Human Immunology 71 (2010) 874–877

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

# Measurement of interleukin-1 receptor antagonist in patients with systemic lupus erythematosus could predict renal manifestation of the disease

Boglarka Brugos [a], Emese Kiss [a], Csaba Dul [b], Wolfgang Gubisch [c], Gyula Szegedi [a], Sandor Sipka [a], Margit Zeher [a,*]

[a] 3rd Department of Internal Medicine, Division of Clinical Immunology, Medical and Health Science Center of University of Debrecen, Debrecen, Hungary
[b] Biomedica Gruppe, Budapest, Hungary
[c] Biomedica Gruppe, Wien, Austria

ARTICLE INFO

*Article history:*
Received 23 January 2010
Accepted 1 June 2010
Available online 9 June 2010

*Keywords:*
Active SLE
Creatinine clearance
Interleukin-1 receptor antagonist
Lupus nephritis
Methylprednisolone

ABSTRACT

Interleukin-1 receptor antagonist (IL-1Ra) is a good indicator of disease activity in patients with systemic lupus erythematosus (SLE). Glucocorticosteroids are the most frequently used drugs in SLE. Our goal was to compare IL-1Ra activity in SLE patients with and without renal involvement and to determine the effect of different dosage of glucocorticosteroids used in 17 patients with active SLE without nephritis, 7 patients with inactive lupus nephritis (LN), and 8 patients with active LN, along with 10 healthy controls. IL-1Ra levels were measured in the serum of SLE patients by Human Luminex [100] analyzer. Both in patients with active SLE without nephritis and in patients with LN, serum levels of IL-1Ra ($p<0.001$) were significantly higher compared with those in the controls. IL-1Ra was significantly higher in patients with active LN than in patients with inactive LN ($p = 0.028$). The use of methylprednisolone was significantly higher in the active LN group compared with the inactive LN group ($p = 0.013$). SLE patients with higher IL-1Ra are at lower risk for developing nephritis. The higher doses of glucocorticosteroids needed in active LN could be due to steroid resistance and IL-1Ra polymorphism. Measurement of IL-1Ra levels in SLE patients could help to predict future renal involvement.



## 1. Introduction

The interleukin (IL)–1 family of cytokines includes two major agonist molecules (IL-1$\alpha$ and IL-1$\beta$) with proinflammatory effects and a specific antagonist for the IL-1 receptor named interleukin-1 receptor antagonist (IL-1Ra) [1–4]. IL-1Ra is the natural antagonist of IL-1$\beta$. IL-1Ra exists in four isoforms; one isoform is the secreted form (sIL-1Ra), and the other three forms remain intracellular [5]. The secreted form (sIL-1Ra) is produced mainly by monocytes, macrophages, neutrophils, and fibroblasts [6], and acts as an anti-inflammatory agent as competitors of IL-1. The balance between IL-1 and IL-1Ra is important in the regulation of inflammatory responses [7,8].

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by dysregulation of the immune system as a whole, culminating in the production of pathologic autoantibodies [9–11]. Several cytokines have also been implicated in the pathogenesis of SLE, as tumor necrosis factor–$\alpha$ (TNF-$\alpha$), interferon-$\gamma$ (IFN-$\gamma$), IL-18, IL-6 and IL-1 forming a rationale also for therapeutic intervention in the disease [12]. IL-1As a proinflammatory cytokine is implicated in the pathogenesis of lupus nephritis (LN). IL-1 is expressed by mesangial cells in the kidneys *in vivo*. It was shown that IL-1 is a mesangial growth factor in experimental mesangioproliferative nephritis and IL-1Ra treatment reduced mesangial cell proliferation [13]. Both secreted and intracellular forms of IL-1Ra are produced by the kidneys [14]. In SLE patients with renal involvement an increased frequency of IL-1Ra allel 2 (IL-1RN*2) has been found, mediating a pro-inflammatory IL-1b/IL-1Ra balance [15].

Suzuki et al. found that high serum levels of IL-1Ra were a good indicator of disease activity [16]. Sturfelt et al. demonstrated that IL-1Ra levels varied within the disease course of SLE. Very high concentrations of IL-1Ra were detected in patients with extrarenal manifestations (4847 pg/ml), whereas in patients with kidney involvement, levels of IL-1Ra were only moderately elevated (363 pg/ml) [7]. Although IL-1 may play role in the pathogenesis of LN, pharmacologic inhibition of IL-1 with IL-1Ra in animals was without effect [17]. Treatment with methylprednisolone reduced plasma IL-1Ra levels [18].

In the current study, we analyzed the changes in the serum levels of IL-1Ra, creatinine, creatinine clearance, and glomerular filtration rate (GFR) in patients with active SLE and with extrarenal signs, and in patients with renal involvement with active LN (ALN; proteinuria >1 g/day and active urinary sediment) and inactive LN

S. Sipka and M. Zeher contributed equally to this work.
* Corresponding author.
E-mail address: zeher@iiibel.dote.hu (M. Zeher).

0198-8859/10/$32.00 - see front matter © 2010 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2010.06.004

(IALN; proteinuria <1 g/day and inactive urinary sediment), along with healthy controls. In addition, proteinuria, correlation with C-reactive protein (CRP) level, and classical parameters of disease activity such as serum C3, C4, nucleosome, anti–double-stranded DNA, β2-glycoprotein, and anticardiolipin levels were also tested in all groups. Correlation and effect of methylprednisolone administration on IL-1Ra level were also analyzed.

