Distinct cytokine profiles of systemic-onset juvenile idiopathic arthritis-associated macrophage activation syndrome with particular emphasis on the role of interleukin-18 in its pathogenesis

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Abstract

Objectives. To compare the pro-inflammatory cytokine profiles and the cytokine kinetics in patients with secondary macrophage activation syndrome (MAS) due to systemic-onset juvenile idiopathic arthritis (s-JIA) and in both active and inactive disease states of s-JIA (but no MAS), with those demonstrated in EBV-induced haemophagocytic lymphohistiocytosis (HLH) and Kawasaki disease (KD), and to investigate the significance of IL-18 in the pathogenesis of s-JIA.

Methods. Five patients with MAS complicating s-JIA (MAS/s-JIA), 10 with HLH due to EBV infection (EBV-HLH), 22 with KD and 28 healthy controls were analysed. Cytokine concentrations (IL-18, IL-6, neopterin and TNF-α receptor Types I and II) were quantified in serum by ELISA. Results were compared with clinical features of MAS/s-JIA, including ferritin concentrations.

Results. Serum IL-18 concentrations in MAS/s-JIA patients were significantly higher than those in EBV-HLH or KD patients (P < 0.05). Serum IL-6 concentrations in KD patients were significantly higher than those in EBV-HLH or MAS/s-JIA patients. Serum neopterin concentrations in EBV-HLH patients were significantly higher than those in MAS/s-JIA or KD patients. Serum IL-18 correlated positively with the following measurements of disease activity: CRP, ferritin, lactate dehydrogenase and other cytokines (P < 0.05). Serum concentrations of IL-18 in s-JIA patients remained elevated in the inactive phase of disease, whereas clinical parameters and other cytokines normalized.

Conclusions. IL-18 may be an important mediator in s-JIA. Although serum Il-18 concentrations correlated with markers of the disease activity, IL-18 concentrations remained elevated even when other markers of disease activity normalized. Serum IL-18 concentration may be a promising indicator of the disease activity. The cytokine release pattern in MAS/HLH is different among patients with different aetiologies. Monitoring the cytokine profile, including IL-18, may be useful for differentiation of MAS/HLH and evaluation of disease activity in s-JIA.

Key words: Macrophage activation syndrome, Systemic juvenile idiopathic arthritis, Interleukin-18.

Introduction

Macrophage activation syndrome (MAS) is a severe, potentially life-threatening complication of childhood systemic inflammatory disorders [1, 2]. It is clinically characterized by fever, hepatosplenomegaly, lymphadenopathy, profound depression of all the three blood cell lines, deranged liver function, intravascular coagulation and CNS dysfunction. Among paediatric rheumatic
diseases, MAS occurs most often in children with systemic-onset juvenile idiopathic arthritis (s-JIA) and is less common in those with other rheumatic diseases, including polyarticular JIA, SLE and Kawasaki disease (KD) [3, 4]. MAS accounts for much of the significant morbidity and mortality observed with s-JIA. A variety of triggers have been implicated in the pathogenesis of MAS associated with s-JIA, including viral infections, NSAID therapy, MTX and etanercept [1, 3, 5]. The hallmark of this syndrome is excessive activation and proliferation of T lymphocytes and macrophages [6]. MAS bears a close resemblance to a group of haemophagocytic lymphohistiocytosis (HLH) syndromes and should be considered among the secondary causes of HLH [7, 8]. Massive hypercytokinaemia is strongly associated with the pathogenesis of MAS/HLH [3]; however, the kinetics of cytokine release in patients with MAS/HLH is still unclear.

IL-18 was originally described as an INF-γ-inducing factor mainly produced by the activated macrophage lineage cells [9, 10]. IL-18 stimulates a variety of inflammatory responses, enhances proliferation and activity of T cells and NK cells and shifts Th-cell balance towards the Th1 response [11, 12]. IL-18 has been reported to enhance the production of Th2 cytokines and IgE and recruitment of eosinophils, suggesting that IL-18 can also regulate allergic inflammation [13]. Some reports have recently shown that serum concentrations of IL-18 are highly elevated in patients with s-JIA [14–16].

To assess the kinetics of cytokine release during MAS/HLH, we measured the concentrations of serum cytokines, including IL-18, IL-6, neopterin and TNF-α receptor Types I (sTNF-RI) and II (sTNF-RII) in patients with MAS/s-JIA. We compared them with the concentrations in patients with HLH due to EBV infection (EBV-HLH) and KD, which are both characterized by prominent and systemic inflammation in children. We determined the correlation between the concentrations of such markers of cytokine release with measures of disease activity and severity in order to clarify the importance of IL-18 in the pathogenesis of not only MAS but also s-JIA.

