Increased serum levels of interleukin-15 in rheumatoid arthritis with long term disease

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ABSTRACT

Objective. *To study the serum levels of IL-15 in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), seronegative spondyloarthropathies (SSd) and healthy donors.*

Methods. *The IL-15 serum levels were measured by ELISA in sera from 50 RA patients, 30 patients with SLE, 30 patients with SSd and 30 healthy donors. In RA patients, several clinical and demographic parameters were also obtained at the time of sample collection. IL-15 levels were compared in different RA subpopulations (positive or negative rheumatoid factor [RF], long term or recent onset disease, high or low disease activity). In addition, the possible association with other demographic and clinical parameters (gender, age, disease duration, etc) was also analysed.*

Results. *RA patients had significantly higher serum levels of IL-15 (102.4 ± 150 pg/ml; p = 0.0001) than SLE patients (9.8 ± 15.3 pg/ml), SSd patients (7.9 ± 14.6 pg/ml) and healthy donors (5.2 ± 11.6 pg/ml). RA patients with a disease evolution less than 2 years showed lower IL-15 levels (33.7 ± 62.2 pg/ml) than those with long-term disease (152.4 ± 64.6 pg/ml; p = 0.004). In addition, a significant correlation between IL15 in serum and the number of disease-modifying antirheumatic drugs (DMARDs) prescribed was detected in RA patients (r = 0.42; p = 0.002). No association between IL-15 levels and age, gender, RF or disease activity was observed in this group.*

Conclusion. *IL-15 is elevated in RA patients, specially in those with long term disease, compared to other rheumatic disorders. This finding supports that IL-15 is involved in the perpetuation of RA synovitis.*

Introduction

Interleukin-15 (IL-15) is a macrophage-derived cytokine that has biological functions similar to IL-2 (1). Interestingly, unlike IL-2, IL-15 has been detected in synovial membranes and fluids from patients with rheumatoid arthritis (RA) (1). This cytokine has chemotactic activity for memory peripheral blood lymphocytes (PBL), thus promoting their recruitment into inflamed tissues (1). IL-15-activated lymphocytes express surface molecules, such as CD69 or CD40L (2, 3), and promote TNF secretion by macrophages through interaction with their ligands (1). On the other hand, it has been described that TNF-activated synoviocytes can produce soluble IL-15 (4). All of these findings support the notion that this cytokine could play an important role in the perpetuation of TNF production in the RA synovium.

Recently, it has been described that IL-15 can be detected in the sera from RA patients (5-7). By contrast, other studies suggest that this cytokine can be detected in the sera of patients suffering from systemic lupus erythematosus (SLE), but not in that from RA patients (8). In this latter work, the authors detected even lower IL-15 levels in RA patients than in healthy controls.

We studied the serum levels of IL-15 in patients with RA, SLE, seronegative spondyloarthropathies (SSd) and healthy donors (HD), in order to provide new information that could help to resolve this discrepancy.

Materials and methods

Patients

Sera were obtained, with previous oral or written informed consent, from three groups of patients and 30 HD. The samples were stored at -40º C until assayed.

The RA group was composed of 50 patients who fulfilled the ACR criteria (9). Table I shows the clinical characteristics of these patients, both the whole group and different subgroups defined by disease duration, RF positivity or disease activity. Briefly, the mean age was 69 ± 2 years, 42 patients were females and the percentage of seropositive disease was 80%. The following data were obtained at the time of sample collection: global disease assessment by the patient, 28 joint counts (tender and swollen), pain on a visual analogical scale, ESR, RF (by nephelometry) and the disease-modifying antirheumatic drugs (DMARDs) prescribed during RA evolution. The disease activity score was obtained

from the 28-joint counts (DAS28) (10). Sera from 30 consecutive SLE patients undergoing systematic autoantibody detection at the Immunology Unit of H.U. La Paz were used as the SLE group. The mean age was 26 ± 7 years, and 90% were females.

The SSd group included 30 patients with the following diagnosis according to the European Spondylarthropathy Study Group criteria (11): 8 ankylosing spondylitis, 13 psoriatic arthritis, 4 reactive arthritis and 5 undifferentiated spondylarthropathies. The mean age was 50 ± 14 years, and 73% were males.

The mean age in HD group was 39 ± 14 years, and the percentage of female donors was 75% (n=30).