## 2. Subjects and methods

### 2.1. Patients

The study subjects were as follows: 17 consecutively selected SLE patients who were treated and followed up by the 3rd Department of Internal Medicine, Division of Clinical Immunology, University of Debrecen, Hungary. The subjects included 17 patients (16 female and 1 male) with active SLE without LN (mean age ± SD: 47.58 ± 11.9 years, range 24–59 years); 15 SLE patients (14 female and one male) with histologically proven LN, comprising seven patients (all female) with clinically inactive LN (ILN; mean age 38.4 ± 11.62 years, range 21–52 years) and eight patients (seven female and one male) with clinically active LN (ALN; mean age 39.16 ± 7.9 years, range 19–46 years); and 10 healthy control subjects (5 female and 5 male; mean age 32.4 ± 9.5 years, range 22–38 years). All patients fulfilled four or more of the classification criteria for SLE [19]. In the ILN group, five patients had diffuse LN (group IV), which was found to be the most frequent subgroup among our treated patients [20], and two patients membranous LN. In the ALN group, six patients had diffuse LN (group IV), one mesangial (group II), and one patient membranous LN (group V). The histologic findings were recorded based on the classification of the International Society of Nephrology and the Renal Pathology Society [21]. Clinical disease activity was measured based on the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score [22]. The values of the median SLEDAI score were 4.5 ± 3.6 for active SLE patients, 2.66 ± 3.2 for ILN patients, and 6.85 ± 1.78 for ALN ($p$ between groups = 0.089, by analysis of variance). Details of the current clinical/laboratory characteristics, and immunosuppressive treatment of the patients are listed in Table 1.

### 2.2. Methods

Peripheral blood samples of active patients were obtained during hospitalization, for inactive patients during routine SLE visits. Serum was separated and frozen at −70°C until assayed for IL-1Ra, complement, and antibody levels.

IL-1Ra level was determined by Fluorokine MAP cytokine multiplex kits designed for Luminex 100 analyzer using analyte-specific antibodies (Biomedica Hungária Kft. Budapest, Hungary). The serum was used undiluted in the assay.

C3 and C4-levels were detected by BN II. Dade-Behring Nephelometer (normal values C3: 0.9–1.8 g/l; C4: 0.1–0.4 g/l), anti-dsDNA antibody levels were determined with enzyme-linked immunosorbent assay (ORGENTEC Diagnostika, Mainz, Germany; cut-off 25 U/ml) according to the manufacturer's instructions. Anticardiolipin antibodies (cut-off 10 U/ml) and anti–β2-glycoprotein (cut-off 10 U/ml) levels were detected with ELISA (ORGENTEC Diagnostika, Mainz, Germany) (normal range for both antibodies <10 U/ml). Values for classical disease activity markers are shown in Table 2.

Routine laboratory measures for the patients were also recorded: serum creatinine level (normal range 44–106 μmol/l), creatinine clearance (normal range: 95–160 ml/min), GFR (normal range 90–120 ml/min/1.73m$^2$), urine protein (<0.1 g/day), serum CRP level (normal range 0–5 ng/l). Infection was excluded in patients with high CRP levels.

### 2.3. Statistical methods

All statistical analyses were carried out using SPSS version 13.0 software (SPSS Inc., Chicago, IL), and values were expressed as the mean ± SD. The comparison of data from multiple groups was made using analysis of variance, from two groups by independent-sample $t$ test. IL-1Ra, sCRP, anti–β2-glycoprotein, anticardiolipin levels were not distributed normally and so the significance of differences between levels of patients compared with controls were assessed using the Mann–Whitney test. Correlation analysis was made using Pearson's parametric and Spearman's non-parametric tests. Values of $p < 0.05$ were considered statistically significant.

## 3. Results

### 3.1. IL-1Ra levels in SLE patients

IL-1Ra levels were measured in serum samples taken at a single time-point from consecutively selected SLE patients with active disease without renal involvement (mean duration of SLE 9.68 ± 7.74 years) and in patients with ALN and ILN (mean duration of SLE 8.42 ± 5.53 and 8.87 ± 6.7 years, respectively). The mean duration of LN was 4±2.88 years for ALN patients and 3.25 ± 4.74 years for patients with ILN.

Patients with active SLE had the highest IL-1Ra levels (1025 ± 948 pg/ml); the difference was significant compared with all other groups and controls (Fig. 1).

### 3.2. Correlations of IL-1Ra and measurements of disease activity markers

To determine the relationship between levels of IL-1Ra and other disease activity markers, we carried out Pearson's and Spear-

**Table 1**
Baseline clinical/laboratory characteristics and immunosuppressive therapy of patients with systemic lupus erythematosus (SLE)

| Patients groups<br>Clinical laboratory characteristics | Active SLE ($n$ = 17) | ALN ($n$ = 8) | ILN ($n$ = 7) |
|---|---|---|---|
| Age, y (mean ± SD) | 47.58 ± 11.9 | 39.16 ± 17.9 | 38.4 ± 11.6 |
| SLEDAI score | 4.52 ± 3.6 | 6.85 ± 1.78 | 2.66 ± 3.25 |
| Rash | 3 | — | — |
| Arthritis | 7 | — | — |
| Cerebrovascular accident | 1 | — | — |
| Pleuritis | 1 | — | — |
| Hypocomplementemia | 7 | 5 | 4 |
| Increased DNA binding | 12 | 2 | 4 |
| Proteinuria (>0.5 g/day) | — | 7 | 3 |
| Thrombocytopenia/leukopenia | 1 | 3 | — |
| Therapy | 17 (mean 16 ± 8 mg) | 8 (22.85 ± 10.4 mg) | 6 (11.3 ± 4.67 mg) |
| Methylprednisolone | — | 4 (mean 550 mg/month) | 1 (1.5 mg/kg/day) |
| Cyclophosphamide | | | |

ALN, active lupus nephritis; IAN, inactive lupus nephritis; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.