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Soluble ST2 as a marker of disease activity in systemic juvenile idiopathic arthritis

Sayaka Ishikawa, Masaki Shimizu *, Kazuyuki Ueno, Naotoshi Sugimoto, Akihiro Yachie

Department of Pediatrics, School of Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Japan

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ABSTRACT

To assess the role of interleukin (IL)-33 and ST2, the receptor for IL-33, in the pathogenesis of systemic juvenile idiopathic arthritis (s-JIA), we sequentially measured the serum levels of IL-33 and soluble ST2 (sST2) in patients with s-JIA and determined their correlation with measures of disease activity and severity. Twenty-four patients with s-JIA, 5 with rheumatoid factor positive polyarticular JIA (RF + poly-JIA), and 20 age-matched healthy controls (HCs) were analyzed. IL-33 and sST2 levels were quantified in serum by enzyme-linked immunosorbent assays. Serum IL-33 levels in most patients with active s-JIA were below the lowest detection limit. Serum IL-33 levels in patients with RF + poly-JIA were significantly higher than those in patients with s-JIA and HC. Serum sST2 levels in patients during the active phase of s-JIA were much higher than those in patients with poly-JIA and HC. Serum sST2 levels in patients with s-JIA were significantly elevated even in the inactive phase, when other clinical parameters were normalized. Serum sST2 levels correlated positively with the clinical parameters of disease activity. These findings indicate that ST2 may be an important mediator in s-JIA. Serum sST2 levels in patients with s-JIA correlated with disease activity, suggesting a potential role as a promising indicator of disease activity.

1. Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous and multifactorial autoimmune disease characterized by chronic joint inflammation in children with onset ages younger than 16 years. It is the most common chronic rheumatic disease of childhood and an important cause of short-term and long-term disability. JIA has different subtypes that are defined based on the number of joints involved in the first 6 months of disease and the extra-articular involvement [1]. The subtypes include oligoarticular JIA (<5 joints), polyarticular JIA (poly-JIA) (⩾5 joints) and systemic JIA (s-JIA). s-JIA is defined by arthritis with spiking fever persisting for more than 2 weeks and at least one of the following clinical features of systemic inflammation: skin rash, lymphadenopathy, hepatosplenomegaly or serositis (pleuritis or pericarditis) [1]. Markedly distinct clinical and laboratory features of poly-JIA and s-JIA indicate their distinct pathogenesis and immunologic abnormality. The pathogenesis of JIA is not fully understood but likely includes genetic and environmental factors which show some commonality to various adult rheumatic diseases e.g. rheumatoid factor positive (RF+)poly-JIA and adult RA, s-JIA and adult Still's disease [2,3]. High numbers of autoreactive T cells within the joint of poly-JIA patients indicate an antigen-driven activation of the adaptive immune system [4]. However, the typical clinical signs of s-JIA are rather associated with granulocytosis, thrombocytosis and increase of acute-phase reactants in the peripheral blood, indicating an uncontrolled activation of the innate immune system. Recent studies have shown that inflammatory cytokines, including interleukin (IL)-1, IL-6, and IL-18, play pathogenic roles in the disease processes of s-JIA [5]. Furthermore, the treatment with biologic therapies to block these cytokines has a dramatic effect for s-JIA patients [5]. These findings indicate that autoinflammatory mechanisms seem to play a major role in the pathogenesis of s-JIA.

Macrophage activation syndrome (MAS) is a severe, potentially life-threatening complication of s-JIA. It is clinically characterized by fever, hepatosplenomegaly, lymphadenopathy, profound depression of all three blood cell lines, deranged liver function, intravascular coagulation, and central nervous system dysfunction [6]. The excessive activation and proliferation of T lymphocytes and macrophages are observed in MAS. Massive hypercytokinemia is strongly associated with the pathogenesis of MAS [6].

Interleukin-33 (IL-33) is a novel IL-1 family cytokine that plays a major role in inflammatory, infectious, and autoimmune diseases [7,8]. IL-33 was identified as the ligand for the orphan receptor, ST2 [9]. ST2 molecule is a member of the IL-1 receptor family that exists in two forms: a transmembrane full-length form and a soluble,

Abbreviations: s-JIA, systemic juvenile idiopathic arthritis; MAS, macrophage activation syndrome; sST2, solubleST2; IL, interleukin; LPS, lipopolysaccharide; RF, rheumatoid factor.

* Corresponding author. Address: Department of Pediatrics, School of Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-8641, Japan. Tel: +81 76 265 2314; fax: +81 76 262 1866.

E-mail address: shimizum@staff.kanazawa-u.ac.jp (M. Shimizu).

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secreted form (sST2) [10]. sST2 acts as a decoyreceptor for IL-33 [11].

IL-33 affects the function of cells that express ST2 molecule. IL-33 polarizes naive T cells to produce Th2-associated cytokines IL-4, IL-5 and IL-13 [7] and functions as a chemoattractant for Th2 cells in vitro and in vivo [12], but also induces secretion of proinflammatory cytokines and chemokines bymast cells [13], basophils [14] and Th1 type cytokines from NK and NKT cells [14,15]. Also, IL-33 amplifies polarization of alternatively activated M2 macrophages [16], induces maturation of dendritic cells [17] and may promote Th1-type response [18].

Besides the regulation of disease outcome through the modulation of Th1/Th2 bias, there is some evidence to suggest that ST2 may also be involved in inflammatory responses. A previous report revealed that the sST2-Fc fusion protein suppressed inflammatory responses that were induced by lipopolysaccharide (LPS) both in vitro and in vivo [19]. In normal conditions, the serum concentration of sST2 is below the detectable level, but elevated level of sST2 has been reported in patients with autoimmune diseases [20], asthma [11], idiopathic pulmonaryfibrosis [23]. The sST2 levels were found to correlate with the activity and severity of these conditions [21–24].

