ORIGINAL ARTICLE

The presence of anti-citrullinated protein antibodies (ACPA) does not affect the clinical response to adalimumab in a group of RA patients with the tumor necrosis factor (TNF) α-308 G/G promoter polymorphism

Lilian Soto · Francisca Sabugo · Diego Catalan · Pamela Wurmann · Tomás Cermenatti · Héctor Gatica · Octavio Aravena · Lorena Salazar · Juan Carlos Aguillón · Miguel Cuchacovich

Abstract The introduction of antitumor necrosis factor (TNF) agents has improved the outcome for many patients with rheumatoid arthritis (RA). To date, the only replicated genetic predictor of anti-TNF response is the −308 G > A single-nucleotide polymorphism in the TNF promoter region. The presence of the −308 TNF G/G genotype appears to be a marker of good response to anti-TNF treatment. Anti-citrullinated protein antibodies (ACPA) have been linked with erosive disease, and have been established as the single most reliable prognostic factor in clinical practice. To test the hypothesis that the ACPA status may affect the −308 G/G patients rate of response to TNF blockade, we prospectively investigated a group of 52 RA patients with the −308 G/G genotype who were ACPA (+) or ACPA (−). All patients were treated with adalimumab, and the clinical response was studied using the Disease Activity Score in 28 joints (DAS28) at 24 weeks of treatment. Over 85% of patients were DAS28 responders in both groups. No significant differences were found between patients from both groups, according to the DAS28 criteria of response at week 24 ($p=0.79$). In conclusion, our findings suggest that the ACPA status does not affect the clinical response to anti-TNF therapy in −308 TNF G/G patients.

Keywords Adalimumab · Anti-TNF · Rheumatoid arthritis · TNF polymorphism

L. Soto · F. Sabugo · P. Wurmann · H. Gatica · M. Cuchacovich
Rheumatology Section, Department of Medicine,
Clinical Hospital University of Chile,
Santiago, Chile

D. Catalan · O. Aravena · L. Salazar · J. C. Aguillón
Disciplinary Program of Immunology, ICBM, Faculty of
Medicine, University of Chile and Millenium Institute on
Immunology and Immunotherapy,
Santiago, Chile

T. Cermenatti
Department of Medicine, Clinical Hospital, University of Chile,
Santiago, Chile

M. Cuchacovich (✉)
Hospital Clínico de la Universidad de Chile,
Santos Dummont 999, Santiago Norte, Santiago, Chile
e-mail: mcuchaco@gmail.com

Introduction

Rheumatoid arthritis (RA) is a multisystem inflammatory disease which affects synovial joints. It causes persistent synovitis, pain, joint destruction, and functional disability [1]. The introduction of antitumor necrosis factor (anti-TNF) agents has improved the outcome for many patients with RA. However, 30–40% of patients fail to respond to treatment for unknown reasons [2]. Thus, screening patients prior to initiation of the TNF antagonists may help select patients with a higher likelihood of response.

Polymorphisms in genes encoding TNF, the HLA region, and the anti-citrullinated protein antibody (ACPA) titers and their ability to predict response to anti-TNF therapies have been the focus of recent studies [3, 4]. To date, the only replicated genetic predictor of anti-TNF response is the −308 G > A single-nucleotide polymorphism (SNP) in the TNF promoter region. The presence of the TNF −308 G/G genotype appears to be a marker of response to anti-TNF treatment [5, 6]. The TNF gene is

located in the class III HLA locus, in proximity with *HLA-DR* genes [7]. Based on the linkage disequilibrium that exists between these two regions and on the strong association between *HLA-DRB1* alleles and susceptibility and severity of RA, it is possible that a relationship may be found between these factors and the response to anti-TNF therapy [8].

The group of *HLA-DRB1* shared epitope (SE) alleles specifically predispose to ACPA-positive RA [9]. ACPAs have been linked with erosive disease and have been established as the single-most reliable prognostic factor in clinical practice [10]. Some reports suggest that a decrease in ACPAs titers might be a useful adjunct in assessing the efficacy of anti-TNF treatment [11–14]. The use of multiple prognostic factors such as *HLA-DRB1*, ACPA, and the −308 G > A SNP in the TNF promoter region may interact to define a final outcome in response to anti-TNF therapy. To test the hypothesis that the ACPA status may affect the prognostic value of −308 TNF SNP on the response to TNF blockade in RA patients, we prospectively investigated a group of 52 RA patients carrying the −308 G/G genotype who were ACPA (+) or ACPA (−). All patients were treated with a human anti-TNF monoclonal antibody (adalimumab), and the clinical response was studied using the Disease Activity Score in 28 joints (DAS28) and the American College of Rheumatology (ACR) definition of improvement in RA.
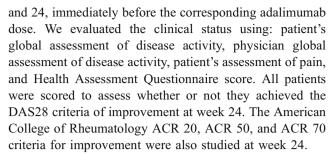
Patients and methods