Materials and methods

Patients and samples

Serum samples were obtained from 5 patients with MAS as a complication of s-JIA, 10 with EBV-HLH, 22 with KD and 28 age- and sex-matched healthy controls (HCs) [age [MAS/s-JIA: 5.8 (6.8) years; control: 8.8 (7.3) years]]. Samples from MAS/s-JIA patients were also obtained during both the active and inactive phases of the s-JIA disease, but where MAS was not present. Diagnosis of s-JIA was based on the ILAR criteria [17]. MAS was diagnosed based on the combination of cytopenias affecting at least two cell lines, coagulopathy and liver dysfunction (Table 1), according to the guidelines proposed by Ravelli et al. [18]. The criteria for the active phase of s-JIA was defined as follows: active arthritis, fever, rash, hepatosplenomegaly, generalized lymphadenopathy, active uveitis and serositis, as well as increased ESR and CRP concentrations, but where criteria noted in the guidelines for MAS proposed by Ravelli et al. [18] were not fulfilled. The criteria for the inactive phase of s-JIA on medication were as follows: no clinical symptoms that can be seen in active phase as well as normal ESR and CRP concentrations. All patients of EBV-HLH fulfilled the diagnostic criteria for EBV-HLH [19], positivity for the EBV genome in the blood and bone marrow and other tissues (determined by PCR, Southern blot and/or in situ hybridization for EBV encoded RNA) and positive anti-viral capsid antigen-specific-IgG. Diagnosis of KD was based on the classic clinical criteria [20]. 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 the receipt of informed consent.

Quantification of serum cytokines

Serum concentrations of IL-18, IL-6, neopterin, sTNF-RI and sTNF-RII were evaluated by commercial ELISA according to the manufacturer’s instructions (IL-18: MBL, Nagoya, Japan; IL-6, sTNF-RI and sTNF-RII: R&D Systems, Minneapolis, MN, USA; neopterin: IBL, Hamburg, Germany).

Statistical analysis

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

Results

Cytokine release in MAS patients

We determined serum concentrations of cytokines, including IL-6, IL-18, sTNF-RI and sTNF-RII, in patients
with MAS complicated with s-JIA (MAS/s-JIA) and compared them with the concentrations in patients with EBV-HLH or KD. It is noteworthy that the magnitude of the difference in serum IL-18 concentrations between patients with MAS/s-JIA and the other patient groups was overwhelming in comparison with that of other cytokine concentrations. As shown in Fig. 1, serum IL-18 concentrations in patients with MAS/s-JIA (median 122,500, range 101,000–830,000 pg/ml) and active phase of s-JIA (median 130,000, range 56,500–203,000 pg/ml) were significantly higher than those in patients with EBV-HLH (median 3825, range 1720–14,800 pg/ml), KD (median 279.5, range 180–560 pg/ml) and HCs (median 140.5, range 76–255 pg/ml) (P < 0.05). Serum IL-18 concentrations in patients with s-JIA were markedly elevated even in the inactive phase of s-JIA (median 6025, range 3730–12,000 pg/ml). Other cytokines that were elevated during active disease in the MAS/s-JIA group normalized when patients were in clinical remission. Serum IL-18 concentrations were significantly higher in active s-JIA patients compared with the elevated concentrations also seen in patients with active EBV-HLH (P < 0.05). These findings indicate that abnormal production of IL-18 appears to be highly specific for s-JIA.

Serum neopterin concentrations in patients with EBV-HLH (median 68, range 46–135 nmol/l) were higher than those in patients with MAS/s-JIA (median 46, range 10.5–122 nmol/l), KD (median 13.5, range 7–50 nmol/l) and HCs (median 4.35, range 1.8–9.5 nmol/l). Serum IL-6 concentrations in patients with KD (median 57, range 22–310 pg/ml) were higher than those in patients with MAS/s-JIA (median 8.7, range 5–22 pg/ml), EBV-HLH (median 14.3, range 0.5–106 pg/ml) and HCs (<3.0 pg/ml). Interestingly, serum neopterin and sTNF-RII concentrations in patients with MAS/s-JIA were significantly higher than those in patients with active phase of s-JIA. Because many inflammatory cytokines are associated with the pathogenesis of MAS/HLD, we believe that monitoring the cytokine profile in combination with these cytokines might be more useful for evaluating disease activity. Consequently, we tried to represent the cytokine profile with a radar chart (Fig. 2). The pattern of the cytokine profile was characteristic in each background (Fig. 2).

