

# Elevated Serum Interleukin-15 Levels in Systemic Lupus Erythematosus

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## Abstract

Interleukin-15 (IL-15) has multiple biological properties, including the induction of other cytokine production and the inhibition of T cell apoptosis. Recently, IL-15 was reported to have a major role in synovial inflammation of rheumatoid arthritis, and that it provokes and amplifies the inflammatory process through the activation of TNF- $\alpha$  production. In systemic lupus erythematosus (SLE), the dysregulation of apoptosis and various cytokine production were observed and have been implicated in the pathogenesis of SLE. Thus, we tried to determine serum IL-15 levels in SLE patients and to assess the relationship among IL-15 levels, TNF- $\alpha$  levels and disease activity of SLE. Twenty SLE patients and 10 controls were studied. Paired serum samples were collected from all SLE patients at the time of presentation with active disease and at 4 weeks after institution of treatment. IL-15 levels were determined by ELISA and compared with the disease activity indices in SLE. The disease activity of SLE was measured using the SLE Disease Activity Index (SLEDAI) and laboratory parameters such as circulating immune complex (CIC), C3, C4, anti-DNA antibody, IgG, IgM, and IgA. The IL-15 levels in SLE patients were significantly higher than those of controls ( $5.38 \pm 4.89$  vs.  $1.04 \pm 1.26$  pg/ml). However, elevated IL-15 levels did not correlate with the SLEDAI, nor did they correlate with other laboratory activity indices. The changes in serum IL-15 levels did not correlate with the changes in serum TNF- $\alpha$  in the disease course of SLE patients, whereas TNF- $\alpha$  reflected the changes in disease activity of SLE. Serum levels of IL-15 are elevated in SLE patients, but IL-15 did not correlate with the disease activity of SLE. TNF- $\alpha$  production in SLE patients was unlikely to be related with IL-15.

**Key Words:** Systemic lupus erythematosus, interleukin-15, TNF- $\alpha$

## INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease which is characterized by aberrant immune regulation, B cell hyperactivity, and exuberant autoantibody production. Studies of cytokines in SLE patients have suggested that cytokines may play an important role in the pathogenesis of SLE.<sup>1,2</sup> Pro-inflammatory cytokines such as, IL-2, IL-6, IL-8, INF- $\gamma$  have been found to be increased in the active stage of SLE.<sup>3-6</sup>

IL-15 is a novel 14–15 kDa cytokine expressed in

numerous normal human tissues, including skeletal muscle and kidney, as well as in cell types such as activated monocytes and fibroblast.<sup>7-9</sup> The biologic functions of IL-15 are similar to those of IL-2 but with no significant sequence homology.<sup>8</sup> It mediates its functions through the  $\beta$ - and  $\gamma$ -chains of the IL-2 receptor and its own unique  $\alpha$ -chain.<sup>10</sup> The multiple biological effects of IL-15 have been described. It induces T cell proliferation, chemotaxis, cytokine production and cytotoxicity, as well as reducing T cell apoptosis.<sup>11,12</sup> Neutrophil activation, cytoskeletal rearrangement, and protection from apoptosis by IL-15 have been reported, as has induction of mast cell proliferation.<sup>13</sup> IL-15 induces natural killer cell cytotoxicity and antibody-dependent cell-mediated cytotoxicity, upregulates production of NK cell-derived cytokines,<sup>14</sup> and can costimulate proliferation and differentiation of B cells activated with anti-immunoglobulin M.<sup>15</sup> IL-15 represents a mechanism whereby host tissues can contribute to the early phase of immune responses, providing enhancement of polymorphonuclear and NK-cell responses, and

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subsequently T cell responses, prior to optimal IL-2 production. The corollary to such pleiotropic activity may be a propensity to chronic, rather than self-limiting, inflammation, which showed IL-15 synthesis to be aberrantly regulated.<sup>16</sup>

Pathological IL-15 expression has now been described in several chronic diseases, including pulmonary sarcoid,<sup>17</sup> leprosy,<sup>18</sup> ulcerative colitis<sup>19</sup> and rheumatoid arthritis (RA). In RA, IL-15 could play a pivotal role in RA pathogenesis, acting upstream of TNF- $\alpha$  in orchestrating the induction of a cascade of inflammatory cytokines.<sup>16</sup>

With these observations in mind, we were concerned about the role of IL-15 in SLE in which the dysregulation of apoptosis<sup>20</sup> and the production of various cytokines exist. This study was designed to determine serum IL-15 levels in SLE patients and to assess the relationship among IL-15 levels, TNF- $\alpha$  levels and the disease activity of SLE.