Patients

Fifty-two patients fulfilling the 1987 revised ACR criteria for classification of RA were studied [15]. All had active disease despite treatment with methotrexate, leflunomide, or sulfasalazine, defined by the presence of six or more swollen joints, nine or more tender joints, and morning stiffness greater than 45 min. The dosages of disease-modifying antirheumatic drugs (DMARDs) were stable for at least 8 weeks before enrolling in the study.

All patients received 40 mg adalimumab (Abbot Laboratories, Chicago, IL, USA) subcutaneously every other week during 24 weeks. Patients were allowed to continue the same dose of non-steroidal anti-inflammatory drug, oral glucocorticoids, and DMARDs they had been taking at the beginning of this study. The study was approved by Ethical Committees at each study site, and all patients gave their written informed consent.

Clinical and laboratory assessment

The number of tender joints and swollen joints were evaluated by an assessor at baseline and at weeks 8, 16,

and 24, immediately before the corresponding adalimumab dose. We evaluated the clinical status using: patient's global assessment of disease activity, physician global assessment of disease activity, patient's assessment of pain, and Health Assessment Questionnaire score. All patients were scored to assess whether or not they achieved the DAS28 criteria of improvement at week 24. The American College of Rheumatology ACR 20, ACR 50, and ACR 70 criteria for improvement were also studied at week 24.

Laboratory analyses included determinations of erythrocyte sedimentation rate, blood count, and blood chemistry at each visit. All clinical evaluations and laboratory tests were performed blindly, with respect to the ACPA status. Blood samples for studies were drawn at 9–10 A.M. on weeks 0, 8, 16, and 24 immediately before the corresponding adalimumab dose was given and stored at −70°C until use.

TNF promoter polymorphism genotyping

The −308 TNF promoter genotype was determined by polymerase chain reaction restriction fragment length polymorphism [15].

ACPA measurement

ACPA status was determined by measuring anti-CCP levels with a commercial anti-CCP2 enzyme linked immunosorbent assay kit (Euro-diagnostica, Sweden), following the manufacturer's instructions. The results of the anti-CCP test were considered positive if the antibody levels were ≥25 IU/ml (cutoff value).

IgM RF measurement

IgM rheumatoid factor (RF) was assayed by ELISA on a 96-well plate coated with highly purified Fc fragments of human IgG, prepared by digestion of human IgG with papain and then purification by chromatography on Sephadex G-75 and protein A-Sepharose. For assays, serum aliquots (200 μl, 1:100 dilution) were added in triplicate in a 200 μl final volume of PBS-Tween and incubated 2 h at 37°C, followed by rinsing in PBS-Tween and incubation with specific anti-human IgM F(ab')$_2$ fragments conjugated to alkaline phosphatase for 1 h at 37°C. Plates were rinsed with PBS-Tween and incubated with 200 μl alkaline phosphatase substrate (1 mg/ml *p*-nitro-phenylphosphate) in 0.1 M glycine, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4. Absorbance was monitored at 405 nm.

Statistical analysis

The main outcome was an improvement in the DAS28 score, which is defined as a decrease of at least 1.2. All other results,

such as ACR 20, ACR 50, and ACR 70 criteria for improvement, were considered secondary outcomes. All the study variables were tested for normality with the Shapiro–Wilks test. Since the serum anti-CCP titers were not normally distributed, all comparisons were made by means of non parametric statistics: Kruskal–Wallis ANOVA for comparison between groups and post hoc analyses using the Mann–Whitney U test when appropriate. Normally distributed variables were analyzed by means of one-way ANOVA or ANCOVA when appropriate. Post hoc analyses were performed using t test. Differences in proportions were analyzed with the Pearson chi square test, the Fisher exact test or the McNemar test according to the number of events/cases involved and the nature of the comparison.

Results

Baseline characteristics of patients

Eighty-two patients were previously screened for the presence of the −308 TNF α promoter polymorphisms. Fifty-two of them had the G/G genotype and were selected for further analysis. All were included in the safety analysis. Thirty-nine patients were ACPA (+) and 13 were ACPA (−).