Markedly elevated concentrations of serum IL-18 in patients with the active phase of s-JIA and MAS

To investigate the relevance of IL-18 to the pathogenesis of s-JIA, serum concentrations of IL-18 were serially monitored in all five cases of s-JIA (Fig. 3A–E). The concentration of serum IL-18 both rapidly and markedly rose with the development of the complication of MAS, but gradually reduced after this manifestation resolved with immunosuppressive therapy including corticosteroid and ciclosporin. However, even a few weeks after normalization of other indicators of the inflammatory reaction such as LDH, IL-18 concentrations were still well above the value of HC. In Case 1, MAS was frequently complicated in this phase with high concentrations of serum IL-18 (Fig. 3A). Since serial monitoring of serum concentrations of IL-18 was started, the patient suffered three relapses but could be treated before MAS was complicated (Fig. 3A). The pattern of cytokine profile of MAS/s-JIA is similar in all cases. Serum concentrations of IL-18 in patients with s-JIA were markedly elevated even in the inactive phase. The other cytokines were detected at significant concentrations in patients with MAS/s-JIA but was undetectable during remission (Fig. 4).

Correlation between serum IL-18 concentrations and measures of disease activity in clinical course of five cases of s-JIA

Since the concentrations of serum ferritin, LDH, aspartate aminotransferase and CRP are clinically used as indicators for disease activity of s-JIA, their concentrations were compared with those of IL-18. The concentrations of serum IL-18 correlated positively with each of these indicators (P < 0.0001; Fig. 5A–D). However, even during the clinically inactive phase after remission from MAS, concentrations of serum IL-18 remained extremely elevated, although other clinical parameters were normalized.

Correlation between serum IL-18 and other cytokines in the clinical course of five cases of s-JIA

The concentrations of serum IL-18 correlated positively with the concentrations of serum neopterin, IL-6, sTNF-RI and sTNF-RII (P < 0.0001; Fig. 5E–H). Although the concentrations of serum IL-6 and neopterin were normalized in the inactive phase, concentrations of serum IL-18 remained significantly elevated.

Discussion

MAS is a severe, potentially life-threatening complication characterized by excessive activation of well-differentiated macrophages, resulting in fever, hepatosplenomegaly, lymphadenopathy, severe cytopenia, serious liver disease, intravascular coagulation and neurological involvement [1, 2]. MAS bears close resemblance to a histiocytic disorder, namely secondary HLH. HLH is a better defined entity observed in a heterogeneous group of diseases, including infections, neoplasms, haematological conditions and autoimmune disorders. It has been suggested that MAS should be replaced with the term autoimmune disease-associated reactive HLH [7, 8]. The hallmark of this syndrome is excessive activation and proliferation of T lymphocytes and macrophages [6]. Massive hypercytokinaemia produced by activated inflammatory cells is strongly associated with the pathogenesis of MAS/HLD; however, the kinetics of cytokine release in patients with MAS/HLD have not been analysed completely.

In the present study, the concentrations of serum IL-18 in patients with s-JIA were markedly increased. In contrast, the concentrations of serum neopterin and IL-6 were significantly increased in patients with EBV-HLH and KD, respectively. These findings show that the pattern
of cytokine release is different among patients with different conditions, although clinical characteristics bear a close resemblance.

Because many inflammatory cytokines are associated with the pathogenesis of MAS/HLH, we proposed that monitoring the cytokine profile in combination with the individual cytokines might be more useful for evaluating the disease activity. Consequently, we tried to represent the cytokine profile with a radar chart (Fig. 2). The pattern of the cytokine profile was characteristic for each disease
In the acute phase of MAS/HLH, it is often difficult to differentiate the patients’ primary disease, but monitoring the cytokine profile might be very useful in achieving early diagnosis and therapeutic decision making.

In the present study, it is noteworthy that the magnitude of the difference in serum IL-18 concentrations between patients with MAS/s-JIA and all other patients was significantly elevated in comparison with that of all the other cytokine concentrations. Serum IL-18 concentrations in patients with MAS/s-JIA and during active disease flares of s-JIA were significantly higher than those in patients with EBV-HLH and KD; these concentrations positively correlated with the measures of disease activity as well as other cytokines. Interestingly, the concentrations of serum IL-18 in patients with s-JIA dropped in the inactive phase of the disease but remained elevated compared with controls and patients with resolved EBV-HLH. Based on serial monitoring of serum IL-18 concentrations in Case 1, relapses of acute flares of s-JIA and the complication of MAS occurred in this phase, indicating that careful monitoring was needed for withdrawal of immunosuppressive drugs until the concentration of serum IL-18 normalized. The concentrations of serum IL-18 increased before other clinical indicators for disease activity of s-JIA including ferritin, LDH, ASP and CRP start to increase. These findings show that serum IL-18 may be a useful biomarker to predict impending disease flares. Interestingly, the concentrations of neopterin and sTNF-RII were significantly higher in MAS/s-JIA phase than in the acute-phase s-JIA alone. These concentrations were also extremely high in EBV-HLH, which shows these might be a useful marker to predict the transition to MAS/s-JIA from the acute phase of s-JIA.

