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Disruption of vascular endothelial homeostasis in systemic juvenile idiopathic arthritis-associated macrophage activation syndrome: The dynamic roles of angiopoietin-1 and -2

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ABSTRACT

To assess the role of angiopoietin (Ang)-1 and Ang-2 and to investigate the clinical significance of serum levels of them in systemic juvenile idiopathic arthritis (s-JIA)-associated macrophage activation syndrome (MAS), we determined these levels in 51 patients with s-JIA, 11 patients with polyarticular JIA (poly-JIA), 12 patients with virus associated hemophagocytic syndrome (VAHS), 12 patients with Kawasaki disease (KD), and 15 age-matched healthy controls (HC). The results were compared with clinical features of MAS. During the MAS phase, serum Ang-1 levels were significantly decreased compared with those during the active and inactive phases. Serum Ang-2/1 ratio were significantly elevated during the MAS phase, compared with those during the active and inactive phases. There was a rapid increase in the Ang-2/1 ratio at the onset of MAS. Serum Ang-1 and the Ang-2/1 ratio significantly correlated with measures of disease activity, including AST and LDH. Ang-2/1 dysregulation was also observed in patients with VAHS, whereas not observed in most cases of KD. The homeostasis of vascular endothelial function by Ang-1 and Ang-2 is disrupted in MAS. Serum Ang-1 levels and the Ang-2/1 ratio might represent promising indicators of disease activity for MAS.

1. Introduction

Systemic juvenile idiopathic arthritis (s-JIA) is a unique subtype of JIA, characterized by arthritis and other systemic features including spiking fever, salmon colored skin rash, hepatosplenomegaly, generalized lymphadenopathy, and polyserositis [1]. Recent studies have shown that s-JIA might be driven by innate proinflammatory cytokines. In particular, interleukin (IL)-1, IL-6, IL-18 play an important roles in the pathogenesis of s-JIA [2,3]. Furthermore, biological therapies that block these cytokines have dramatic effects in patients with s-JIA [4,5]. These findings support the hypothesis that s-JIA is an autoinflammatory condition.

Macrophage activation syndrome (MAS) is a potentially fatal condition of s-JIA, characterized by fever, cytopenias, hepatosplenomegaly, lymphadenopathy, liver dysfunction, coagulopathy, and central nervous system dysfunction [6]. MAS is a secondary form of hemophagocytic lymphohistiocytosis (HLH) and massive hypercytokinemia induced by excess activation of macrophages and proliferation of T lymphocytes is closely associated with the development of MAS [6].

Vascular endothelial cells activated by inflammatory cytokines act as a procoagulant surface and contribute to intravascular coagulation of s-JIA [2]. Angiopoietins (Ang)-1 and -2 are key regulators of endothelial cell function [7,8]. In inflammatory conditions including sepsis and hemolytic uremic syndrome, the Ang-2/1 ratio increases because of decreased Ang-1, increased Ang-2 [9–12]. We speculated that dysregulation of the Ang-2/1 is also present in s-JIA, and that the homeostasis of vascular endothelial function by Ang-1 and Ang-2 is disrupted in MAS.

To assess the role of Ang-1 and Ang-2 in endothelial damage in the pathogenesis of s-JIA and MAS, we measured serum Ang-1 and Ang-2 levels in patients with s-JIA. We compared them with the

Abbreviations: Ang, angiopoietin; s-JIA, systemic juvenile idiopathic arthritis; MAS, macrophage activation syndrome; poly-JIA, polyarticular juvenile idiopathic arthritis; VAHS, virus associated hemophagocytic syndrome; KD, Kawasaki disease; HC, healthy control; IL, interleukin.

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levels in patients with virus associated hemophagocytic syndrome (VAHS), a secondary form of HLH as well as MAS and Kawasaki disease (KD), a popular pediatric vasculitis which complicates the vascular endothelial damage. We determined the correlation between the levels of serum Ang-1and Ang-2 levels, and the Ang-2/1 ratio with measures of disease activity and severity in order to clarify the clinical significance of these levels as indicators of disease activity for MAS.

2. Materials and methods

2.1. Patients and samples

Serum samples were obtained from 51 patients with s-JIA, 11 patients with polyarticular JIA (poly-JIA), 12 patients with Kawasaki disease (KD), and 15 age-matched healthy controls (HC) (mean age s-JIA: 7.9 ± 5.4 years, mean age poly-JIA: 11.8 ± 5.0 years, mean age KD: 1.5 ± 1.4 years, mean age HC: 9.3 ± 7.8 years). Plasma samples were obtained from 12 patients with virus associated hemophagocytic syndrome (VAHS) (mean age VAHS: 5.0 ± 4.0 years). Eleven patients with s-JIA had MAS, and seven of these had already developed MAS by the time they were referred to us at the onset of s-JIA. Four patients with s-JIA developed MAS during the active phase after beginning immunosuppressive therapy with steroids and/or tocilizumab. Samples from these four patients were obtained during the active and inactive phases of s-JIA. The clinical characteristics of the patients with active s-JIA are shown in Table 1. The clinical characteristics of the patients with MAS are shown in Supplementary Table 1. Furthermore, samples from 16 patients with s-JIA were obtained during the active and inactive phases of s-JIA. The diagnoses of s-JIA and poly-JIA were based on the criteria of the International League of Associations for Rheumatology [13]. MAS was diagnosed based on the guidelines proposed by Ravelli et al [14]. The criteria for the active phase of s-JIA were defined as follows: active arthritis, fever, salmon colored rash, hepatosplenomegaly, generalized lymphadenopathy, polyserositis, increased erythrocyte sedimentation rate, and increased serum C-reactive protein (CRP) level. The criteria for the inactive phase of s-JIA on medication were as follows: the absence of clinical symptoms observed in the active phase of s-JIA, normal erythrocyte sedimentation rate, and normal serum CRP level. The diagnosis of VAHS was made according to established HLH diagnostic criteria [15]. The diagnosis of KD was based on the classic clinical criteria [16].