## MATERIALS AND METHODS

### Subjects

We studied 20 Korean patients (2 males and 18 females, mean age  $26.5 \pm 5.9$  years; range 18 to 37) who met the 1982 criteria of the American College of Rheumatology for SLE. All patients were treated at Severance Hospital, Yonsei University Medical Center, Seoul, Korea. Ten healthy donors were used as controls (1 male and 9 females, mean age  $27.5 \pm 5.3$  years; range 18 to 37). We used the SLE Disease Activity Index (SLEDAI)<sup>21</sup> as a measure of clinical disease activity. The disease activity of each SLE patient was assessed at initial presentation and at post-treat-

ment with steroid and/or immunosuppressive agents. Blood samples were taken at each point. Informed consent was obtained from the patients and controls included in this study. The mean duration of treatment was one month. Patients at initial presentation were in a flare-up state of disease. Patients at post-treatment were treated with an increased steroid dose or by adding immunosuppressive agents, and most of them were in an improved state after treatment. At initial presentation, 13 patients were taking steroid only, while 2 patients were taking steroid in combination with immunosuppressive agents. The remaining 5 patients were presenting for the first time or were not taking any treatment. The mean dose of daily prednisolone intake for patients taking steroid was  $15.9 \pm 22.7$  mg (range 0 to 60) (Table 1).

### Serum IL-15 level

The serum IL-15 level was determined by using a commercial IL-15 ELISA kit (Endogen, Woburn, MA, U.S.A). 100  $\mu$ l of IL-15 standard concentrate and each patient's sera were put onto a plate precoated with rat anti-IL-15 monoclonal antibody, and the plate was incubated for 1 hour at room temperature. After washing 3 times, 100  $\mu$ l of biotinylated anti-IL-15 antibody was added into each well and incubated for 1 hour at room temperature, and then washed again 3 times. 100  $\mu$ l of horseradish peroxidase-conjugated streptavidin was put into each well and the plate was incubated for 30 minutes at room temperature and washed again 3 times. 100  $\mu$ l of 3,3',5,5' tetramethylbenzidine and hydrogen peroxide substrate were put into each well and an enzymatic color reaction was allowed to develop at room temperature for 15 minutes with a plate sealer. Then

Table 1. Characteristics of SLE Patients and Controls

	SLE † (n=20)	Controls (n=10)
Age* (years)	$26.5 \pm 5.9$ (18–37)	$27.5 \pm 5.3$ (18–37)
Sex (male/female)	2/18	1/9
Duration of disease* (month)	$22.8 \pm 24.1$ (0–65)	
Daily prednisolone intake* (mg)	$15.9 \pm 22.7$ (0–60)	
SLEDAI*	$13.3 \pm 7.3$ (3–33)	

\* mean  $\pm$  value SD (range).

† initial presentation of SLE patients.

the reaction was stopped by adding 100  $\mu$ l of 1 N hydrochloric acid. Absorbance at 450 nm was measured by a Spectra Max 340 Microplate Reader (Molecular Device Co., Sunnyvale, CA, U.S.A.). The IL-10 level in serum was calculated according to the concentration curve of absorption. The assay range of this IL-15 ELISA was from 0 to 500 pg/ml and it did not cross-react with other human cytokines.

### Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )

The serum TNF- $\alpha$  level was determined by using a commercial TNF- $\alpha$  ELISA kit (Endogen, Woburn, MA, U.S.A.). 50  $\mu$ l of TNF- $\alpha$  standard concentrate and each patient's sera were put onto a plate pre-coated with anti-human TNF- $\alpha$  monoclonal antibody. 50  $\mu$ l of biotinylated anti-TNF- $\alpha$  antibody was added into each well and incubated for 2 hours at room temperature, and then washed again 3 times. 100  $\mu$ l of horseradish peroxidase-conjugated streptavidin was put into each well and the plate was incubated for 30 minutes at room temperature and washed again 3 times. 100  $\mu$ l of 3,3',5,5' tetramethylbenzidine and hydrogen peroxide substrate were put into each well and an enzymatic color reaction was allowed to develop in the dark at room temperature for 30 minutes. Then the reaction was stopped by adding 100  $\mu$ l of stop solution. Absorbance at 450 nm was measured by a Spectra Max 340 Microplate Reader (Molecular Device Co., Sunnyvale, CA, U.S.A.). The TNF- $\alpha$  level in serum was calculated according to the concentration curve of absorption. The assay range of this TNF- $\alpha$  ELISA was from 0 to 1,000 pg/ml and it did not cross-react with other human cytokines.

### Circulating immune complex (CIC)

CIC was measured by modified Singh's method (solid phase C1q ELISA method).<sup>22</sup>

### Other serologic tests

Anti-dsDNA antibody was measured using a commercial Fluoro nDNA test (MBL Co., Nagoya, Japan) which detected anti-dsDNA antibody by indirect immunofluorescence. Complement C3, C4 and immunoglobulin IgG, IgM, IgA were measured by Equil Nephelometry (Behring Co., Behring, Germany).

### Statistics

All data were analyzed by the SPSS package program. The comparison of IL-15 levels and TNF- $\alpha$  levels between SLE and controls were done using Mann-Whitney test. The correlations between parameters were analyzed by Spearman's rank correlation test. A level of  $p < 0.05$  was considered statistically significant.

## RESULTS

### Serum IL-15 level

The mean serum IL-15 level in SLE patients was  $5.38 \pm 4.89$  pg/ml (mean  $\pm$  SD; range: 0–18.2 pg/ml), and the mean serum IL-15 level in controls was  $1.04 \pm 1.26$  pg/ml (range: 0–3.26 pg/ml). The serum IL-15 levels in SLE patients were significantly higher than controls ( $p < 0.01$ ) (Fig. 1). However, the mean serum IL-15 level in SLE patients at initial presentation was not higher than at post-treatment ( $5.2 \pm 4.8$  vs  $5.6 \pm 5.1$ , respectively).

### Serum IL-15 level and disease activity

There was no significant correlation between IL-15 levels and SLEDAI, or between IL-15 and laboratory activity indices (Table 2). To evaluate the change in serum IL-15 levels in relation to the change of disease

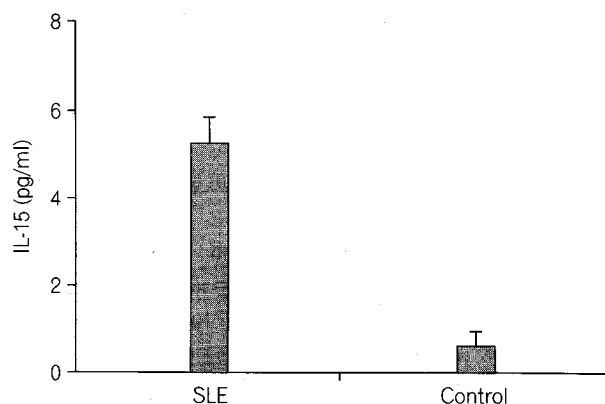


Fig. 1. Serum IL-15 levels in SLE patients and controls. The mean level of IL-15, indicated by the horizontal line and error bar, was significantly higher in SLE patients than in controls (mean  $\pm$  SE,  $5.38 \pm 0.77$  vs  $1.04 \pm 0.45$  pg/ml,  $p < 0.01$ ).

