study.

The frequencies of the genotypes of BAFF-871C>T in this population were CC = 34%, CT = 47% and TT = 19% and were comparable to the frequencies of the genotypes of the HapMap database for Caucasians (33, 42 and 25%, respectively). Patients’ baseline characteristics according to BAFF-871C>T genotypes are presented in Table 1.

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The analysis showed a significantly better response to RTX across the three genotypes according to the number of copies of the C allele carried (Cochran–Armitage trend test; P = 0.006) and 92% of patients with BAFF-871C>T CC genotype experienced a response (good or moderate) to the first course of RTX, while the response rate was lower in the TT genotype (64% of responders, P = 0.012).

EULAR response rates according to BAFF-871C>T genotypes

The BAFF genotyping was in Hardy–Weinberg equilibrium (P = 0.669). Twenty-four weeks after the first course of RTX, 93 patients (81%) were responders, of whom 31 (27%) were good responders. The EULAR response rate 24 weeks after the first course of RTX according to the BAFF-871C>T genotypes is presented in Fig. 1.

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Fig. 1 EULAR response rate according to BAFF-871C>T genotype distribution.

*Comparison of responders (good and moderate) between CC and TT genotypes with Fisher’s exact test; P = 0.01. aComparison of responders (good and moderate) with Cochran-Armitage trend test across the three genotypes; P = 0.006.

**TABLE 1** Patients’ baseline characteristics according to BAFF-871C>T genotypes distribution

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>CC (n = 39; 34%)</th>
<th>TC (n = 54; 47%)</th>
<th>TT (n = 22; 19%)</th>
<th>Whole sample (n = 115)</th>
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<tr>
<td>Age, females, n (%)</td>
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<td>Disease duration, median (IQR), years</td>
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<td>RF, n (%)</td>
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<td>ACPA, n (%)</td>
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<td>IgG, mean (s.d.), g/l</td>
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<td>Presence of erosions, n (%)</td>
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<td>DAS28-CRP, median (IQR)</td>
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<td>Prednisone (&lt;10 mg/day), n (%)</td>
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<td>MTX dosage, median (IQR), mg/week</td>
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<td>Time since last anti-TNF-α use, median (IQR), months</td>
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<tr>
<td>Anti-TNF-α duration before inclusion in SMART, median (IQR), months</td>
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</table>
EULAR response rates according to BAFF-871C>T allele

C allele carriage was significantly associated with EULAR response (OR = 3.2; 95% CI 1.1, 9.2; P = 0.0271) in univariate analysis and was then tested in a multivariate analysis to investigate whether it was an independent factor of response to RTX through a logistic regression where the response to RTX was the outcome assessed. Univariate analyses showed that baseline DAS28-CRP > 5.1, oral CS therapy and high serum IgG rate (>12.66 g/l) were associated with RTX response with P < 0.15. Oral CS therapy, high serum IgG rate and C allele carriage were then included in the model, adjusted on DAS28. The results of the univariate analyses and the logistic regression are presented in Table 2. The final model confirmed that BAFF-871C>T C allele carriage independently increased the chances of response to RTX (OR = 4.1; 95% CI 1.3, 12.7; P = 0.017).

Association between BAFF-871C>T polymorphism and serum BAFF level

The comparison of baseline serum BAFF level measured at baseline according to the different genotypes showed a trend towards a higher level of serum BAFF for patients carrying the TT genotype compared with CC and CT genotypes [mean serum BAFF level for TT genotype 772.14 pg/μl (s.d. 879.45 pg/μl), mean serum BAFF level for CT and TC genotypes 556.82 pg/μl (s.d. 193.38 pg/μl)], but the difference was not statistically significant (P-value of the global test = 0.16) (Fig. 2).

Association between BAFF-871C>T polymorphism and serious adverse events

The number of patients with at least one serious adverse event in this study was 18 (16%), of whom 4 (3.5%) had a serious adverse event related to RTX. No association between BAFF-871C>T polymorphism and the risk of serious adverse events was observed (Fisher’s exact test P = 1.0 for comparison of C allele carriers vs non-carriers).

