Cytokine 61 (2013) 345–348

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Short Communication

Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles

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ARTICLE INFO

Article history:
Received 29 July 2012
Received in revised form 8 November 2012
Accepted 27 November 2012
Available online 29 December 2012

Keywords:
Systemic juvenile idiopathic arthritis
Interleukin-6
Interleukin-18
Cytokine profiles

ABSTRACT

To assess the serum interleukin (IL)-6 and IL-18 levels in patients with systemic juvenile idiopathic arthritis (s-JIA) and to identify the clinical features of patient subsets with different cytokine profiles, we analyzed the serum levels of IL-6 and IL-18 in patients with s-JIA and compared them with the clinical features of s-JIA. Eighteen patients were analyzed. IL-6 and IL-18 levels were quantified in serum by enzyme-linked immunosorbent assays. Interestingly, two distinct s-JIA patient subsets based on their serum IL-6 and IL-18 levels were identified: an IL-6 dominant and an IL-18 dominant. The serum IL-6 and IL-18 levels were consistent both at relapse and at the onset of s-JIA in each subset. The IL-6-dominant subset had a significantly greater number of joints with active disease and higher serum levels of matrix metalloproteinase-3, whereas the IL-18-dominant subset was more likely to develop macrophage activation syndrome (MAS). These findings indicate that two subsets of patients with s-JIA, one which is prone for arthritis and another with prone for MAS, can be identified on the basis of their serum IL-6 and IL-18 levels. These two subsets appear to be characterized by certain distinct clinical features.

1. Introduction

Systemic juvenile idiopathic arthritis (s-JIA) is characterized by the clinical features of remitting fever, typical skin rash, and arthritis. Recent investigations into the pathophysiology of s-JIA have focused on mediators of the innate immune system. In particular, interleukin (IL)-1, IL-6, and IL-18 levels are correlated with disease activity and secondary complications [1,2]. Biological agents that inhibit these pivotal inflammatory cytokines (specifically, IL-1 and IL-6) have already changed the approach for the treatment of s-JIA [3,4]. There is accumulating evidence that inhibition of IL-1 or IL-6 is highly efficacious in a significant number of patients with s-JIA, with improvements seen in both systemic symptoms and arthritis [3,4].

A recent study showed that there were two subsets of s-JIA that had some distinct clinical features that could be identified on the basis of patients' responses to IL-1 blockade [5]. We previously reported that some patients with s-JIA had incomplete responses to IL-6 blockade and had macrophage activation syndrome (MAS), the most devastating complication of s-JIA [6]. In these patients, serum IL-18 is a promising marker of s-JIA disease activity [6].

On the basis of these findings, we hypothesized that there are different subsets of s-JIA with distinct cytokine profiles and clinical features. In this study, we examined serum IL-6 and IL-18 levels in patients with s-JIA during the active phase of the disease. We identified two subsets of patients with s-JIA having certain distinct clinical features. These subsets could be identified on the basis of IL-6 and IL-18 levels.

2. Methods

2.1. Patients and samples

Serum samples were obtained from 18 patients with s-JIA. Samples were obtained during the active phase of s-JIA. The diagnosis of s-JIA was made on the basis of the criteria of the International League of Associations for Rheumatology [7]. MAS was diagnosed on the basis of a combination of clinical features, including cytopenia or sudden decrease in white blood cell counts and/or platelet counts, coagulopathy, and liver dysfunction, according to the guidelines proposed by Ravelli et al. [8]. The criteria for the active phase of s-JIA were active arthritis, fever, rash, hepatosplenomegaly, generalized lymphadenopathy, and serositis, as well as increased C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR). The clinical characteristics of these patients are shown in Table 1. Some patients had minimal joint disease at the onset of s-JIA and the presence of arthritis was confirmed later.

Abbreviations: s-JIA, systemic juvenile idiopathic arthritis; MAS, macrophage activation syndrome; IL, interleukin.

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http://dx.doi.org/10.1016/j.cyto.2012.11.025

Table 1
Clinical features of the 2 s-JIA subsets based on serum IL-6 and IL-18 levels.