In this way, IL-33 and ST2 have important functions in host defense, immune regulation, and inflammation. However, its role in the pathogenesis of s-JIA and a causal relationship with disease activity are still unclear. To assess the role of IL-33 and ST2, in the pathogenesis of s-JIA, we sequentially measured serum levels of IL-33 and sST2 in patients with s-JIA and determined their correlation with measures of disease activity and severity.

2. Methods

2.1. Patients and samples

Serum samples were obtained from 24 patients with s-JIA, 5 patients with RF + poly-JIA, and 20 age- and sex-matched healthy controls (HC) [age, s-JIA: 8.9 ± 6.5 years and HC: 10.5 ± 7.4 years]. Eleven patients were evaluated longitudinally on a second occasion when their disease was in an inactive phase. Four patients with MAS were evaluated serially from the phase of MAS to remission. The clinical characteristics of the patients with s-JIA are shown in Table 1.

Diagnoses of s-JIA and RF + poly-JIA were based on the International League of Associations for Rheumatology criteria [1]. MAS was diagnosed based on the combination of cytopenias affecting at least two cell lines, coagulopathy, and liver dysfunction, according to the guidelines proposed by Ravelli et al. [24]. The criteria defining the active phase of s-JIA were active arthritis, fever, rash, hepatosplenomegaly, generalized lymphadenopathy, and serositis as well as increased erythrocyte sedimentation rates and C-reactive protein (CRP) levels. The criteria for the inactive phase of s-JIA on medication were as follows: the first time after the recovery from MAS with no clinical symptoms that were observed in the active phase, as well as normal erythrocyte sedimentation rates (<5 mm/h) and CRP levels (<0.1 mg/dL). The criterion for remission of patients with s-JIA on medication was six continuous months of inactive disease while on medication [25].

Table 1
Clinical characteristics of patients with systemic juvenile idiopathic arthritis during the active phase. CRP, C-reactive protein; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; PSL, prednisolone; MTX, methotrexate.

	S-JIA
Patients	24
Sex (male:female)	11:13
Age (years)	10 (1–26)
Disease duration (years)	0.1 (0–11)
Laboratory finidings	
CRP (mg/dl) (n = 24)	8.41 (1.6–25.8)
AST (IU/l) (n = 24)	39 (11–136)
LDH (IU/l) (n = 24)	344 (162–1359)
Ferritin (ng/mL) (n = 17)	864 (250–17,484)
Treatment	
PSL (mg/day) (n = 9)	17.5 (5–37.5)
MTX (mg/m^2) (n = 2)	10

Serum was separated from cells, divided into aliquots, frozen, and stored at −80 °C until use. This study was approved by the Institutional Review Board at Kanazawa University, and all specimens were used after informed consent was obtained.

2.2. Quantification of serum cytokines

Levels of IL-33 were evaluated by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Human IL-33 DuoSet® ELISA Development System, R&D Systems, Inc., Minneapolis, MN, USA). Levels of sST2 were evaluated by ELISA according to the manufacturer's instructions (Human ST2/IL-1R4DuoSet® ELISA Development System, R&D Systems, Inc., Minneapolis, MN, USA). The range of ELISA for IL-33 was from 3.6 to 1500 pg/mL. The range of ELISA for sST2 was from 31.5 to 2000 pg/mL. RF positivity did not interfere with the ELISA assay.

2.3. Statistical analysis

Within-group comparisons were analyzed by the Mann–Whitney test. Correlations were expressed using the Spearman rank correlation coefficient. For the analyzed measures, $P < 0.05$ was considered significant.

3. Results

3.1. Serum levels of IL-33 and sST2

We determined the serum levels of IL-33 and sST2 in patients with s-JIA and compared them with the levels in patients with RF + poly-JIA and HC. Serum IL-33 levels in most patients with active s-JIA were found to lie below the lowest detection limit of the assay (Fig. 1A). IL-33 was detected in 4 out of 24 s-JIA patients (17%) 9 out of 20 control subjects (45%). The differences in the serum IL-33 levels among these s-JIA patients (median, 68; range, 4–702 ng/mL), and HC (median, 50; range, 4–169 ng/mL) were not statistically significant. On the other hand, serum IL-33 levels in RF + poly-JIA patients (median, 155; range, 61–533 ng/mL) were significantly elevated compared with those in active s-JIA patients and HC ($P < 0.01$). These patients did not demonstrate symptoms that were suggestive of allergic diseases or atopy at the time of the study.

In contrast, serum sST2 levels in patients with MAS (median, 19,500; range, 3680–61,000 pg/mL) and in patients in the active phase of s-JIA (median, 2205; range, 496–43,000 pg/mL) were much higher than those in patients with RF + poly-JIA (577, 338–1120 pg/mL) and HC (354, 102–1052 pg/mL)(Fig. 1B). Serum sST2 levels in patients with s-JIA were significantly elevated even in the inactive phase (839, 360–4550 pg/mL), and they normalized in the remission phase (402, 267–512 pg/mL). The serum sST2 levels in RF + patients were not significantly different compared to HC.

3.2. Markedly elevated concentrations of serum sST2 in patients in the active phase of s-JIA and MAS

To investigate the relevance of sST2 to the pathogenesis of s-JIA and MAS, serum sST2 levels were serially monitored in four cases