Table 1
Clinical characteristics of patients with active systemic juvenile idiopathic arthritis. CRP, C-reactive protein; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; FDP-DD, fibrin degradation product D-dimer; PSL, prednisolone; CyA, cyclosporine; MTX, methotrexate; TCZ, tocilizumab.

Patients	44
Sex	Male 20 female 24
Age (years)	8.4 ± 5.5
Disease duration (months)	11.2 ± 39.0
Laboratory findings	
CRP (mg/dl) (n = 43)	10.1 ± 7.1
AST (IU/L) (n = 48)	47.5 ± 33.1
LDH (IU/L) (n = 48)	367.1 ± 150.2
Ferritin (ng/ml) (n = 48)	1669.4 ± 2926.7
FDP-DD (n = 34)	6.1 ± 7.0
Treatment	
PSL (mg/kg/day) (n = 13)	0.77 ± 0.55
CyA (mg/kg/day) (n = 5)	2.7 ± 1.6
MTX (mg/m^2/week) (n = 1)	10
TCZ (mg/kg/2 weeks) (n = 5)	8

Sera and plasma were separated from the 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 only after informed consent was obtained according to the Declaration of Helsinki.

2.2. Quantification of serum cytokines

Levels of Ang-1 and Ang-2 were evaluated using enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (RayBio Human Angiopoietin-1, -2 ELISA kit, RayBiotech, Norcross, GA, USA). The levels of Ang-1 and Ang-2 could be measured equally well in plasma and serum samples.

2.3. Statistical analysis

Within-group comparisons were analyzed using the Mann–Whitney test or paired t-test. Correlations were analyzed using the Spearman rank correlation coefficient. Analyzed measures with p values <0.05 were considered to be statistically significant.

3. Results

3.1. Disruption of vascular endothelial homeostasis by Ang-1 and Ang-2 in s-JIA and MAS

The serum Ang-1 and Ang-2 levels were determined in patients with s-JIA, and compared with the levels in patients with poly-JIA, VAHS, KD and in HC. As shown in Fig. 1A, during the MAS phase in patients with s-JIA, the serum Ang-1 levels were significantly decreased (41,583 ± 27,773 pg/ml) compared with those during the active (100,866 ± 42,949 pg/ml, $p = 0.0001$) and inactive phases (120,553 ± 33,076 pg/ml, $p < 0.0001$) in patients with s-JIA, and compared with those in patients with poly-JIA (101,850 ± 25,951 pg/ml, $p < 0.01$), in KD (114,517 ± 23,080 pg/ml, $p < 0.001$) and in HC (100,740 ± 31,718 pg/ml, $p < 0.001$). Serum Ang-1 levels in patients with VAHS were also significantly decreased (41,583 ± 27,773 pg/ml) compared with those during the active and inactive phases in patients with s-JIA, and compared with those in patients with poly-JIA, in KD and in HC (100,740 ± 31,718 pg/ml, $p < 0.001$). As shown in Fig. 1B, during the MAS phase in patients with s-JIA, serum Ang-2 levels were significantly elevated (8750 ± 7488 pg/ml) compared with those during the active (5514 ± 5392 pg/ml, $p < 0.05$) and inactive phases (2836 ± 1341 pg/ml, $p < 0.01$) in patients with s-JIA, and compared with those in patients with poly-JIA (2631 ± 1554 pg/ml, $p < 0.05$), in VAHS (5225 ± 6784 pg/ml, $p < 0.05$), in KD (4191 ± 3704 pg/ml, $p < 0.01$) and in HC (2317 ± 1035 pg/ml, $p < 0.001$). As shown in Fig. 1C, during the MAS phase in patients with s-JIA, the Ang-2/1 ratio was significantly elevated (0.272 ± 0.254 pg/ml) compared with that during the active (0.066 ± 0.071 pg/ml, $p < 0.001$) and inactive phases (0.026 ± 0.012 pg/ml, $p < 0.0001$) in patients with s-JIA, and compared with those in patients with poly-JIA (0.028 ± 0.019 pg/ml, $p < 0.001$), in KD (0.036 ± 0.028 pg/ml, $p < 0.0001$) and in HC (0.023 ± 0.007 pg/ml, $p < 0.0001$). Furthermore, as shown in Fig. 1C, during the active phase, the Ang-2/1 ratio was significantly elevated compared with that during the inactive phase in patients with s-JIA ($p < 0.01$), and compared with that in patients with poly-JIA ($p < 0.05$) and in HC ($p < 0.01$) (Fig. 1C). However, the Ang-2/1 ratio during the active phase of s-JIA were significantly decreased compared with those in patients with VAHS (0.165 ± 0.196 pg/ml, $p < 0.05$).