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 8

IL2/IL21 region polymorphism influences response to rituximab in systemic lupus erythematosus patients

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Abstract

To determine whether the *IL2/IL21* region, a general autoimmunity locus, contributes to the observed variation in response to rituximab in patients with systemic lupus erythematosus as well as to analyze its influence in a cohort including other autoimmune diseases. rs6822844 G/T polymorphism at the *IL2–IL21* region was analyzed by TaqMan assay in 84 systemic lupus erythematosus (SLE) and 60 different systemic autoimmune diseases Spanish patients receiving rituximab. Six months after the first infusion patients were classified, according to the EULAR criteria, as good responders, partial responders and non-responders. A statistically significant difference was observed in GG genotype frequency between responder (total and partial response) (83.56%) and non-responder (45.45%) SLE patients ($p = 0.010$, odds ratio (OR) = 6.10 [1.28–29.06]). No association with the response was evident in the group of patients with autoimmune diseases other than lupus. Furthermore, when both groups of patients were pooled in a meta-analysis, a reduced statistical significance of the association was observed ($p = 0.024$, OR = 3.53 [1.06–11.64]). Our results show for a first time that *IL2–IL21* region seems to play a role in the response to rituximab in SLE patients but not in other autoimmune diseases.

INTRODUCTION

Rituximab is an anti-CD20 monoclonal antibody that suppresses inflammation effectively in multiple autoimmune diseases (AD) (1). It was initially approved by FDA for the treatment of B cell lymphomas and later for rheumatoid arthritis (RA) refractory to anti-tumor necrosis factor therapies (2, 3). The precise mechanisms by which rituximab exerts its effects are not fully understood. Different mechanisms have been proposed for explaining the therapeutic action of this drug in AD. On the one hand, rituximab is hypothesized to suppress disease injury by promoting rapid and long-term elimination of circulating and possibly lymphoid-tissue-associated B cells (4-6). On the other hand, rituximab-opsonized B cells may act as decoy immune complexes that effectively divert monocytes or macrophages from interactions with tissue associated immune complexes (7).

Recently, studies in the research field of pharmacogenetics have reported potential markers associated with clinical response on treatment with rituximab. In this way, polymorphisms located in *FcGR3A*, *IL6* and *TGFB1* genes seem to act as predictors of response in patients with RA (8-10).

Certain clinical factors have also been associated with a better response to rituximab, including the presence of positive rheumatoid factor in RA patients, positive Epstein-Barr virus in bone marrow, depletion of B cells after first infusion (11, 12) low levels of B lymphocyte stimulator (BLyS) and low levels of type I interferons (13, 14).

Several early clinical investigations of the combination of interleukin-2 (IL2) and rituximab have reported an increased efficacy of this drug by expansion of circulating NK cells, leading to an increased antibody-dependent cellular cytotoxicity (ADCC) (15, 16), therefore evidencing the key role played by this cytokine in rituximab response.

The *IL2/IL21* region at 4q27 is a susceptibility locus for multiple autoimmune diseases (17). Both genes, *IL2* and *IL21*, are plausible functional candidates as genetic modifiers of autoimmunity (18, 19). IL2 stimulates T cell proliferation and activation and regulates the adaptive immune response by stimulating both T-regulatory cells and activation-induced cell death in antigen-activated T cells. Although different polymorphisms in this region have been associated with autoimmunity, rs6822844 has been the most consistently replicated in independent studies and different populations (17, 20-25).

Polymorphisms in susceptibility genes for RA have been shown to be associated with treatment response (26, 27); we hypothesized that rs6822844, known to have a role in several autoimmune diseases, may also influence the response to rituximab therapy. Our main aim was to analyze the role of this genetic variant in the rituximab response in a

cohort of SLE patients and, additionally, to check whether this polymorphism is a common factor influencing the response in different autoimmune disorders.

MATERIALS AND METHODS

Patients and treatment

In the present study were included 84 SLE patients and 60 patients with other systemic autoimmune diseases (16 patients (26.7%) presented different inflammatory myopathies including polymyositis and dermatomyositis, 16 (26.7%) were ANCA-mediated vasculitis patients including Wegener's granulomatosis, Churg–Strauss syndrome and microscopic polyangiitis and the remaining 29 (48.3%) patients presented other systemic autoimmune diseases, such as systemic sclerosis, Sjögren's syndrome, rheumatoid arthritis and autoimmune haemolytic anemia), all of them Spanish Caucasian patients treated with rituximab. Patients were recruited from three university medical centers (Hospital Universitario San Cecilio, Granada; Hospital Carlos Haya, Málaga; Hospital Virgen del Rocío, Sevilla). The main characteristics of the patients enrolled in this study are shown in Table 8.1.

