

## CONCISE REPORT

# Raised serum interleukin 15 levels in Kawasaki disease

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**Background:** Interleukin (IL)15 is a novel cytokine that induces T cell proliferation, B cell maturation, natural killer cell cytotoxicity, and may have a pivotal role in the pathogenesis of inflammatory disease, acting upstream from tumour necrosis factor  $\alpha$  (TNF $\alpha$ ). Kawasaki disease (KD) is an inflammatory disease, in which serum levels of inflammatory cytokines such as TNF $\alpha$  and IL6 are increased.

**Objective:** To examine the serum levels of IL15 in KD and to evaluate the role of IL15 in estimating the severity of inflammation in KD.

**Results and conclusion:** There was a significant increase in the mean (SD) serum levels of IL15 measured in the acute stage of KD (11.5 (5.8) pg/ml) compared with those in the subacute stage (1.3 (0.9) pg/ml) ( $p < 0.01$ ) and normal controls (0.9 (1.0) pg/ml) ( $p < 0.01$ ). The increase in IL15 correlated with the increase in TNF $\alpha$  ( $r_s = 0.66$ ,  $p < 0.01$ ); however it did not correlate with the levels of erythrocyte sedimentation rate and C reactive protein, suggesting that IL15 may not be a useful marker in estimating the severity of inflammation in KD.

Since Kawasaki disease (KD) was first reported in Japan by Dr Kawasaki,<sup>1</sup> the clinical manifestations and natural history of KD have been studied extensively. However, its aetiology and pathophysiology still remain unclear. With medical progress and the development of antibiotics, improved living standards, and education on hygiene, the incidence of rheumatic heart disease has been reduced remarkably, and KD is beginning to be recognised as a major cause of acquired heart disease these days. KD can be considered an inflammatory disease because there are changes in T and B cell levels in patients with the disease, and an increase in inflammatory cytokines, such as tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin (IL) 6.

IL15 is a novel cytokine of 14–15 kDa that is found in activated monocytes, fibroblasts, normal muscle cells, and human epidermal keratinocytes.<sup>2–4</sup> IL15 has similar features to those of IL2,<sup>5</sup> and requires the  $\beta$ ,  $\gamma$  chain of IL2 receptor (IL2R) for its combination and signal transduction.<sup>6</sup> IL15 is especially involved in natural killer cell cytotoxicity, increasing the secretion of cytokines from natural killer cells such as interferon  $\gamma$ , granulocyte macrophage colony stimulating factor, and TNF $\alpha$ ,<sup>7–9</sup> and thus acting as one of the proinflammatory cytokines.

Clinically, an increase in IL15 levels was reported in several inflammatory diseases such as rheumatoid arthritis<sup>10</sup> and systemic lupus erythematosus.<sup>11</sup> This strongly suggests that IL15 may be related to KD also. In this study we measured the levels of IL15 during the acute and subacute phases of KD. We also aimed to show whether IL15 might be useful in assessing the degree of inflammation in KD by comparing and analysing other inflammatory indicators and IL15 levels.

## SUBJECTS AND METHODS

### Subjects

Forty serum samples were obtained from 20 patients (12 male) both in the acute (0–2 weeks) and subacute stages (2–4 weeks) of KD. These patients were all admitted to the Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. All patients fulfilled at least five of the six criteria for the diagnosis of KD. Atypical KD was excluded in this study. The control sera were obtained from 10 age matched children (six male) who were having routine blood samples taken before elective surgical procedures. The mean (SD) age of patients and control groups was 2.1 (0.7) years and 2.7 (0.9) years, respectively. There was no difference in the age and sex ratio between the patients and the control groups. Informed consent was obtained from the parents of the children included in the study.

### IL15 and TNF $\alpha$ levels by enzyme linked immunosorbent assay (ELISA)

Serum was stored at  $-70^\circ\text{C}$  until measured for levels of IL15 and TNF $\alpha$  by an ELISA kit (Endogen, Woburn, MA, USA). ELISA was performed according to the manufacturer's manual.

### Measurement of erythrocyte sedimentation rate (ESR) and C reactive protein (CRP)

CRP was measured by TDx equipment (Abbott Inc, North Chicago, IL, USA). ESR was measured by the Westergren method.

### Statistical analysis

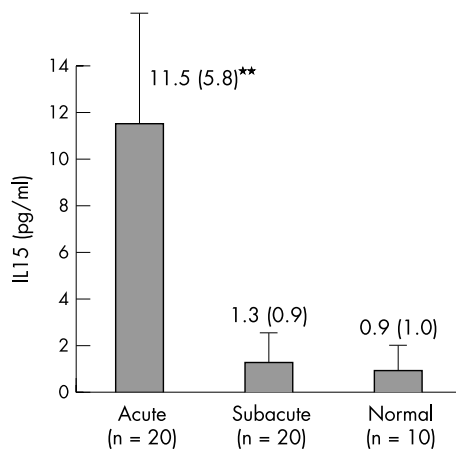
A paired *t* test was used to compare the paired levels of IL15 and TNF $\alpha$  in the acute and subacute phase serum samples. The Wilcoxon signed rank test was used to compare the values of the IL15 levels in the acute phase of KD groups and control groups. The correlation between IL15 level and TNF $\alpha$ , ESR, and CRP was measured by the Spearman's rank correlation test. A value of  $p < 0.05$  was regarded as significant.