Quantitation of IL-15 serum levels

The IL-15 concentration in serum was measured using a sandwich enzyme immune assay (EIA). Briefly, 96-well high binding EIA plates (Costar, Cambridge, MA) were coated overnight at 4ºC with 50 µL/well of MAB647 MAb (anti-IL-15; R&D Systems Europe Ltd., Abingdon, UK) at 4 µg/ml in PBS, pH 7.4. Then, each well was washed twice with 200 µl of wash buffer (0.05% Tween 20 in PBS, pH 7.4) and blocked by adding 200 µl of PBS containing 2% BSA for 1 hour at 37ºC. Between each step, wells were washed three times with 200 µl of wash buffer. Then, 50 µl/well of diluent buffer (0.1% BSA, 0.05% Tween20, 20 mM Trizma base, 150 mM NaCl, pH 7.3) plus 50 µl of each sample and standard dilutions for recombinant human IL-15 (500 to 6.2 pg/ml; R&D Systems) were added to their respective wells (by duplicate) and incubated at room temperature (RT) for 2 h. Bound IL-15 was detected using 50 µl/well of BAM247 (biotynilated anti-IL-15 MAb; R&D Systems) diluted at 200 ng/ml in diluent buffer for 1 h at RT. After washing, 100 µl/well streptavidin HRP (Calbiochem, San Diego, CA) 1/5000 in diluent buffer were added for 20 min at RT, and then developed with 100 µl/well 3,3',5,5'-tetramethylbenzidine (Chemicon International Inc., Temecula, CA). The optical density of each well was determined using a microtiter plate reader LP400 (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) set to 450 nm, with wavelength correction set to 550 nm. Cytokine values were calculated from the standard curve. Samples that generate values higher than the highest standard were diluted (1:1) in diluent buffer and assayed again.

To exclude the possible interference of RF in the immune assay, every sample was analysed using both diluent buffer alone and supplemented with normal mouse immunoglobulin at 1 mg/ml (Calbiochem). No significant differences were observed in the IL-15 levels between samples processed in presence or absence of murine immunoglobulins. In addition, rheumatoid factor was measured by nephelometry in the same samples and no linear correlation was observed between IL-15 serum levels and rheumatoid factor titres.

The intra-assay variability was 15.4 ± 21.7% and the inter-assay variability was 21.9 ± 33.4% (mean ± standard deviation [SD]).

Statistical analysis

The IL-15 serum levels in the four groups (RA, SLE, SSd and HD) were compared by the Kruskal-Wallis test and between groups by the Mann-Whitney test with Bonferroni correction. The chi-square test was used to analyse the comparisons between groups for the categorical variables, and the Spearman correlation to assess the association between continuous variables without a normal distribution. Data are displayed as the mean ± SD.

Table I. Characteristics of patients with rheumatoid arthritis.

	Total	Rheumatoid factor			Disease duration			DAS28		
		Positive	Negative	p	RO	LT	p	< 3.2	≥ 3.2	p
N	50	40	10	-	21	29	-	9	41	-
Age	63 ± 14	64 ± 14	59 ± 15	ns	61 ± 15	65 ± 13	ns	54 ± 14	65 ± 14	0.02
Female	75.6%	72.7%	87.5%	ns	83.3%	72.4%	ns	62.5%	78.8%	ns
Disease duration (months)	86 ± 125	90 ± 24	46.6 ± 60	ns	9 ± 1	143 ± 27	< 0.001	45 ± 35	92 ± 138	ns
No. of DMARDs	1.9 ± 1.9	1.9 ± 2.1	1.1 ± 1.7	ns	0.2 ± 0.4	3.1 ± 1.7	< 0.001	1.5 ± 1.1	1.9 ± 2.2	ns
RF+	80%	100	0	-	80.9%	79.3%	ns	77.8	80.5	ns
CRP	2.6 ± 4	2.7 ± 4.3	2.1 ± 2.2	ns	1.1 ± 1.3	3.6 ± 4.8	< 0 .05	0.4 ± 0.3	3.1 ± 4.2	ns
ESR	35 ± 25	37 ± 26	29 ± 18	ns	32 ± 26	38 ± 25	ns	18 ± 9	39 ± 26	0.02
GDA	52 ± 29	55 ± 29	42 ± 26	ns	52 ± 28	53 ± 30	ns	28 ± 20	58 ± 28	0.003
TJC (28)	7.6 ± 7.1	7.7 ± 7.2	7.1 ± 6.8	ns	10 ± 7.4	5.8 ± 6.5	0.047	0.2 ± 0.7	9.2 ± 6.9	< 0.001
SJC (28)	7 ± 5.4	6.7 ± 5.4	8.3 ± 5.8	ns	7.8 ± 5.2	6.5 ± 5.7	ns	0.8 ± 2.3	8.4 ± 4.9	< 0.001
Pain (VAS)	56 ± 29	55 ± 31	60 ± 20	ns	54 ± 31	57 ± 29	ns	31 ± 28	62 ± 27	0.004
DAS28	5 ± 1.7	5.1 ± 1.7	4.8 ± 1.6	ns	5.3 ± 1.6	4.8 ± 1.8	ns	2.5 ± 0.5	5.6 ± 1.3	< 0.001

RF: rheumatoid factor; LT: long term; RO: recent onset; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; GDA: global disease assessment by the patient; TJC: tender joint count; SJC: swollen joint count; VAS: visual analogue scale; DAS28: disease activity score from 28 joint counts.