The administered intravenous dose of rituximab was 375 mg/m^2 weekly for 4 weeks in most cases, although some patients received 1,000 mg twice at an interval of fifteen days. Clinical response was evaluated 6 months after the first infusion of rituximab, according to the ACR and EULAR recommendations (28-30). The criteria used to evaluate the response to rituximab in different autoimmune diseases have already been described in detail elsewhere (1). Complete response was defined as disappearance of all symptoms and signs of the systemic disease that recommended the use of rituximab. Partial response was defined as a significant improvement (at least 50%) of initial disease activity, based on clinical judgment. Responders included complete responders and partial responders; no response was defined as no significant improvement or worsening of the disease. Concomitant and previous treatments are shown in Table 8.1. The study was approved by an ethic committee, and all patients gave written informed consent before participation.

Genotyping

DNA was isolated from whole peripheral blood, using standard procedures. *IL2–IL21* SNP (rs6822844) genotyping was performed using the Taqman allelic discrimination assay technology in a 7,500 real-time PCR system, from applied biosystems (Foster City, California, USA). The genotype call rate was 100% for the tested genetic variant. The probes were labeled with the fluorescent dyes VIC and FAM and PCR reaction was carried.

Table 8.1: Main characteristics of systemic autoimmune diseases patients treated with rituximab included in this study

Characteristics	n (%)
Female	114 (79.16)
Male	30 (20.83)
Systemic autoimmune diseases	
Systemic lupus erythematosus	84 (57.64)
Inflammatory myopathies	16 (11.11)
ANCA-mediated vasculitis	16 (11.11)
Sjo¨gren'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 (95.14)
Cyclophosphamide	89 (61.80)
Methotrexate	52 (36.11)
Mycophenolate	50 (34.72)
Intravenous immunoglobulins	49 (34.02)
Antimalarials	38 (26.38)
Azathioprine	38 (26.38)
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 = 144)	
Complete	87 (60.42)
Partial	40 (27.77)
No response	17 (11.81)

Statistical analysis

Statistical analysis for allelic and genotypic distributions was calculated by Chi squared test or 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-value lower than 0.05 was 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). Multivariate logistic regression analysis was performed using STATA v. 10.

RESULTS

The clinical response of the treatment with rituximab was evaluated at month 6 after the first infusion, according to the EULAR and ACR criteria (1, 28-30). In the group of SLE patients, 49 patients (58.3%) responded well to the treatment, 24 (28.6%) were considered as partial responders and 11 (13.1%) were considered non responders. In patients with other autoimmune disorders, 38 patients (63.3%) showed a good response, 16 patients (26.7%) were considered as partial responders and 6 patients (10%) did not respond to the treatment. The response rate observed in patients including in our study is similar to those described in other studies (31, 32).

Genotypic and allele frequencies of the *IL2/IL21* polymorphism observed in responder and non-responder autoimmune diseases patients are summarized in Table 8.2. These frequencies were not significantly different from those previously described in Caucasian populations (22).

In SLE patients, both GG genotype and G allele frequencies were increased in responders compared with non-responders (83.6% vs 45.5%; p = 0.010, OR = 6.10 [1.28–29.06] and 91.8% vs 72.7%; p = 0.016, OR = 4.19 [1.12–14.06], respectively) (Table 8.2). Nevertheless, no differences between responder and non-responder patients were observed in the group of non-SLE patients (Table 8.2).

Subsequently, different systemic autoimmune diseases patients were pooled and homogeneity of odds ratio between SLE and the remaining autoimmune diseases patients was verified by Breslow-Day test (p > 0.05). In responders, GG genotype frequency was significantly increased with respect to non-responders (83.5% vs 58.82%; p = 0.024, OR = 3.53 [1.06–11.64]). Significant differences were also observed in the allelic frequencies between responder and non-responder patients (91.7% vs 79.4%; p = 0.032, OR = 2.88 [1.00–8.01]) (Table 8.2).

Table 8.2: Distribution of rs6822844 *IL2/IL21* genetic variant in systemic lupus erythematosus, non-systemic lupus erythematosus and autoimmune diseases patients

		Genotype, n (%)			Genotype test		G allele	Allele test	
Disease	Subgroup (n)	GG	GT	TT	p-value[a]	OR (95% CI)	frequency (%)	p-value	OR (95% CI)
SLE	Non-responders (n = 11)	5 (45.45)	6 (54.55)	0			72.70		
	Responders (n = 73)	61 (83.56)	12 (16.44)	0	**0.010**	6.10 (1.28–29.060	91.78	**0.016**	4.19 (1.12–14.06)
Non-SLE	Non-responders (n = 6)	5 (83.33)	1 (16.67)	0			91.67		
	Responders (n = 54)	45 (83.33)	9 (16.67)	0	0.683	1.00 (0.02–10.67)	91.69	0.666	1.00 (0.02–8.55)
Autoimmune diseases pooled	Non-responders (n = 17)	10 (58.82)	7 (41.18)	0			79.41		
	Responders (n = 127)	106 (83.46)	21 (16.54)	0	**0.024**	3.53 (1.06–11.64)	91.73	**0.032**	2.88 (1.00–8.01)

[a] P-values have been calculated comparing responders carrying GG versus non-responders carrying GG.
SLE, systemic lupus erythematosus.
Bold text denotes significant p-values.

