Association Between IL-23 And Coronary Arterial Lesions In Children With Kawasaki Disease

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Research Article

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Abstract

The pathogenesis of coronary artery lesions (CALs) in KD patients has been thought of as an unknown stimulus that triggers an inflammatory cascade with activation of the immune system. However, interleukin-23 (IL-23) is supposed as a key cytokine in inflammatory and autoimmunity diseases. The role of IL-23 in the pathogenesis of CALs has not been fully elucidated. This study explored the relationship between the serum IL-23 levels and CALs in patients with KD. We collected blood specimens from 90 children with KD before intravenous immunoglobulin (IVIG) therapy. Levels of IL-23, IL-6, IL-17A, IL-10, MCP-1 and VEGF were measured in 190 cases, including 4 groups: KD with CALs (n = 46), KD without CALs (n = 44), febrile control group, (FC, n = 40) and normal control group, (NC, n = 60). Clinical parameters were tested in all subjects. IL-23 was significantly elevated in the KD group compared with the febrile and normal control groups, especially increased in the KD patients with CALs. Serum levels of IL-23 in KD patients were positively associated with WBC, CRP, IL-6, IL-17A, IL-10, MCP-1, and VEGF in children with KD. IL-23 may be involved in the pro-inflammatory process and the pathogenesis of CALs in KD patients.

1. Introduction

Kawasaki disease (KD) is a self-limited autoimmune systemic vasculitis disease predominantly in children under 5 years [1]. Its most serious complication is coronary arteritis lesions (CALs) or aneurysms that may cause ischemia, myocardial infarction, and sudden cardiac death [2]. Although the pathogenesis of CALs in KD patients has not been fully elucidated, an unknown stimulus triggers an inflammatory cascade with activation of the immune system is thought to be a cause of the disease [3]. There are increased inflammatory cytokines (IL-6, IL-17A, IL-10, and tumor necrosis factor (TNF) - α) and dysregulations of T-cells that CD8⁺ T cells decrease and CD4⁺ T cells increase, which involved in the acute and subacute phase of KD [4–6].

Interleukin-23(IL-23) is a recently discovered cytokine of the IL-12 family and a heterodimeric protein composed of an IL-23-specific p19 subunit and a p40 subunit shared with IL-12 [7]. IL-23 is mainly produced by activated macrophages and dendritic cells [8, 9]. IL-23 is supposed as a key cytokine in inflammatory and autoimmunity diseases such as atherosclerosis, rheumatoid arthritis (RA), and asthma [10, 11], which promotes Th17 cell differentiation and activation to produce inflammatory factors(IL-6, IL-17, TNF- α , and GM-CSF). Moreover, IL-23 can produce IL-1, TNF- α , and IL-23 itself through activating inflammatory macrophages[12, 13]. However, whether IL-23 involve in the pathogenesis of KD is still unclear. Thus, we examined serum IL-23 levels and the correlation with other inflammatory cytokines (IL-6, IL-17A, IL-10, MCP-1, and VEGF) to explore whether IL-23 has a role in developing CALs in KD patients.

2. Materials And Method

2.1. Subjects recruitment

All patients diagnosed with KD were from the Children's Hospital of Chongqing Medical University, Chongqing, China. A total of 90 KD patients (48 males and 42 females, 2.55 ± 1.72 years old) were enrolled in the study, all of whom met the Japanese Kawasaki Disease Research Committee's clinical diagnostic criteria for KD [14]. 60 healthy children (32 males and 28 females, 2.19 ± 2.22 years old) as the normal control group (NC) and 40 children with fever of pneumonia (20 males and 20 females, 2.84 ± 1.63 years old) as the febrile control group (FC).

KD patients were divided into 2 groups based on the presence or absence of CALs: KD with CALs ($n=46$) and KD without CALs ($n=44$). CALs were defined by having an actual internal diameter of 3 mm or more in a child under 5 years; 4 mm or more in a child 5 years or older, or if the internal diameter of the segment was at least 1.5 times greater than that of an adjacent segment according to examination by echocardiography [15]. Echocardiography was obtained before administering intravenous immunoglobulin (IVIG) or within 2 weeks of the onset. We collected blood specimens from KD patients before IVIG therapy within 1 week after symptom onset. Serum was stored at -80°C for future analysis.

