

Original Article

The Therapeutic Blockade of TNF Reduces Serum Levels of Interleukin 15 in Patients With Rheumatoid Arthritis

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ABSTRACT

Objective: To analyze the effect of the TNF blocking agents (aTNF) on the serum levels of interleukin 15 (IL-15). To determine whether baseline IL-15 serum levels or their response to aTNF therapy can predict the TNF antagonist's clinical response to this treatment.

Patients and method: We studied 75 patients suffering from rheumatoid arthritis that were selected to start aTNF therapy. Serum samples were obtained at baseline visit and after 3 months of aTNF treatment. Measurement of IL-15 serum concentration was performed through immune-enzyme assay. We collected the clinical and analytical parameters needed to calculate DAS28 both at baseline and final visit, as well as sociodemographic variables and other such as rheumatoid factor, previous disease modifying anti-rheumatic drugs (DMARD), etc. We defined remission as a DAS28 <2.6 and clinical response when the decrease in DAS28 value was higher than 1.2.

Results: There was a significant correlation between IL-15 serum level and the number of previous DMARD. We also detected a significant decrease in the concentration of serum IL-15 after 3 months of treatment with aTNF. However, neither the baseline IL-15 serum level nor the decrease in the concentration of IL-15 was associated with a specific pattern of response to aTNF.

Conclusions: Our data seem to support previous in vitro findings suggesting that TNF is involved in the regulation of IL-15 expression. Nevertheless, the measurement of IL-15 serum levels does not seem to be a useful tool to select those patients that should be treated with aTNF therapy.

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El bloqueo terapéutico del factor de necrosis tumoral disminuye la concentración sérica de interleucina 15 en pacientes con artritis reumatoide

RESUMEN

Objetivo: Analizar el efecto de la terapia con agentes inhibidores del factor de necrosis tumoral (TNF) en la concentración sérica de interleucina 15 (IL-15) y determinar si los valores basales de ésta o su variación con el tratamiento predicen la respuesta clínica a los anti-TNF.

Pacientes y método: Se estudió a 75 pacientes con artritis reumatoide que iban a iniciar tratamiento con anti-TNF. Se recogieron muestras de suero previas y a los 3 meses de tratamiento. La concentración de IL-15 se cuantificó mediante enzimoanálisis. Tanto en la visita basal como en la final se recogieron parámetros clínicos y analíticos que permitieran calcular el DAS28. También se recogieron variables sociodemográficas y otras relacionadas con la enfermedad, como factor reumatoide, número de fármacos previos, etc. Se definió remisión como un DAS28 < 2,6 y respuesta clínica relevante, como una disminución del DAS28 > 1,2.

Resultados: La concentración de IL-15 se relacionó de forma significativa con un mayor uso de fármacos modificadores de la enfermedad durante el seguimiento de los pacientes. También se observó una disminución significativa de la IL-15 a los 3 meses de tratamiento con anti-TNF. Sin embargo, los valores

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basales de IL-15 y su disminución con el tratamiento no se relacionaron con la respuesta a los anti-TNF o la consecución de remisión clínica.

Conclusiones: Nuestros datos parecen confirmar los obtenidos in vitro, que indican que el TNF está implicado en la modulación de la expresión de IL-15. No obstante, la medición de la concentración sérica de IL-15 no parece ser de utilidad para seleccionar a los pacientes candidatas a terapia anti-TNF.

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Introduction

Interleukin (IL) 15 is a cytokine that plays a fundamental role in innate immunity, although it can also modulate other functions in the immune system.^{1,2} Different studies support the fact that IL-15 can be implicated in the pathogenesis of rheumatoid arthritis (RA), because of elevated concentrations of this cytokine, have been detected both in synovial fluid and serum of patients with the disease.³⁻⁶ In addition, IL-15 can be key in the perpetuation of the production of tumor necrosis factor alpha (TNF), because it is involved in different cycles of mutual cell stimulation in which adhesion molecules and cytokines such as IL-15, interferon gamma, and TNF itself intervene.^{5,7,8} However, as additional evidence of the phenotypical variability of the disease, in a previous study we described that not all patients with RA have elevated values of IL-15.⁴ In that work we described that the patients who presented higher IL-15 concentrations had received a larger number of disease modifying anti-rheumatic drugs (DMARD) during the follow up of their disease.⁴

