Genetic variants contribute to differences in response and toxicity to drugs used in autoimmune diseases: Rheumatoid arthritis and systemic lupus erythematosus

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Chapter 6

Association between *-174 Interleukin-6* gene polymorphism and biological response to rituximab in several systemic autoimmune diseases

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Abstract

Rituximab has become a pivotal treatment for systemic autoimmune diseases. The aim of this study was to determine whether the genetic variant *-174 IL-6* contributes to differences in the response to rituximab in patients with systemic autoimmune diseases, including systemic lupus erythematosus, inflammatory myopathies, anti-neutrophil cytoplasmic antibody-mediated vasculitis, systemic sclerosis, Sjöegren's syndrome, rheumatoid arthritis, and autoimmune hemolytic anemia. DNA samples from 144 Spanish patients with different systemic autoimmune diseases receiving rituximab were genotyped for *-174 IL-6* (rs1800795) gene polymorphism using the TaqMan allelic discrimination technology. Six months after the first infusion with rituximab, we evaluated the response to the drug: 60.4% of the patients showed a complete response, partial 27.8%, and 11.8% did not respond to the treatment. The CC genotype frequency was significantly increased in non-responders with respect to responders (23.5% vs. 7.1%, respectively; p = 0.049; odds ratio (OR) = 4.03, 95% confidence intervals (CI) 0.78–16.97). According to the genotype distribution, rituximab was effective in 69.2% of the CC carriers, 91.9% of the CG carriers, and 88.4% of the GG carriers. A similar trend was observed when SLE patients were analyzed separately (27.3% carried CC homozygosis in non-responders and 6.9% in responders; p = 0.066; OR = 5.10, 95% CI 0.65–31.73). Rituximab was effective in 62.5% of the CC carriers, 88.9% of the GC carriers, and 90% of the GG carriers. These results suggest that *-174 IL-6* (rs1800795) gene polymorphism plays a role in the response to rituximab in systemic autoimmune diseases. Validation of these findings in independent cohorts is warranted.

INTRODUCTION

Rituximab is a chimeric monoclonal immunoglobulin G1 antibody against the CD20 protein of B-lymphocytes promoting B cell depletion (1, 2). It has become a crucial therapy against systemic autoimmune diseases, since an aberrant B cell regulation is among the common pathogenic mechanisms of these diseases (3). The Food and Drug Administration has approved the use of rituximab in Non-Hodgkin's lymphoma, Chronic lymphocytic leukemia, and Rheumatoid arthritis (RA) in combination with methotrexate in adult patients with moderately to severely active RA who have an inadequate response to one or more tumor necrosis factor (TNF) antagonist therapies, Wegener's granulomatosis (WG), and microscopic polyangiitis (MPA) in adult patients in combination with glucocorticoids. Recent studies in other systemic autoimmune diseases show the importance of this therapy in refractory patients (4-8). Certain clinical and genetic characteristics, including the presence of positive rheumatoid factor (9), the presence of Epstein-Barr virus (10), low levels of type I interferons (11), and low levels of B lymphocyte stimulator (12), have been associated with a positive response to the drug. Pharmacogenetic studies have been suggested to explain variations in efficiency of biological treatments and predisposition of patients to a nonresponse to rituximab (13). Interleukin-6 (IL-6) is a cytokine expressed by lymphocytes, monocytes, and fibroblasts that plays a key role in B cell maturation and autoantibodies production (14). IL-6 actions are mainly controlled through a complex, including the membrane-bound IL-6 receptor (IL-6R) and two gp130 subunits. However, IL-6 can also signal via a soluble receptor (sIL-6R), which binds to IL-6 and then interacts with gp130 subunits (15). IL-6 acts as a proinflammatory mediator in response to inflammatory stimuli (16, 17). In autoimmunity, IL-6 inhibits the function of T-reg cells and induces the generation of pathogenic Th17 cells, essential in an inflammatory autoimmune response leading to tissue inflammation and destruction (18). During the acute inflammation phase in RA, monocytes and macrophages release IL-6 to serum and can be used as a biomarker of inflammation or disease activity (19). The *-174 G/C* genetic variant (rs1800795), located in the *IL-6* gene promoter region, has been seen associated to autoimmune diseases and involved in increased levels of IL-6 protein in serum in diverse inflammatory diseases, although it is unclear which allele or genotype is involved in these findings (20-22). Recently, Fabris et al. (23) reported a lower response to rituximab in RA patients that presented CC homozygosis in the *-174 IL-6* variation. The aim of our study was to assess the possible involvement of the *-174 IL-6* polymorphism in the clinical response to rituximab in different systemic autoimmune diseases.