Table 1 shows the baseline demographic and disease characteristics in the two groups of the study patients. The methotrexate mean dose was 15.59±7.61 mg/week in the ACPA (+) group and 15.71±3.45 mg/week in the ACPA (−) group. The prednisone mean dose was 8.43±4.45 mg/day in the ACPA (+) group and 9.23±3.44 mg/day in the ACPA (−) group.

Despite long-term therapy with methotrexate and other DMARDs, patients with G/G genotype had a high swollen and tender joint count. Patients from the ACPA (+) group had a higher DAS28 basal score than patients who were ACPA (−).

Clinical response to anti-TNF therapy and ACPA presence in −308 G/G patients

Fifty out of 52 patients received adalimumab for at least 24 weeks and were included in the final evaluation of treatment efficacy (one patient withdrew from the study due to adverse effects and one because of lack of efficacy). Table 2 summarizes the clinical improvement in ACPA (+) and ACPA (−) patients according to DAS28, ACR 20, ACR 50, and ACR 70 criteria. Most patients achieved DAS28 and ACR 20 criteria after 24-week treatment. Over 85% of patients were DAS28 responders in both groups; however, no significant differences were found between ACPA (+) and ACPA (−) patients ($p=0.79$). Although the ACPA (+)

Table 1 Baseline characteristic of patients with RA

Characteristic	ACPA (+) group (n=39)	ACPA (−) group (n=13)	p Value
Age (years)	52.08±1.60	43.69±2.90	0.046
Sex: female	34	12	0.30
Duration of disease (months)	149.22±161.03	125.07±107.55	0.054
Rheumatoid factor (+) (n)	30	9	0.29
DAS28 (basal)	6.07±1.41	5.16±1.18	0.038
SJC	17.69±10.48	14.57±7.04	0.1
TJC	23.16±14.12	15.92±10.35	0.059
PAP	7.08±2.13	5.69±4.33	0.02
PGADA	6.72±1.66	5.42±1.82	0.01
PhGADA	6.69±1.93	5.28±1.68	0.012
HAQ	1.52±0.79	1.18±0.56	0.07
ESR (mm/h)	42±29.94	43.53±34.39	0.56

ACPA anti-cyclic citrullinated peptide antibodies, *RA* rheumatoid arthritis, *SJC* swollen joint count, *TJC* tender joint count, *PAP* patient's assessment of pain, *PGADA* patient assessment of disease activity, *PhGADA* physician assessment of disease activity, *HAQ* Health assessment questionnaire, *ESR* erythrocyte sedimentation rate

group exhibited a higher rate of ACR 20, ACR 50, and ACR 70 achievement than ACPA (−) group, we did not find significant differences between groups.

Clinical response to anti-TNF therapy and RF presence in −308 G/G patients

IgM RF was measured in 50 patients who completed 24 weeks of therapy. Thirty-eight patients were RF (+) and 12 patients were RF (−); 86.8% of RF (+) and 100% of RF (−) patients were DAS28 responders at week 24. No difference was found among responders and nonresponders according to ACR 20, ACR 50, and ACR 70 criteria between RF (+) and RF (−) patients (Table 3).

Table 2 Correlation between adalimumab response and the presence of ACPAs in a group of 50 patients with the G/G polymorphism at position −308 of the TNF α gene

	ACPA (+) (n=37)		ACPA (−) (n=13)		p Value
	n	(%)	n	(%)	
DAS28 responders	32/37	(86.4)	12/13	(92.3)	0.79
ACR 20 improvement (n)	28/37	(75.67)	7/13	(53.8)	0.07
ACR 50 improvement (n)	14/37	(37.83)	4/13	(14.2)	0.32
ACR 70 improvement (n)	2/37	(5.4)	0/13	(0)	0.19

DAS28 response and ACR response at week 24

ACPA anti-cyclic citrullinated peptide antibodies

Table 3 Correlation between adalimumab response and the presence of RF in a group of 50 patients with the G/G polymorphism at position −308 of the TNF α gene

	RF (+) (n=38)		RF (−) (n=12)		p Value
	n	(%)	n	(%)	
DAS28 responders	33/38	(86.8)	12/12	(100)	0.09
ACR 20 improvement (n)	27/38	(70)	9/12	(75)	0.39
ACR 50 improvement (n)	13/38	(34.2)	5/12	(41.6)	0.31
ACR 70 improvement (n)	2/38	(5.2)	0/12	(0)	0.21

DAS28 response and ACR response at week 24

RF rheumatoid factor

RF was also studied in the ACPA (+) and in the ACPA (−) group of patients (Tables 4 and 5). No significant differences were found between RF (+) and RF (−) patients in the ACPA (+) or in the ACPA (−) group of patients.