We currently have different biologic therapies for the treatment of patients with rheumatoid arthritis (RA) who have failed to respond to at least one DMARD. However, not all patients have an optimal response to biologic therapy, making the description of potential response markers to these new treatments important, as they could ease the selection of the most adequate patients for each treatment. In the present study we analyze the effect that treatment with TNF inhibitors have on the concentration of IL-15 in patients with RA. On the other hand, it must be determined if elevated serum concentrations of this cytokine mark a subgroup of patients with different clinical characteristics and whether its treatment with anti-TNF is different than that of patients with low or undetectable concentrations of IL-15.

Patients and Methods

We studied 75 patients who fulfilled the classification criteria for RA as proposed by the American College of Rheumatology,⁹ of which 65 received treatment with adalimumab (ADA) and 10 with infliximab (INF). The clinical characteristics of the patients are shown on Table 1. Serum samples were obtained before starting treatment with anti-TNF (baseline) and after 12 weeks of treatment, in patients treated with ADA or at the fourth infusion of INF (14 weeks). In each visit, the degree of disease activity was evaluated through DAS28.¹⁰ We also collected, through the clinical history of the patient, sociodemographic data, and the number of DMARD received during the course of the disease.

Determination of Serum IL-15

The concentration of IL-15 in the sera of patients was determined through enzyme-linked immunoanalysis (EIA) generated in our laboratory using the monoclonal antibodies MAB647 and BAM247 (R&D Systems Europe Ltd., Abingdon, United Kingdom), as has been described elsewhere.⁴ The inferior limit for detection is 6.2 pg/mL and the upper limit of the curve, 500 pg/mL. Samples in which we obtained a higher number than the upper limit were diluted 1:2 and reanalyzed in order to confirm the number. Intra-analytical variability was 13.5% and inter-analytical variability was 20.7%.

We considered IL-15 to be elevated if values were >30 pg/mL, representing the 95th percentile of a population of 250 healthy controls studied by our laboratory (manuscript under preparation).

Statistical Analysis

The statistical analysis was performed with the Stata statistical software for Windows, version 9.2 (StataCorp LP, College Station, Texas, USA). The Fisher test was used for comparing qualitative variables and for the quantitative variables, Student *t* or Mann-Whitney *U* were employed, according to whether the distribution of the variables was normal or not, respectively. Wilcoxon's test was applied for paired data in order to establish statistical significance of differences between the values of IL-15 before and after treatment with anti-TNF.

The number of prior DMARD was used during follow-up as a proxy variable for disease severity. Because different factors can modify this variable, a multivariate analysis was performed to determine which were associated to a higher prescription of DMARD. For that we used the *ztp* function of Stata, which estimates Poisson's regression through linear generalized models for variables truncated at zero, because there were not any patients who had not received DMARD previously.¹¹ In the initial model, the following variable were included: age, gender, time since onset of disease, rheumatoid factor concentration (RF), baseline IL-15 concentration in the serum, and the center at which the patient was cared for. The final model was reached by eliminating variables with a *P*>.2, except if the general significance of the model worsened.¹¹

We also studied whether the concentration of IL-15 before treatment or the modification in these numbers at 3 months could predict the clinical response to anti-TNF. For that, 2 logistical regression models were carried out, one of them employed remission at 3 months as a dependent variable, considering DAS28 <2.6,¹⁰ and a second one in which the dependent variable was the existence of clinical response, defined as an improvement in the DAS28 of >1.2 after 3 months of treatment.¹² Variables mentioned in the last paragraph were included as independent variables.