MATERIALS AND METHODS

Study population

This study was performed using a Spanish Caucasian cohort comprising a total of 144 patients with systemic autoimmune diseases treated with rituximab, recruited from three university medical centers (Hospital Universitario San Cecilio, Granada; Hospital Carlos Haya, Málaga; Hospital Virgen del Rocío, Sevilla). Table 6.1 shows the main characteristics of the patients enrolled in this study. Systemic autoimmune diseases patients included 83 (57.6%) systemic lupus erythematosus (SLE) patients, 16 (11.1%) with different inflammatory myopathies such as polymyositis and dermatomyositis, 16 (11.1%) anti-neutrophil cytoplasmic antibody associated vasculitis patients, including WG, Churg-Strauss Syndrome, and MPA, and other systemic autoimmune diseases such as Sjögren syndrome, systemic sclerosis, or autoimmune hemolytic anemia in the remaining 29 patients. The most-frequently administered rituximab dose was 375 mg/m^2 of rituximab weekly for 4 weeks in most cases, although some patients received 1000 mg twice at an interval of 15 days. Six months after the first infusion, a clinical response to the drug was evaluated according to the ACR and EULAR recommendations (24-26). The criteria used to evaluate the response to rituximab in different autoimmune diseases have already been described in detail elsewhere (6). The response to rituximab was assessed on the basis of clinical evolution. Responders included complete responders and partial responders depending on improvement of initial disease activity, total or at least 50%, but not reaching complete remission, respectively. Non-responders were defined as patients with no significant improvement or a worsening of the disease. Previous and concomitant treatments are shown in Table 6.1. All patients gave written, informed consent before participation and an ethics committee approved the study protocol.

IL-6 genotyping

DNA was isolated from whole peripheral blood, using standard procedures. *IL-6-174* single nucleotide polymorphism (SNP) (rs1800795) genotyping was performed using the TaqMan allelic discrimination assay technology in a 7500 Real-Time PCR System, both from Applied Biosystems (Foster City, CA). The genotype call rate was 100% for the tested *IL-6* genetic variant. The probes were labeled with the fluorescent dyes 2-chloro-7-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) and 6-carboxyfluorescein (FAM).

Table 6.1: Main characteristics of systemic autoimmune diseases patients treated with rituximab Included in this study

Characteristics	n (%)
Female	114 (79.2%)
Male	30 (20.8%)
Systemic autoimmune diseases	
Systemic lupus erythematosus	83 (57.6%)
Inflammatory myopathies	16 (11.1%)
ANCA-mediated vasculitis	16 (11.1%)
Sjögren's syndrome	4 (2.77%)
Systemic sclerosis	5 (3.47%)
Hemolytic autoimmune anemia	3 (2.08%)
Pemphigus vulgaris	3 (2.08%)
Mixed connective tissue disease	2 (1.38%)
Idiopathic thrombocytopenic purpura	4 (2.77%)
Cryoglobulinemia	2 (1.38%)
Rheumatoid arthritis	3 (2.08%)
Axonal polyneuropathy associated with HCV	1 (0.69%)
Autoimmune trombocytopenia	1 (0.69%)
Sarcoidosis + RA	1 (0.69%)
Previous therapies	
Corticosteroids	137 (95.1%)
Cyclophosphamide	89 (61.8%)
Methotrexate	52 (36.1%)
Mycophenolate	50 (34.7%)
Intravenous immunoglobulins	49 (34.0%)
Antimalarials	38 (26.4%)
Azathioprine	38 (26.4%)
Cyclosporine A	11 (7.6%)
Leflunomide	5 (3.4%)
Other biologic therapies	5 (3.4%)
Plasma exchange	3 (2%)
Thalinomide	2 (1.38%)
Other therapies	11 (7.63%)
Concomitant therapies	
Corticosteroids	136 (94.4%)
Antimalarials	13 (9%)
Methotrexate	8 (5.5%)
Azathioprine	8 (5.5%)
Mycophenolate	7 (4.86%)
Cyclophosphamide	8 (5.5%)
Cyclosporine A	2 (1.38%)
Intravenous immunoglobulins	3 (2.08%)
Other therapies	2 (1.38%)
Response (n = 132)	
Complete	87 (60.4%)
Partial	40 (27.8%)
No response	17 (11.8%)