## RESULTS

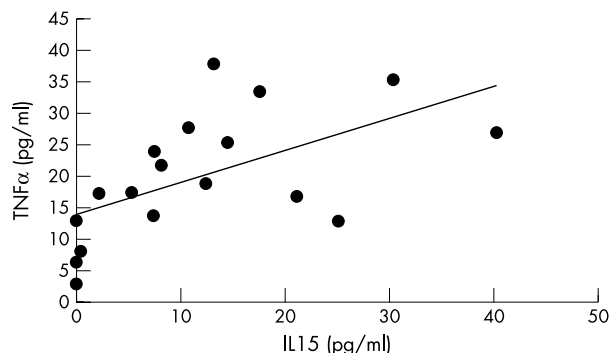
The mean (SD) IL15 level was 11.5 (5.8) pg/ml in the acute phase, higher than in the subacute phase (1.3 (0.9) pg/ml) ( $p < 0.01$ ). The serum IL15 level of control groups was 0.9 (1.0) pg/ml, lower than in the acute phase ( $p < 0.01$ ); however there was no significant difference between controls and the subacute phase of disease (fig 1). The mean (SD) TNF $\alpha$  level was 24.1 (9.4) pg/ml in the acute phase, higher than in the subacute phase (11.8 (5.8) pg/ml) and in controls (10.4 (4.9) pg/ml) ( $p < 0.01$ ).

The serum IL15 level correlated with the TNF $\alpha$  level, which means that IL15 level was high in patients whose TNF $\alpha$  level was high ( $p < 0.01$ ,  $r_s = 0.66$ ) (fig 2). On the other hand, the

**Abbreviations:** CRP, C reactive protein; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; IL, interleukin; KD, Kawasaki disease; TNF $\alpha$ , tumour necrosis factor  $\alpha$ .



**Figure 1** Serum levels of IL15 in patients in the acute phase of KD, compared with the subacute phase and normal controls \*\* $p < 0.01$ .



**Figure 2** Correlation between IL15 and TNF $\alpha$  levels in patients with KD.

serum IL15 level in the acute phase did not correlate with the CRP level and the ESR level ( $p > 0.05$ ) (data not shown).

## DISCUSSION

The clinical manifestations of KD have been studied extensively; however, its aetiology and pathophysiology remain unclear. Various cytokines such as IL1, TNF $\alpha$ , interferon  $\gamma$ , IL6, and IL10 have been reported to increase during the acute phase of KD.<sup>12-14</sup> TNF $\alpha$  has a central role in the pathogenesis of vasculitis in KD.<sup>15</sup> The TNF $\alpha$  308 A/G genotype was overrepresented among white subjects with KD who had coronary artery abnormalities compared with those with normal echocardiograms.<sup>16</sup>

IL15, a proinflammatory cytokine, activates TNF $\alpha$ , thereby inducing inflammation. It has been reported that IL15 levels increase in some inflammatory diseases. In inflammatory bowel diseases IL15 levels increase as the disease progresses, and decrease when the disease improves.<sup>12</sup> In rheumatoid arthritis, IL15 in synovium activates T cells and worsens inflammatory reactions by inducing TNF $\alpha$ .<sup>13</sup> In recent studies, it has been reported that IL15 levels increase in patients with systemic lupus erythematosus, showing the role of IL15 in inflammatory diseases.<sup>11</sup>

These findings strongly suggest that IL15 levels may increase in KD also. In this study, we tried to observe changes in the levels of the inflammatory precursor IL15, and to find out how effective IL15 might be in assessing the degree of inflammatory reaction in KD.

Upon comparing IL15 levels in the acute phase, subacute phase, and controls, we found that IL15 levels were higher in the acute phase than in the subacute phase and controls. Considering the immunological changes in KD, we suggest that T

cell activation and B cell proliferation are related to the increase in IL15.

An increase in the inflammatory precursor IL15 would lead to an increase in other factors involved in inflammation, especially TNF $\alpha$ . So, we compared TNF $\alpha$  levels with IL15 levels and found that IL15 levels were related to TNF $\alpha$  levels. As IL15 levels increased, so did TNF $\alpha$  levels. According to our results, the inflammatory precursor IL15 increases in the acute phase of KD, which induces TNF $\alpha$  proliferation and leads to an inflammatory reaction.

Besides TNF $\alpha$ , other inflammatory indicators such as CRP and ESR were measured in order to determine whether IL15 might be used as an effective indicator for assessing the degree of inflammation in KD.

Contrary to our expectations, there was no correlation between IL15 and either CRP or ESR. The acute phase reactants were affected by TNF $\alpha$ , IL6, or other various inflammatory cytokines. IL6 was especially more closely related to the acute phase reactants.<sup>16</sup> For this reason, it was thought that the IL15 level did not correlate with both indicators.

Because coronary artery disease is an important inflammatory reaction in KD, the clinical and immunological role of IL15 in coronary artery disease must be researched. Among the 20 subjects of our study, however, only one patient had coronary artery disease. Therefore it was impossible to determine the correlation between IL15 levels and coronary artery disease.

With a larger number of patients, we should define the pathological role of IL15 in KD by comparing IL15 levels in patients with and without cardiovascular complications. Also the source of the increased serum IL15 levels must be found and the role of IL15 in KD elucidated.

## CONCLUSION

Serum IL15, which induces inflammation in KD, increases in the acute phase and decreases in the subacute phase. We found a correlation between IL15 and TNF $\alpha$  levels. However, there was no significant correlation between IL15 levels and the acute phase reactants CRP and ESR. Thus, IL15 cannot be an effective indicator in assessing the degree of inflammation in KD.

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