Discussion

TNF blockade has emerged as an important therapy for patients with RA; however, a substantial proportion of treated patients still demonstrate partial or no response to these therapies [2]. Previous studies have suggested that the effect of clinical factors alone in predicting anti-TNF therapy response is relatively modest [16–18].

Potter et al. studied a large cohort of patients treated with anti-TNF agents and tested the hypothesis that confirmed RA susceptibility factors, namely RF, ACPA, SE, and the PTPN22 620 W polymorphism, were associated with clinical response. They concluded that the presence of RF

or ACPAs was associated with a reduced response to anti-TNF drugs. However, these antibodies only account for a small proportion of the variance in treatment response. They hypothesized that genetic factors may additionally contribute to treatment response [19]. Hence, the present study was undertaken to address the potential associations of different genetic and serological markers with poor or good response to anti-TNF therapy in patients with RA. We investigated the value of different combinations of two variables including the −308 TNF polymorphism and ACPAs for predicting clinical response to anti-TNF treatment in a group of patients with long-standing RA. We had previously demonstrated patients carrying −308 TNF G/G genotype displayed a better response to adalimumab than those with the G/A genotype [5]. In the current study, G/G patients were classified according to the ACPA status in ACPA (+) or ACPA (−) in order to evaluate whether the presence of these autoantibodies could influence the predictive property of the −308 TNF SNP. Even though patients from the ACPA (+) group had a higher basal DAS28 than ACPA (−) patients, no significant differences were found between groups according to the DAS28 or ACR criteria of response at 24 weeks of treatment. However, the ACR criteria suggest a trend for a better response in the ACPA (+) group of patients. New studies with increased numbers of patients should be done in the future to clarify this subject.

It has been previously reported that the presence of ACPAs is associated with a significantly reduced improvement in the DAS28 score following 6 months of anti-TNF therapy [14]. However, none of those patients was typified for the −308 G/A TNF-gene promoter polymorphism.

We previously studied if the presence and the RF or ACPA levels correlate with adalimumab efficacy in a group of RA patients who had an inadequate response to

Table 4 Correlation between adalimumab response and the presence of RF in a group of 37 ACPA (+) patients with the G/G polymorphism at position −308 of the TNF α gene

	RF (+) (n=30)		RF (−) (n=7)		p Value
	n	(%)	n	(%)	
DAS28 responders	26/30	(86.6)	7/7	(100)	0.15
ACR 20 improvement (n)	26/30	(86.6)	7/7	(100)	0.15
ACR 50 improvement (n)	12/30	(40)	4/7	(57.1)	0.20
ACR 70 improvement (n)	2/30	(6.6)	0/7	(0)	0.24

DAS28 response and ACR response at week 24

ACPA anti-cyclic citrullinated peptide antibodies, RF rheumatoid factor.

Table 5 Correlation between adalimumab response and the presence of RF in a group of 13 ACPA (−) patients with the G/G polymorphism at position -308 of the TNF α gene (DAS28 response and ACR response at week 24)

	RF (+) (n=8)		RF (−) (n=5)		p Value
	n	(%)	n	(%)	
DAS28 responders	5/8	(62.5)	5/5	(100)	0.11
ACR 20 improvement (n)	4/8	(50)	3/5	(60)	0.36
ACR 50 improvement (n)	1/8	(12.5)	1/5	(20)	0.35
ACR 70 improvement (n)	0/8	(0)	0/5	(0)	

DAS28 response and ACR response at week 24

ACPA anti-cyclic citrullinated peptide antibodies, *RF* rheumatoid Factor.

methotrexate or to other DMARDs [11]. The −308 G > A SNP in the TNF promoter region was not studied in this group of patients. We found a significant decrease in the RF and ACPA levels after 24 weeks of adalimumab therapy. We also found that patients who were ACPA-positive before treatment were also better responders than ACPA negative patients. It is possible that ACPA-positive RA patients might display a more active disease associated with a higher response to adalimumab therapy in comparison with patients negative for ACPAs.

In the present study, all of our patients had the G/G responder polymorphism, and interestingly, the presence of RF or ACPA antibodies did not influence the clinical outcome in this group of patients.

In conclusion, our findings, as well as others using different anti-TNF α blockers, suggest that the presence of the −308 G/ G genotype may help to define a subgroup of RA patients with a better response to this treatment. Our data also suggest that the RF or ACPA status does not affect the clinical response to anti-TNF therapy in this group of patients.

Disclosures None

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