Statistical analysis

A statistical analysis for allelic and genotypic distributions was calculated by the chi-squared test or the Fisher's exact test, when necessary, using the Statcalc software packages (Epi Info 2002; centers for Disease Control and Prevention, Atlanta, GA); p-values, odds ratio (OR), and 95% confidence intervals (CI) were calculated according to this software. p-values lower than 0.05 were considered as statistically significant. The presence of heterogeneity between SLE and the remaining autoimmune diseases patients was tested on the basis of the Breslow-Day test using a significance level of 0.05 (StatsDirect, v. 2,6,6). A multivariate logistic regression analysis was performed using STATA v. 10. Statistical power of our study is 80% to detect an association of -174 G/C with the OR reported in previous studies (OR = 3) (23, 27).

RESULTS

According to the EULAR and ACR criteria (6, 24-26), clinical evaluation of the response to rituximab was carried out at month 6 after first infusion with the therapy. There were 87 (60.4%) good responders (complete remission of the symptoms and clinical characteristics that recommended the use of the drug), 40 (27.8%) partial responders (reduction in at least 50% of the disease activity), and 17 (11.8%) non-responders (reduction in less than 50% of the disease activity). Interestingly, when we stratified by sex, 10.6% of the women (12/113) were non-responders, whereas 17.2% of the men (5/29) did not respond, although this difference did not become significant. Main characteristics of systemic autoimmune diseases patients including in this study are shown in Table 6.1. Genotype frequencies of the IL-6 polymorphism in Spanish systemic autoimmune diseases patients were not significantly different from those previously described in RA and SLE studies in Caucasian populations (23, 28). Of the 144 patients analyzed, 69 (47.9%) were homozygous for GG, 62 (43.1%) were heterozygous GC, and 13 (9%) were homozygous for C allele. Different systemic autoimmune diseases patients were pooled, and homogeneity of odds ratios between SLE and the remaining autoimmune diseases patients was verified by the Breslow-Day test ($p > 0.05$). Table 6.2 shows genotypic and allelic frequencies in patients stratified into two groups, according to the response to rituximab. In the subgroup of patients presenting total or a partial response to rituximab, 61 (48%) were GG, 57 (44.9%) were GC, and 9 patients (7.1%) were CC. In non-responders, the CC genotype frequency was significantly increased with respect to responders (four patients = 23.5%, CC in non-responders vs. nine patients = 7.1%, CC in responders: $p = 0.049$; OR = 4.03, 95% CI 0.78–16.97), whereas GC and GG genotypes frequencies were diminished with respect to responders (five patients = 29.4% and eight patients = 47.1%, respectively), although these differences did not reach statistical significance. No significant differences in allelic frequencies

for the *-174 IL-6* gene promoter polymorphism were observed when patients that responded to the treatment were compared against non-responders (p = 0.301). A logistic regression analysis was performed to evaluate if the concomitant therapies could be confounding the observed association. This analysis found no confounding factors (data not shown).

In correlation with these results, only 69.2% (9/13) of the patients carrying the CC genotype were responders to the treatment with rituximab, whereas this drug was effective in 89.9% (118/131) of the patients carrying the GC or GG genotype (p = 0.049; OR = 4.03, 95% CI 0.78–16.97) (Table 6.2). On the other hand, when we analyzed separately SLE patients (84/144, 58.3% of the patients), we found a trend in the same direction, although it did not reach statistical significance (Table 6.3). The efficiency level of rituximab was lower in

Table 6.2: Distribution of *-174 IL-6* rs1800795 SNP and efficiency in systemic autoimmune diseases patients treated with rituximab

	Non-responders n = 17 n (%)	Responders n = 127 n (%)	Efficiency rituximab %	p-value	OR (95% CI)
Genotype					
GG	8 (47.1)	61 (48.0)	88.4	0.940	0.96 (0.31–2.94)
GC	5 (29.4)	57 (44.9)	91.9	0.226	0.51 (0.13–1.68)
CC	4 (23.5)	9 (7.1)	69.2	0.049[a]	4.03 (0.78–16.97)
Allele					
G	21 (61.8)	179 (70.5)	89.5	0.301	0.68 (0.30–1.52)
C	13 (38.2)	75 (29.5)	85.2	0.301	1.48 (0.66–3.28)

P-value comparing frequency and efficiency in non-responders versus responders. Significant p-values are in bold. [a] Fisher's exact test. OR, odds ratio; 95% CI, 95% confidence interval; SNP, single nucleotide polymorphism.