Table 1
Patient's Characteristics

	Total	IL-15 High	IL-15 Low	<i>P</i>
Patients, n	75	24	51	
Women, n (%)	61 (81.3)	19 (79)	42 (86)	NS
Age, mean (SD), y	56 (14)	59 (12)	54 (14)	NS
Duration of disease, y	8 [4-15]	6 [4-15]	8 [4-15]	NS
Positive RF, n (%)	65 (87)	22 (92)	43 (84)	.08
Number of DMARD	3 [2-4]	4 [2-4]	3 [2-3]	.08
DAS28 baseline	5.9 (1)	5.5 (1.1)	6.1 (1)	.06
HAQ baseline	1.6 [1.1-2.1]	1.5 [1.12-2.25]	1.75 [1.12-2.12]	NS
PGA baseline	61 (16)	60 (19)	62 (14)	NS
CRP baseline, g/L	15 [9-24]	17 [9-25]	14 [8-22]	NS

Abbreviations: CRP, C-reactive protein; DAS28, disease activity score 28 joints; DMARD, disease-modifying anti-rheumatic drugs; HAQ, Health Assessment Questionnaire; IL, interleukin; PGA, physicians global assessment; RF, rheumatoid factor

Results

Characteristics of Patients With Elevated Serum Concentrations of IL-15

The population of this study is a subgroup of patients with severe RA who had an indication for treatment with anti-TNF. It is a population with long standing disease, predominantly women, with a larger percentage of seropositive disease than populations with prevalent RA¹³ and with elevated DAS28 and HAQ scores (Table 1). After classifying this population according to their IL-15 serum concentrations, we did not find major differences in the clinical profile of these patients according to whether the patients had high or low concentrations of this cytokine (Table 1). Differences in the percentage of patients with positive RF, number of previous DMARD and baseline DAS28 almost reached statistical significance, but it was unable to establish if one of the groups had a more severe RA than the other (Table 1).

In a prior study we had observed that in patients with long-standing RA there was a relationship between the numbers of IL-15 and the number of DMARD used in their follow-up. Therefore, because we found no significant differences in the evaluation of the disease by the physician, the DAS28, HAQ, and CRP in the baseline visit between the subgroups with high and low concentrations of IL-15 (Table 1), the number of previously employed DMARD was used as a proxy measure of severity. In the bivariate analysis we observed a tendency towards a greater use of DMARD in patients with elevated IL-15, which did not attain statistical significance (Table 1). Given that the number of DMARD could clearly be conditioned by disease duration, which oscillated between 7 months and 37 years in this population, we decided to carry out a multivariate analysis in which different factors that could affect the use of DMARD were included. The best model turned out to be the one that included the variables: hospital center, time since onset of disease, and serum concentration of IL-15 (Table 2). Patients from hospital 2 had received a significantly lower number of DMARD than those from hospital 1, although the incidence rate ratio (IRR) was only 40% less. Also, in a significant manner, for every 100 pg/mL of increase in the serum concentration of IL-15, there was a 20% increase of the IRR of DMARD use.

Table 2

Factors That Play a Role in the Number of Disease Modifying Drugs in Patients Within the Study

	Bivariate Number of DMARD	P	Multivariate IRR	P
Gender				
Women	3.1 (1.6)	NS	-	-
Men	2.8 (1.2)		-	-
Age, y	r=0.24	.06	-	-
Time since onset, y	r=0.17	.24	1.014	.22
RF, U/mL	r=0.12	.33	-	-
IL-15 baseline, pg/mL	r=0.21	.11	1.002	.03
Center 1	3 [3-5]	NS	Reference	-
Center 2	3 [1-4]		0.62	.04
Center 3	3 [2-4]		0.77	.24

Abbreviations: DMARD, disease-modifying anti-rheumatic drugs; IL, interleukin; IRR, incidence-rate ratio; RF, rheumatoid factor.

Serum Concentration of IL-15 Reduces With Anti-TNF Therapy, but Is Not Useful in Predicting Response to this Treatment

Values of IL-15 diminished in a significant manner after treatment with anti-TNF (Figure 1A). This effect was more manifest in patients with elevated concentrations of IL-15 (Figure 1B), with a clear correlation between the intensity of the reduction in these values related to those obtained in the baseline visit (Figure 1C).