s-JIA (*n*, % or mean ± SD)	Group A (IL-6 dominant group) *n* = 10	Group B (IL-18 dominant group) *n* = 8	*P* value
Age	7.8 ± 4.2	10.6 ± 8.2	0.9797
Sex (male/female)	3/7	5/5	0.6499
Dosage of prednisolone (mg/kg/day)	0.05 ± 0.13(0–0.4)	0.14 ± 0.30(0–0.84)	0.7604
Clinical symptoms			
Fever	10(100)	8(100)	
Rash	6(60)	6(75)	0.6380
Lymphadenopathy	0(0)	1 (12.5)	1.0000
Hepatosplenomegaly	0(0)	1 (12.5)	1.0000
Arthritis (number of affected joints)	6.3 *t* 8.6	1.1 ± 1.1	0.0104
Macrophage activation syndrome	0(0)	4(50)	0.0229
Laboratory findings			
WBC (/mm^3)	15,189 ± 4436	16,391 ± 8940	0.9052
CRP (mg/dl)	13.2 ± 6.0	7.5 ± 3.4	0.0343
Ferritin (ng/ml)	1151 ± 821	4897 ± 6735	0.3964
MMP-3 (ng/ml)	343.3 ± 500.8	57.4 ± 75.8	0.0321
IL-6 (pg/ml)	133.9 ± 96.3	23.7 ± 42.6	0.0029
IL-18 (pg/ml)	20,420 ± 16,112	160,188 ± 105,344	0.0031

We serially determined the serum IL-6 and IL-18 levels of 4 patients. We measured their levels in active disease at relapses during tapering of the dosage of steroid. Serum was extracted from blood samples, divided into aliquots, frozen, and stored at −80 °C until use. This study was approved by the Institutional Review Board of Kanazawa University and all the patients provided informed consent.

2.2. Serum cytokine level measurements

Serum IL-18 and IL-6 levels were determined using commercial enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions (IL-18: MBL, Nagoya, Japan; IL-6: R&D Systems, Inc, Minneapolis, MN, USA). We also determined serum TNF-α, IFN-β and IL-1β levels using commercial ELISA kit according to the manufacturers' instructions (TNF-α, IFN-β, IL-1β: R&D Systems, Inc.).

2.3. Statistical analysis

Results are given as means ± standard deviations. Comparisons between groups were made by the Mann–Whitney *U*-test or Fisher's exact probability test, as appropriate. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Different subsets of patients with s-JIA based on their serum IL-6 and IL-18 levels

As shown in Fig. 1A, there were two subsets of patients with s-JIA based on their serum IL-6 and IL-18 level: IL-6 dominant ($n = 10$) (Group A) and IL-18 dominant ($n = 8$) (Group B). As shown in Table 1, serum IL-6 levels in Group A patients (mean ± SD, 133.9 ± 96.3 pg/ml) were significantly higher than those in Group B patients (23.7 ± 42.6 pg/ml) ($P < 0.01$). On the other hand, serum IL-18 levels in Group B patients (mean ± SD, 160,188 ± 105,344 pg/ml) were significantly higher than those in Group A patients (20,420 ± 16,112 pg/ml) ($P < 0.01$). We could serially determine the serum IL-6 and IL-18 levels of only two patients in each subset. In both subsets, the serum IL-6 and IL-18 levels at relapse were similar to those at the onset of s-JIA (Fig. 1B).

3.2. Distinct clinical features of the 2 s-JIA subsets based on their serum IL-6 and IL-18 levels

Next, we compared the clinical features of each subset (Table 1). The patients in Group A had a significantly greater number of active joints ($P < 0.05$). Serum matrix metalloproteinase (MMP)-3 levels and C-reactive protein levels were significantly higher in Group A than in Group B. Four of 10 patients in Group B had the complication of MAS, whereas no patients in Group A experienced MAS during their clinical courses. Serum TNF-α, IFN-β and IL-1β levels were not detectable in both groups.

4. Discussion

Inflammatory cytokines are critical for perpetuating the inflammatory processes in s-JIA. Previous studies suggested pivotal roles for the three proinflammatory cytokines IL-6, IL-1, and IL-18 [1,2]. IL-6 has an important role in the systemic manifestations and arthritis observed in s-JIA. IL-6 is markedly elevated in both the peripheral blood and synovial fluid of patients with s-JIA and its expression appears to be correlated with disease activity and certain clinical features, such as fever patterns, growth retardation, and osteoporosis [9].

Recently, the important role of IL-1β in s-JIA has become appreciated. Increased IL-1β can result in fever, anorexia, and pain hypersensitivity, and the dysregulation of its levels can lead to the clinical and laboratory findings of s-JIA. Pascual et al. showed that culturing peripheral blood mononuclear cells from healthy persons with serum from patients with s-JIA increased the IL-1β secretion of these cells; increased production of IL-1β protein by mononuclear cells from patients with active s-JIA was also observed [10].

IL-18 was originally described as an IFN-γ-inducing factor that was primarily produced by activated cells of the macrophage lineage. IL-18 stimulates a variety of inflammatory responses. Serum IL-18 levels are increased in patients with s-JIA, as we previously reported [11]. Another report showed that an imbalance between IL-18 and its natural inhibitor, IL-18-binding protein, resulted in Th-1 lymphocyte and macrophage activation, which escaped the control by natural killer (NK) cell-mediated cytotoxicity and may have allowed for secondary hemophagocytic syndrome [12]. A relationship between high IL-18 levels and impaired NK cell func-