Our data indicate that abnormal production of IL-18 appears to be highly specific for s-JIA. However, it is still unknown what causes the induction of extremely high IL-18 concentrations in the serum of patients with s-JIA. IL-18 is the most effective at regulating NK cell activity [21, 22] and it has been reported that decreased NK cell function is found in s-JIA [23–25]. Recently, it was reported that the mechanism of the impaired NK cell function in s-JIA involves a defect in IL-18 receptor β phosphorylation [26]. Further study will be required, but non-functional IL-18/NK cell axis might be associated with the pathogenesis of s-JIA.

Some reports have recently shown that serum concentrations of IL-18 are also highly elevated in patients with adult-onset Still’s disease (AOSD) [27–29]. It is still controversial whether s-JIA and AOSD are identical. It has been reported that no significant difference in clinical features such as systemic manifestations or joint lesions, or in prognosis exists between these two diseases [30]. In addition to these observations, our findings in the present study would be consistent with these two diseases being pathogenically identical.
During the clinically inactive phase after remission from MAS, the concentrations of serum IL-6 and neopterin normalized. However, interestingly, the concentrations of sTNF-RI and sTNF-RII increased in the inactive phase. These findings suggest that TNF-α is also associated with the pathogenesis of the inflammatory process in s-JIA, not only during exacerbation but also during the clinically silent phase of this disease.
Tocilizumab (TCZ) is a humanized mAb recently developed against the human IL-6 receptor [31]. Although the introduction of TCZ has brought about a paradigm shift in the treatment of s-JIA [31], it has been reported that some cases were complicated with MAS during treatment with TCZ (Dr Syuji Takei, personal communication). This finding indicates that IL-6 blocking cannot prevent the onset of MAS. It is important to analyse the kinetics of cytokine release during MAS in these cases, and the results of such analysis may give us findings that are useful in understanding the role of each cytokine in the pathogenesis of MAS and s-JIA.

In the majority of patients with active s-JIA, coagulation abnormalities and greatly elevated serum ferritin concentrations are observed. Some rheumatologists suggest that MAS and s-JIA are included in the same spectrum [32]. Our findings suggest that MAS and s-JIA are at different ends of the same spectrum, which is based on the significant production of IL-18 by activated macrophages [14]. The clinical course at later stages of s-JIA is highly variable. Systemic features such as fever, rash and polyserositis tend to subside during the initial months and up to years of the disease. About half of the children with s-JIA recover almost completely, but the other half continue to show progressive involvement of additional joints. To address whether these two subpopulations have the same spectrum or not, further analysis of the kinetics of cytokine release may be useful.

In spite of the limitations, our results suggest that IL-18 plays a key role in the complex network involved in the inflammation of s-JIA and that serum IL-18 concentration is a promising indicator of the disease activity. The pattern of cytokine release in MAS/HLH is different among patients with different backgrounds. Monitoring of the cytokine profile may be useful for differentiation of the primary underlying disease in patients with MAS/HLH and evaluation of disease activity in s-JIA. Some other potentially useful markers to predict MAS in s-JIA, including soluble CD163 and soluble IL-2 receptor, have been reported [33]. Inclusion of some of these markers in cytokine profiling may improve the quality of the analysis. Further studies are needed to assess what combination of markers are the most useful for the monitoring of MAS/HLH.

**Rheumatology key messages**

- IL-18 is an important mediator in s-JIA.
- The cytokine release pattern is different among patients with different aetiologies of their in MAS/HLH.
- Monitoring the cytokine profile may be useful for the evaluation of disease activity in s-JIA.

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Positive correlations between IL-18 and other measures of the disease activity. Serum IL-18 concentrations were compared with other serum markers and cytokines, in five cases of s-JIA. (A) CRP, (B) AST, (C) LDH, (D) ferritin, (E) neopterin, (F) IL-6, (G) sTNF-RI and (H) sTNF-RII. Red boxes indicate the areas where IL-18 concentrations are increased whereas other measures remain within normal limits.
References


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