Discussion

Biologic DMARDs have led to remarkable benefits for the treatment of RA, either in patients who have had inadequate response to MTX or to a TNF inhibitor. After TNF inhibitor failure, there is evidence for the efficacy of switching to another TNF inhibitor or to another class of biologic DMARD such as abatacept, RTX or tocilizumab [12]. Since there are several therapeutic options, with no clear differences in efficacy or safety concerns, clinicians need predictive factors for the response to a biologic DMARD in an individual patient to make a tailored decision [13]. In the present study we confirm, as has already been reported in a study of 120 RA cases where the CC genotype of BAFF-871C>T was associated with a higher response rate to RTX [14], that BAFF-871C>T C allele carriage is an independent factor associated with the response to a first course of RTX associated with MTX in patients who have had inadequate response or a contraindication to a TNF inhibitor.

In this study, no statistically significant correlation between the BAFF-871C>T polymorphism and baseline BAFF serum level before RTX course was identified, but a trend of higher BAFF serum level was observed in the TT genotype compared with CC and TC genotypes. Previous studies showed that BAFF-871C>T polymorphism was associated with BAFF serum level in familial lymphoproliferative disorders [4], idiopathic thrombocytopenic purpura [15], SS [6, 7] and mixed cryoglobulinaemia.

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analyses, OR (95% CI), P-value</th>
<th>Multivariate analysis, OR (95% CI), P-value</th>
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<tbody>
<tr>
<td>C allele carriage</td>
<td>3.2 (1.1, 9.1), 0.027</td>
<td>4.1 (1.3, 12.7), 0.017</td>
</tr>
<tr>
<td>High serum IgG rate</td>
<td>3.6 (1.1, 11.5), 0.030</td>
<td>5.4 (1.5, 19.9), 0.011</td>
</tr>
<tr>
<td>Positive RF</td>
<td>1.9 (0.7, 4.9), 0.210</td>
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<tr>
<td>Positive ACPA</td>
<td>1.4 (0.4, 4.3), 0.563</td>
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<tr>
<td>Oral cortisone</td>
<td>0.3 (0.0, 1.3), 0.081</td>
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<tr>
<td>DAS28-CRP &gt; 5.1</td>
<td>3.2 (1.2, 8.5), 0.017</td>
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*Adjusted on baseline DAS28 level, V: valine.
associated with HCV virus [5]. However, such an association was not identified in two studies involving RA [8, 9]. This discrepancy may be due to much higher serum BAFF levels in SS or haematological diseases. However, after B-cell depletion therapy, where BAFF/BAFF-R components are implicated, we could hypothesize that such a polymorphism could play a role in BAFF expression and in B-cell depletion and/or return, with a lower BAFF increase after B-cell depletion and delayed reconstitution or decreased survival of autoreactive B cells, explaining such differences in clinical outcome.

In the BAFF gene, located on Chs13 (13q32-q34), the position _871 corresponds to the binding site of transcription factor myeloid zinc finger protein (MZF1), which was reported to be preferentially expressed in differentiating myeloid cells, the prominent source of BAFF [16]. Thus it is possible that this single-nucleotide polymorphism may change the binding affinity of MZF1, and hence BAFF expression.

Although a previous study of the SMART trial demonstrated an association between RF or ACPA presence and the response to RTX [10], the RF positivity was not associated with EULAR response in the present ancillary study. In this sample of 115 patients, the difference between patients with and without RF antibodies in terms of EULAR response was only 11% (84% vs 73% of EULAR response, respectively), whereas in the sample of 208 patients, this difference was ~21% (79% vs 58%, respectively). The smaller difference in this sample can explain the absence of a statistically significant difference. Another explanation could be a selection bias of patients who gave their consent for the genetic study with a better response rate in the subgroup of RF-negative patients. However, we assume that this selection bias did not influence the BAFF-871C>T genotype distribution and its association with EULAR response.

If the association between BAFF-871C>T C allele carriage and the response to RTX could be replicated in independent studies, this pharmacogenetic marker, as well as B-cell activation biomarkers, could help clinicians to make a tailored decision in the choice of a biologic DMARD in an individual RA patient in the perspective of personalized medicine.

Rheumatology key messages
- **BAFF-871C>T** polymorphism influences the outcome after RTX in RA with inadequate response to anti-TNF.
- There was no association between **BAFF-871C>T** polymorphism and BAFF serum level in RA.

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