However, we did not observe significant differences between responder and non-responder patients with respect to the initial serum concentrations, the final serum concentrations and the degree of reduction in the concentration of IL-15 with treatment (Figures 2A and B). Clinical improvement among patients with high IL-15 was 73.7%, versus 65.8% of patients with low IL-15 concentrations, although these differences were not statistically significant.

We did not observe a relationship between the initial numbers, the final numbers or the intensity in the reduction of IL-15, and the development of clinical remission, defined as a value of DAS28 \leq 2.6 (Figures 3A and B).

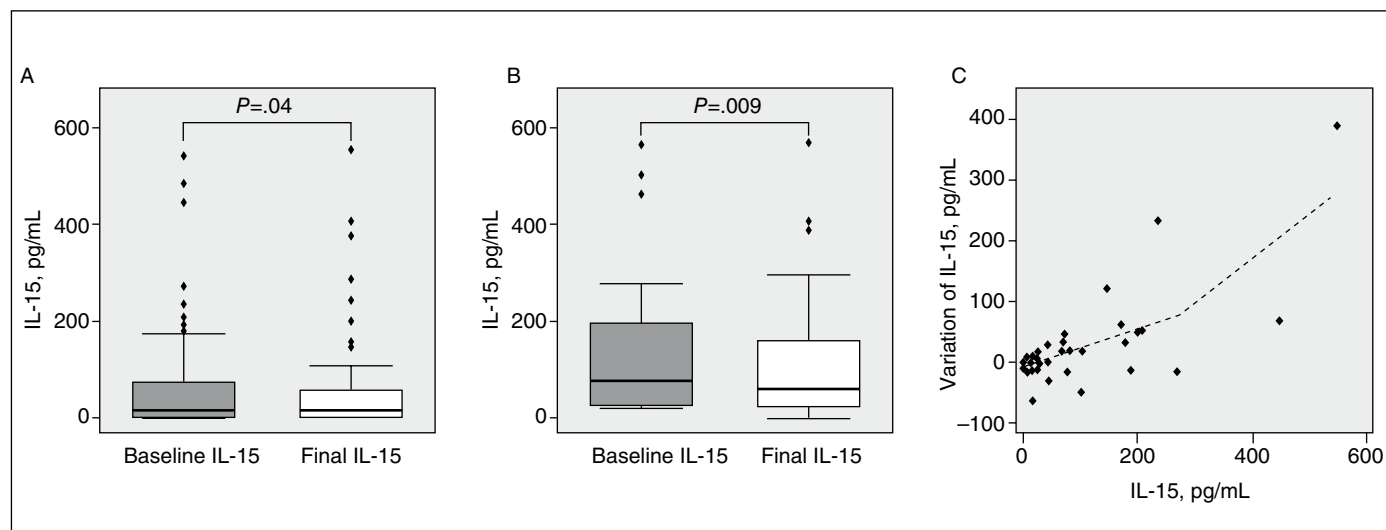


Figure 1. Serum concentrations of interleukin (IL) 15 are reduced after 3 months of treatment with tumor necrosis alpha inhibitors (TNF). A, total population. B, patients with elevated IL-15 (>30 pg/mL). Data are shown as median (middle line in the box) and percentiles 25 and 75 (inferior and superior borders of the box), 5 and 95 (horizontal lines outside the box). Statistical test: Wilcoxon for paired data. C, there is a correlation between the variations of IL-15 concentrations (baseline-final) and the baseline concentration. Data is shown as dispersion with a tendency line (discontinuous black line), estimated by the lowest smoothing function of Stata statistical software.