Table 2. Correlations between Laboratory Activity Indices and Clinical Activity Index

	SLEDAI		IL-15	
	r	p*	r	p*
IL-15	-0.08	0.68	—	
CIC	0.37	0.02	-0.10	0.56
C3	-0.24	0.19	-0.12	0.23
C4	-0.09	0.63	0.01	0.25
Anti-DNA Ab	0.34	0.12	-0.01	0.98
Ig G	0.06	0.85	-0.48	0.11
Ig M	0.24	0.47	-0.49	0.12
Ig A	0.11	0.75	-0.51	0.09
TNF- $\alpha$	0.56	<0.01	-0.22	0.17

\*Calculated by Spearman's rank correlation test; A level of  $p < 0.05$  is considered statistically significant.

activity, we compared changes in the SLEDAI (between initial presentation and post-treatment) with changes in IL-15 levels (between initial presentation and post-treatment) in paired samples of SLE patients. There was no significant correlation between changes in serum IL-15 levels and changes in the SLEDAI ( $r=0.12$ ).

#### Serum TNF- $\alpha$ level

The mean serum TNF- $\alpha$  level in SLE patients was  $76.3 \pm 236.6$  pg/ml (mean  $\pm$  SD; range: 0–1099.6 pg/ml), and the mean serum TNF- $\alpha$  level in controls was  $12.9 \pm 8.5$  pg/ml (range: 2.1–25.8 pg/ml), but the difference was statistically insignificant ( $p=0.40$ ). The mean serum TNF- $\alpha$  level in SLE patients at initial presentation was higher than at post-treatment ( $99.7 \pm 267.2$  vs  $52.9 \pm 206.2$ , respectively), but this was not significant either ( $p=0.54$ ) (Fig. 2).

#### Disease activity and serum TNF- $\alpha$ level

There was a positive correlation between serum TNF- $\alpha$  levels and the SLEDAI ( $r=0.56$ ,  $p < 0.01$ ), as well as a significant correlation between TNF- $\alpha$  and anti-DNA Ab ( $r=0.45$ ,  $p < 0.05$ ). To evaluate the change in serum TNF- $\alpha$  levels in relation to the change of disease activity, we compared changes in the SLEDAI with changes in TNF- $\alpha$  levels in paired samples of SLE patients. There was no significant correlation between changes of serum TNF- $\alpha$  levels and

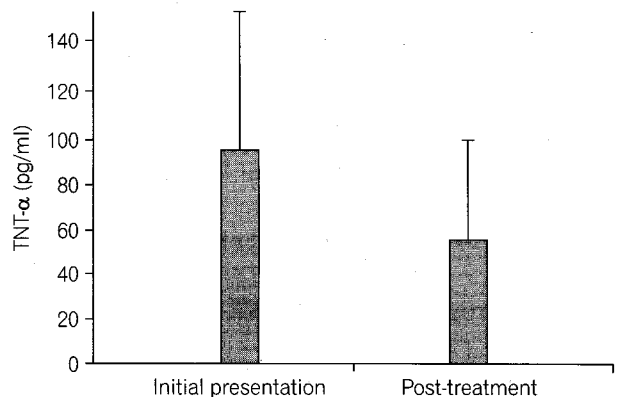


Fig. 2. Serum TNF- $\alpha$  level in SLE patients. The mean serum TNF- $\alpha$  level in SLE patients at initial presentation was higher than at post-treatment ( $99.7 \pm 267.2$  vs  $52.9 \pm 206.2$ , respectively), but this was not significant ( $p=0.54$ ).

changes in the SLEDAI. As well, there was no significant correlation between the change of IL-15 level with the change of TNF- $\alpha$  level in paired samples of our SLE patients (Table 2).

## DISCUSSION

IL-15 has a potent pro-inflammatory property, mainly due to the induction of TNF- $\alpha$ .<sup>16</sup> In active inflammatory bowel disease (IBD) patients, the levels of pro-inflammatory cytokines such as IL-1, IL-6, IL-8 and TNF- $\alpha$  are increased to a greater extent and reflect disease activity.<sup>23</sup> The IL-15 expression is also elevated in active IBD patients and it is reduced with the advent of improvement. That increased production of IL-15 has implied an on-going inflammatory process.<sup>19</sup> In RA, IL-15 was reported to be involved in the regulation of TNF- $\alpha$  production.<sup>16</sup> Locally-produced IL-15 in synovium can recruit and activate synovial T cells, which then amplify and perpetuate inflammation through the induction of monocyte-derived TNF- $\alpha$  via a cell-contact-dependent mechanism.<sup>24-26</sup> Thus, IL-15 could play a pivotal role in RA pathogenesis, acting upstream of TNF- $\alpha$  in orchestrating the induction of a cascade of inflammatory cytokines.<sup>16</sup> Considering these pro-inflammatory properties of IL-15 in several autoimmune diseases, we surmised that IL-15 would be increased as a pro-inflammatory cytokine in SLE patients.