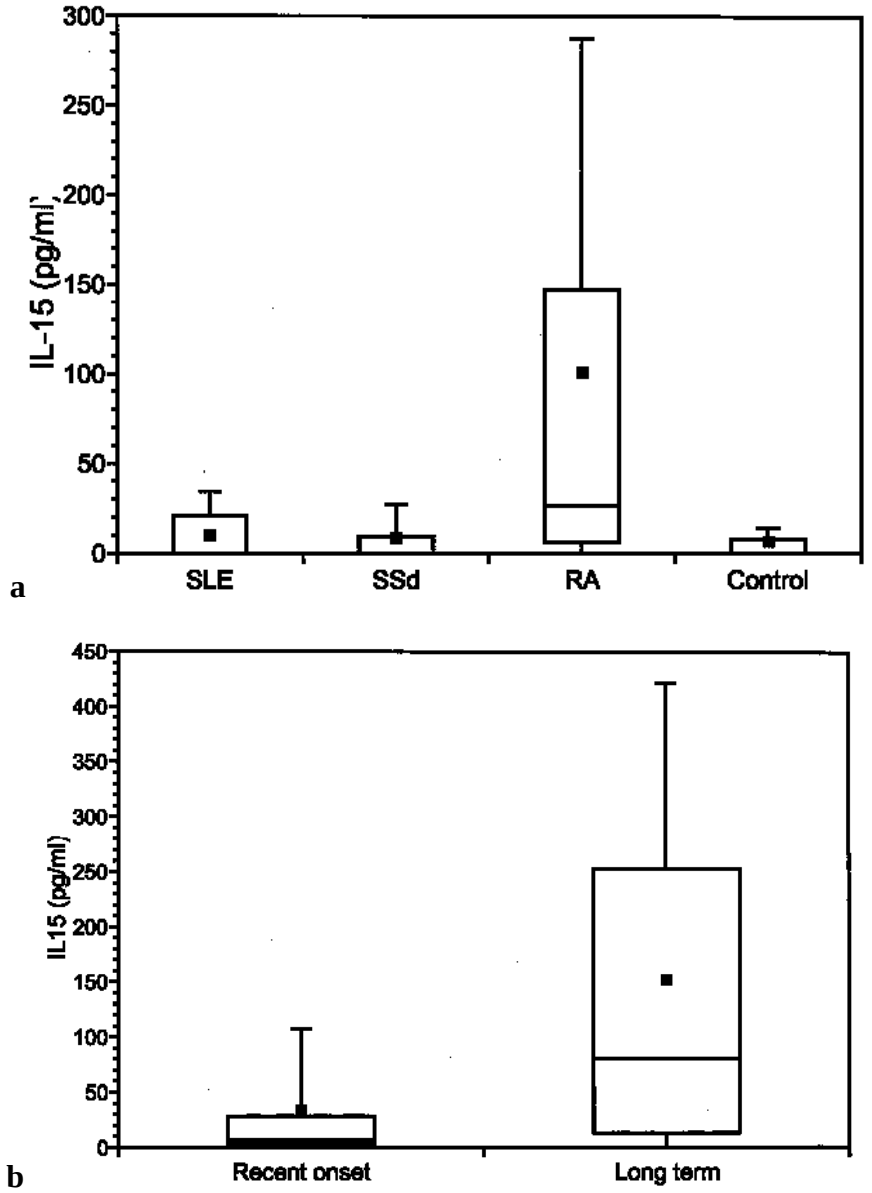

Fig. 1. (**a**) Distribution of serum IL-15 levels in patients with systemic lupus erythematosus (SLE), seronegative spondyloarthropathies (SSd),rheumatoid arthritis (RA) and healthy donors (Control). (**b**) Distribution of serum IL-15 levels in patients with rheumatoid arthritis depending on the disease evolution. The mean is represented by the black solid square and the median by the middle line; the edge of the boxes mark the 25th and 75th percentiles and the error bars the 10th and 90th percentiles for each group of patients.