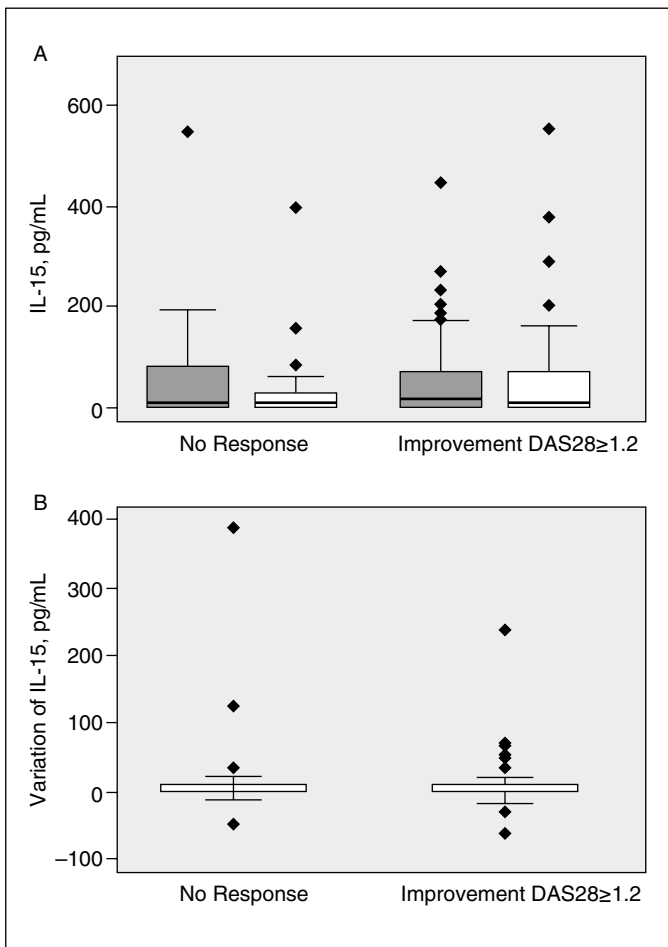


Figure 2. Distribution of interleukin (IL) 15 concentration according to the clinical response after 3 months with treatment based on tumor necrosis factor inhibitors (TNF). A, serum concentrations of IL-15 in the baseline visit (gray box) or final (white box). B, variation of IL-15 between both visits. In all cases, data is shown as a median (middle line of the box) and percentiles 25 and 75 (inferior and superior borders of the box), 5 and 95 (horizontal lines outside the box).

Discussion

The main finding described in the present study is the confirmation *in vivo* that TNF is implicated in the modulation of IL-15 expression. On the other hand, the results of this study seem to back our previous findings that indicated that patients with elevated IL-15 had received a larger number of DMARD throughout the progression of their disease.⁴

Different *in vitro* studies have shown that TNF is implicated in the modulation of IL-15 expression, confirmed by the increase in its mRNA transcription, manifesting an increase on the membrane of skin fibroblasts, synoviocytes of patients with RA, or muscle cells.^{8,14-17} However, because there are no studies that have demonstrated that TNF is capable of inducing the production of soluble IL-15 in cultures, it is probable that other co-adjuvating factors are necessary apart from the effect of TNF, in order to produce the liberation of soluble IL-15.^{2,18} Our data shows that the *in vivo* blockade of TNF was associated with a significant reduction in the serum concentration of IL-15. It may be possible that a reduction in IL-15 is associated with improvement in the disease activity independent of treatment given to the patients. However, this possibility seems remote, because in this study we did not observe a correlation between the intensity of improvement of disease activity and the variation in the concentrations of IL-15. In addition, in the