Finally, to evaluate if socio-demographic variables and concomitant therapies could be confounding the observed association in SLE patients, a multivariate logistic regression analysis, considering the effect of the GG genotype on the rituximab response as the dependent variable and gender, age and concomitant therapies as independent variables, was performed. As shown in Table 8.3, this analysis observed no confounding factors.

Table 8.3: Multivariable model of *IL2/IL21* rs6822844 GG genotype carriage adjusting for potential confounding factors in systemic lupus erythematosus

	p-value	OR (95% CI)
rs6822844 GG genotype unadjusted	0.008[a]	6.1 (1.60–23.26)[a]
Adjusted for individual covariates		
Gender	0.007[b]	6.37 (1.64–24.67)[b]
Age	0.012[b]	6.63 (1.51–29.11)[b]
Corticosteroids	0.005[b]	7.38 (1.80–30.18)[b]
DMARDs	0.006[b]	7.43 (1.78–31.03)[b]
Other therapies	0.006[b]	7.13 (1.74–29.18)[b]
Adjusted for all the covariates	0.016[b]	6.43 (1.42–21.07)[b]

[a] p-value and OR correspond to the effect of the rs6822844 GG genotype in rituximab response.
[b] p-values and OR correspond to the effect of the rs6822844 GG genotype in rituximab response adjusted for different covariates considered individually and altogether.
DMARDS, disease-modifying antirheumatic drugs.

DISCUSSION

Treatment with rituximab results in a reduction of disease activity in most autoimmune diseases patients. However, a percentage of patients do not respond to this therapy and/or experience toxicity (33). The reason for this non-response is unknown, but genetic and environmental factors are thought to be implicated. Given the potential toxicities and the high cost of rituximab therapy, it would be beneficial to predict whether an individual patient will benefit from this treatment, beforehand.

Knowledge about related genetic variants, mostly SNPs, may help to predict drug response or optimal dose in the individual patient. Classically, explorative pharmacogenetic association studies are aimed at finding polymorphisms potentially predictive (34).

Our results indicate that SLE patients homozygous for rs6822844 G allele show a better clinical response to rituximab at month 6 than patients with GT genotype. On the contrary, no association was evident in the group of non-SLE patients. It could be speculated that this lack of association was a consequence of a lower statistical power in the latter analysis;

however, it should be noted that no effect size was suggested in this case (i.e. OR = 1) and, in addition, a reduction of the statistical significance of the association was observed when the non-SLE patients were meta-analyzed with those showing SLE (which increases the statistical power). Taken together, our data suggest that the influence of the *IL2/IL21* rs6822844 polymorphism in the therapeutic response to rituximab is specific of the SLE condition.

Although the mechanism of action of rituximab remains unclear, accumulating data suggest that antibody-dependent cellular cytotoxicity (ADCC) may play a dominant role (35). ADCC is mediated through immune effector cells, mainly NK cells, via expression of an activating receptor for the Fc portion of IgG antibodies (FcGR). The majority of human NK cells are CD16 positive (FcGRIII) and express the intermediate affinity interleukin-2 receptor. It has been described that intermediate doses of IL2 are capable of expanding CD16 positive NK cells and activating cytotoxic effector functions, including ADCC activity (36-40).

Several studies have demonstrated that this ability of IL2 to promote NK cell expansion and cytotoxicity influences the efficiency of rituximab treatment and correlates with the clinical response (15, 16, 41-44). Furthermore, the relationship between IL2 and the efficacy of rituximab is supported by the fact that soluble interleukin-2 receptor is used as a prognostic factor in patients with lymphoma receiving rituximab (45-49).

An alteration of the function of B cells is a key factor contributing to SLE athophysiology; however, some clinical trials with rituximab in this disease have failed to show efficacy. Murine models of SLE based on antibody mediated cellular depletion evidenced that this lack of efficacy can be explained by a defect in macrophage and neutrophil IgG-dependent phagocytosis induced by serum IgG (50). In this context, the role of IL2 promoting rituximab-mediated ADCC could become more critical in the efficacy of rituximab in lupus than in other autoimmune diseases in which this drug acts through all its mechanisms.

The *FCGR3A-158* polymorphism is currently shown to enhance rituximab mediated ADCC and improve clinical response to this drug (51). Similarly, rs6822844 variant could affect the cytotoxic activity of NK cells and, therefore, the efficacy of rituximab in SLE condition; although to date no functional studies analyzing this issue have been published.

In conclusion, we show for a first time that *IL2–IL21* rs6822844 G/T polymorphism influences the clinical efficacy of rituximab in SLE patients. The replication of this association in independent studies could enable the potential use of this variant as a pharmacogenetic marker.

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