Results

Serum IL-15 levels in the different groups of patients

Figure 1a shows the distribution of IL-15 concentration in the four groups studied. RA patients had significantly higher serum levels of this cytokine (102.4 ± 150 pg/ml; p=0.0001) than SLE patients (9.8 ± 15.3 pg/ml), SSd patients (7.9 ± 14.6 pg/ml) and HD (5.2 ± 11.6 pg/ml). No significant differences were observed between these three latter groups. In addition, there were no significant differences in IL-15 levels between the different groups of SSd patients: psoriatic arthritis (11.3 ± 16.2 pg/ml)), ankylosing spondylitis (12.2 ± 19.3 pg/ml), reactive arthritis (1.3 ± 2.7 pg/ml) and undifferentiated spondylarthropathies (0.3 ± 0.8 pg/ml). The distribution of IL-15 levels in each group was variable, although only the RA group had a marked number of patients (50% of the cases) with cytokine concentrations higher than 28 pg/ml (this value represents the mean + 2 SD of the mean IL-15 concentration in HD). No patient in the non-RA groups had an IL-15 concentration higher than 65 pg/ml, while 38% of RA patients exceeded this level.

We did not observe any association between IL-15 levels and age, gender or disease duration.

IL-15 in RA patients

When all RA patients were studied, no association was detected between IL-15 serum levels and the clinical parameters listed in Table I, except for a significant linear correlation between IL-15 and the number of DMARDs prescribed to each patient (r=0.42, p=0.002).

Next we analysed the concentration of this cytokine in the different subgroups of RA patients. No significant differences were observed in IL-15 concentrations between patients with seropositive (113.2 ± 159.3 pg/ml) and seronegative disease (59.3 ± 99.5 pg/ml). As shown in Table I, there were also no significant differences in clinical parameters between these two groups, probably due to the low number of seronegative RA patients in our sample. In addition, no differences were observed when patients were clustered according to low disease activity, defined as a DAS28 score lower than 3.2, or high disease activity (DAS28 ≥ 3.2) (10).

However, as is displayed in Figure 1b, patients with recent onset RA (less than 2 years; RO RA) showed significant lower levels of serum IL-15 (33.7 ± 62.2 pg/ml) than patients with long term (LT) disease (152.3 ± 174.6 pg/ml; p=0.004). Obviously, significant differences between these two groups were observed in the disease duration and number of DMARDs prescribed (Table I). In addition, the CRP levels were significantly higher and the tender joint count lower in LT RA patients than in the RO group (Table I). However, there was no correlation between serum IL-15 levels and clinical parameters in the two groups.

Discussion

It has been proposed that IL-15 can play a remarkable role in the perpetua-

tion of several autoinmune diseases, especially in RA (1, 6). Different authors have described that this cytokine can be detected in the serum of patients with inflammatory bowel disease, RA, SLE and other autoimmune diseases (6-8, 12). We have confirmed that IL-15 can be detected at relevant concentrations in patients with RA, and comparatively low or undetectable levels were measured in HD and patients with SSd or SLE. Regarding this latter disorder, our data are in opposition to that of Aringer *et al*. who described that SLE patients showed higher IL-15 levels than RA (8). However, the RA group in that study was only composed of 10 patients. Since in our study 20% of RA patients had very low IL-15 levels, some of them even below the detection limit, it is feasible that the data on RA patients in the study of Aringer *et al*. might not be representative of a prevalent RA cohort. In addition, consistent with our data, Robak *et al*. have described that IL-15 levels in SLE patients are similar to that in HD (13).

Regarding differences in IL-15 levels between the whole population of RA patients, we did not observe an association between serum concentrations of this cytokine and treatment, disease activity or gender, nor any linear correlation between IL-15 levels and age or disease duration. Thus, it is possible that IL-15 serum levels may define different subsets among all patients who fulfil the RA criteria: some with high IL-15 serum levels and others with low or undetectable IL-15 in serum. We need prospective studies to confirm that the presence of elevated IL-15 serum levels is a sustained feature in a subset of RA patients.

Concerning the differences observed between RO- and LT-RA patients, this could be related to the selection of severe forms of the disease in the LT-RA group, as is suggested by the higher CRP levels and the higher number of DMARDs prescribed in this group compared to RO-RA patients. Furthermore, patients in the early arthritis group, although all of them fulfilled the RA criteria,could represent a more heterogeneous population with a different prognosis and clinical course.

Since IL-15 serum levels correlate with the number of DMARDs prescribed to RA patients, a feature usually associated with a poor evolution, it would be interesting to study whether RA patients with increased serum IL-15 levels are at higher risk of radiological progression and functional disability than those patients with low or undetectable IL-15 levels in their serum. In this regard, as the benefits of IL-15 blocking therapies have been demonstrated in animal models (14), it will be interesting to know the outcomes of current clinical assays in humans.

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