We found that serum IL-15 was significantly higher

in SLE patients than in normal controls. This implied that IL-15 may have a role in SLE, but we found that serum IL-15 levels did not correlate with the SLEDAI in the presence of a significant relationship between the SLEDAI and classic laboratory activity indices<sup>27</sup> such as CIC in this study. These observations suggested that serum IL-15 levels did not reflect the disease activity of SLE. The role of IL-15 in the pathogenesis of SLE is not yet known. In SLE, there is dysregulation in the production of various cytokines and a complex cytokine network. Considering that the levels of IL-15 did not correlate with the disease activity, even though IL-15 is involved in the cytokine network, then IL-15 may not be a major cytokine in SLE. In our study, the level of IL-15 was elevated at both time-points in the disease course, but the levels of IL-15 were not higher at the initial active point than at post-treatment. At the latter point, the inflammation of SLE was not fully suppressed (SLEDAI:  $8.5 \pm 6.7$ ; range: 0–29), so that inflammation still existed then. Therefore, the elevation of IL-15 through the disease process of SLE was most likely thought to be nonspecific elevation during the on-going inflammatory process.

Serum TNF- $\alpha$  levels reflected the disease activity of SLE in our study (Table 2). The role of TNF- $\alpha$  as a pro-inflammatory cytokine has been well documented. However, studies on TNF- $\alpha$  in SLE have led to some controversial results. In murine lupus, the application of low-dose TNF- $\alpha$  apparently accelerated the disease in NZB/NZW F1 mice,<sup>28</sup> whereas high doses retarded it.<sup>29,30</sup> Moreover, the administration of anti-TNF- $\alpha$  monoclonal antibodies to mice treated with anti-IL-10 antibodies was shown to reverse the protective effects of anti-IL-10 treatment.<sup>31</sup> In other animal models, however, TNF- $\alpha$  was ineffective or even exacerbated the disease.<sup>32</sup> Obviously, the effects of TNF- $\alpha$  not only depended on the disease model investigated, but also on the cytokine dosage. Therefore, data from animal models cannot easily be extrapolated to the human situation. In SLE patients, controversial observations have been reported. Slightly-elevated TNF- $\alpha$  serum levels were seen only during chronic infections,<sup>33</sup> or TNF- $\alpha$  was undetected while serum levels of soluble TNF-receptors were found to be increased.<sup>34</sup> Other studies showed that TNF- $\alpha$  levels were elevated and correlated with the disease activity of SLE,<sup>35</sup> or that they were elevated but did not correlate with disease activity.<sup>36</sup>

Our data agreed with Studnicka-Benke, et al.<sup>35</sup> in that TNF- $\alpha$  correlated with disease activity, but that the changes of serum TNF- $\alpha$  levels between paired time-points did not accurately reflect the changes in the SLEDAI. The discrepancy may be explained as follows: Though TNF- $\alpha$  correlated with disease activity, TNF- $\alpha$  did not have a primary pathologic role in provoking inflammation, but only reflected the on-going inflammatory process in SLE. Previous controversial reports of TNF- $\alpha$  in human lupus indirectly support our explanation. Serum TNF- $\alpha$  levels did not show significant mutual correlation with serum IL-15 levels. In contrast to RA, the regulation of TNF- $\alpha$  production in SLE was unlikely to be related to IL-15.

Until now, there has been no report about IL-15 in SLE. This study shows that serum IL-15 levels increased in SLE patients, but that IL-15 does not correlate with the disease activity of SLE.

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