Table 6.3: Distribution of *-174 IL-6* rs1800795 SNP and efficiency in systemic lupus erythematosus patients treated with rituximab

	Non-responders n = 11 n (%)	Responders n = 73 n (%)	Efficiency rituximab %	p-value	OR (95% CI)
Genotype					
GG	4 (36.4)	36 (49.3)	90.0	0.423	0.59 (0.12–2.56)
GC	4 (36.4)	32 (43.8)	88.9	0.449[a]	0.73 (0.14–3.19)
CC	3 (27.3)	5 (6.9)	62.5	0.066[a]	5.10 (0.65–31.73)
Allele					
G	12 (54.6)	104 (71.2)	89.7	0.114	0.48 (0.18–1.32)
C	10 (45.5)	42 (28.8)	80.8	0.114	2.06 (0.76–5.61)

P-value comparing frequency and efficiency in non-responders versus responders. Significant p-values are in bold. [a] Fisher's exact test.

CC carriers (5/8 = 62.5% responded to the treatment) than in GC or GG carriers (68/76 = 89.5% did respond); p = 0.066, OR = 5.10; 95% CI 0.65–31.73.

DISCUSSION

Rituximab has become a pivotal therapy in the treatment of several autoimmune diseases, and the study of the genetic predisposition to a positive or a negative response to this drug has been suggested to be a first step to improve its efficacy.

In the present study, we have analyzed the association of the *-174 IL-6* promoter variation with the response to rituximab in a group of patients that presented diverse systemic autoimmune diseases. Frequencies for this SNP were similar to those previously reported in Caucasian populations (23, 28). Genotypic frequencies for CC were increased in non-responders, which correlates with the fact that patients carrying this homozygosis responded worse to the treatment with rituximab than those carrying GC or GG genotypes (69.2% vs. 90.2%). Fabris et al. (23) found a lower response to rituximab in RA patients that were homozygous for CC. Their findings agree with our results, both in the group of diverse systemic autoimmune diseases patients and in SLE patients analyzed separately, although, in SLE patients, the observed differences are not statistically significant, probably due to the lower statistical power of this stratified analysis. Pathogenesis of systemic autoimmune diseases involves inflammation cytokines IL-1, TNF alpha, and IL-6. Murine models in inflammatory diseases indicate that IL-6 deficiency reduces the severity of an inflammatory response (29). Recent studies have clarified evidence that antagonizing the action of proinflammatory cytokines, including IL-6, may exert a therapeutic effect in patients nonresponsive to other therapies. Tocilizumab, a humanized antibody to the IL-6 receptor, blocks IL-6 signaling and activity and decreases levels of inflammatory markers in RA (30, 31). Previous studies reported that B cell depletion induced by rituximab resulted in a downregulation of proinflammatory cytokines, including IL-6 and, consequently, a decrease of the autoimmune response and re-establishment of the immunotolerance (32). The lower efficiency of rituximab in systemic autoimmune diseases patients carrying the CC genotype, suggests an increase in the number of refractory patients to rituximab in this group. Biological therapies different to rituximab might be had under consideration to get an adequate and more effective response in these patients. According to our data, *-174 IL-6* SNP suggests a pharmacogenetic association with the clinical response to rituximab in systemic autoimmune diseases, and the hypothesis that this variation could be a predictive value, independently of other clinical or environmental factors.

Anyway, as the observed significant associations could be due to a casual finding resulting from multiple comparisons, larger replication studies are needed and still planned by our group to confirm present results.

Currently, there are very few data about genetic markers of prognosis that may be used in the future to facilitate treatment decisions. We herein provide preliminary evidence of a possible new genetic marker, the CC homozygosis of the *-174 IL-6* promoter polymorphism, as a predictor of non-response to rituximab in autoimmune diseases.

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