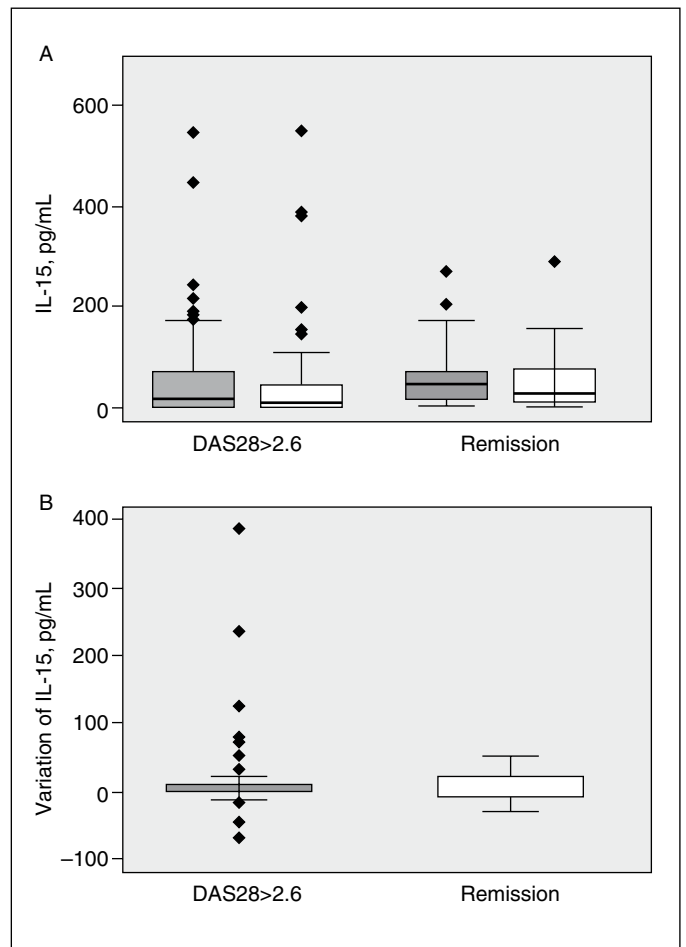


Figure 3. Distribution of the IL-15 concentration in relation to clinical remission after 3 months of tumor necrosis factor inhibitors (TNF). A, serum concentrations of IL-15 during the baseline visit (grey box) or final visit (white box). B, variation of IL-15 between both visits. In all of the cases data is shown as median (middle line of the box) and percentiles 25 and 75 (inferior and superior borders of the box), 5 and 95 (horizontal lines outside the box).

early arthritis registry of the Hospital de La Princesa, we have not seen a reduction in the values of IL-15 in patients undergoing DMARD therapy (manuscript under preparation).

Unfortunately, in this study we did not have information on the progression of disease activity or radiological data or complications of RA that would have allowed us to establish the severity of disease in a more truthful manner. In addition, because they were patients about to undergo therapy with anti-TNF, all of them presented elevated disease activity and functional limitations. Therefore, as a proxy measure of severity, we analyzed DMARD use. Patients with higher concentrations of IL-15 had received previous therapy with a larger number of DMARD in their follow up. Although the number of previously used DMARD depended on the duration of the disease and the variability of the physician's prescription habits,¹⁹ here we have observed that, adjusting for these parameters, the association in the use of DMARD and the concentration of IL-15 is still significant. The only inconvenience is that the motive for the suspension of previous DMARD treatment, whether due to secondary effects of lack of efficacy, went unregistered; this notwithstanding, a similar finding has already been described by our group in a previous study with patients with other characteristics.⁴ In addition, in patients with recent-onset arthritis followed in the database of the Hospital de La Princesa, we have described that patients with elevated IL-15 have worse disease progression in spite of receiving more treatment

than those with low IL-15 (oral communication at the SER 2007 meeting and a poster presentation at the 2007 ACR meeting).

Lastly, different studies have shown that stimulating peripheral lymphocytes with IL-15 allows them to induce the production of TNF by macrophages.^{5,7} Due to this, it would be expected that patients with elevated IL-15 serum concentrations had a spectacular response, or at least a partial one to TNF blockade. Unfortunately, our data does not allow us to establish whether the baseline measurement of IL-15 or the response of this cytokine to treatment with anti-TNF are useful in the prediction of remission, nor allow us to differentiate between partial or complete response, or the response to the absence of treatment with these drugs.

Financing

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Conflict of Interest

We have also received research funding from Abbott Laboratories for the following authors: Isidoro González-Álvaro, Ana M. Ortiz, and Rosario García de Vicuña.

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