2.2. Measurement of serum IL-23, IL-6, IL-17A, IL-10, MCP-1, VEGF levels and clinical parameters

Serum IL-23, IL-6, IL-17A, IL-10, MCP-1, VEGF levels were detected by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (RayBiotech, USA). Various clinical parameters from all subjects were also tested, including White blood cells (WBC), red blood cells (RBC), hemoglobin (HB), Platelet (PLT), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and procalcitonin (PCT). The Ethics Committee of Children's Hospital of Chongqing Medical University approved our study. Informed consent was obtained from the guardians of each child.

2.3. Statistical analysis

All data are expressed as number and percentage ($n, \%$) or mean $\pm$ standard deviation (SD). One-way ANOVA and 2-tailed unpaired t-test were used to analyze the statistical significance of concentrations of cytokines. Correlation between two groups was tested by Pearson's correlation analysis. A two-tailed p -value of <0.05 was considered significant. SPSS version 18 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

3. Results

3.1. Serum IL-23 levels were higher in KD patients than that in normal children and acute febrile infectious patients

The serum samples analyzed in this study are the same samples analyzed in the previous report. It shows that no statistically significant differences in age and gender among three groups: the KD patients group, the normal control group (NC), and the febrile control group (FC) [16]. The detected serum IL-23 levels ranged from 0.4 to 2507.3 pg/ml. As shown in Fig. 1, serum IL-23 levels in the KD groups (446.9

pg/ml [58.0, 2507.3]) were significantly elevated compared with the FC(388.9 pg/ml [1.9, 1039.8]) and NC(187.2 pg/ml [0.4, 820.1]) ($p = 0.017$).

3.2. Serum IL-23 levels were higher in KD patients with CALs than that in KD patients without CALs.

Our previous report has showed that there were no significant differences between the KD with CALs (KD-CALs) group and KD without CALs (KD-NCALs) group in age, sex, WBC, platelet, RBC, HB, CRP, PCT, IL-17A and IL-10 ($p > 0.05$). However, the serum level of ESR, IL-6, MCP-1 and VEGF was markedly higher in the group of KD patients with CALs than in the group of patients without CALs ($p < 0.05$) [16]. Moreover, serum IL-23 levels were significant higher in the KD with CALs group (596.8 pg/ml [103.6, 2507.3]) than that in KD without CALs group (319.2 pg/ml [58.0, 1159.1]) as shown in Fig. 2 ($p < 0.05$).

3.3. The relationship between IL-23 and clinic characteristics and inflammatory factors.

Our previous report has showed that levels of HB and RBC markedly decreased, and levels of WBC, PLT, CRP, ESR and PCT were significantly elevated in the KD group than those in NC and FC groups. Moreover, IL-6, IL-17A, IL-10, MCP-1 and VEGF were markedly increased in the KD group compared with the NC and FC groups ($p < 0.05$) [16]. In this study, we further compare the relationship between IL-23 and clinic characteristics(WBC, RBC, HB, PLT, CRP, ESR and PCT), and inflammatory factors(IL-6, IL-17A, IL-10, MCP-1 and VEGF). We find IL-23 were positively correlated with WBC, CRP, IL-17A, IL-10, IL-6, MCP-1 and VEGF levels in patient with KD ($r = 0.381$, $p = 0.026$; $r = 0.383$, $p = 0.012$; $r = 0.301$, $p = 0.033$; $r = 0.282$, $p = 0.047$; $r = 0.426$, $p = 0.017$; $r = 0.340$, $p = 0.018$, $r = 0.394$, $p = 0.019$, respectively) (Table 1).

Table 1
Compare relationship between IL-23 and clinic characteristics and inflammatory factors

IL-23		
	<i>r</i>	<i>p</i>
WBC(10^3 /uL)	0.381	0.026
HB (g/dL)	0.145	0.311
PLT (10^3 /uL)	-0.021	0.890
RBC(10^6 /mm ³)	-0.028	0.853
PCT	0.184	0.291
CRP(mg/dL)	0.383	0.012
ESR(mm/h)	-0.190	0.183
IL-6(pg/ml)	0.426	0.017
IL-17A(pg/ml)	0.301	0.033
IL-10(pg/ml)	0.282	0.047
MCP-1(pg/ml)	0.340	0.018
VEGF(pg/ml)	0.394	0.019
<i>KD</i> Kawasaki disease, <i>WBC</i> white blood cells counts, <i>RBC</i> red blood cells counts, <i>PLT</i> Platelet, <i>HB</i> Hemoglobin, <i>CRP</i> C-reactive protein, <i>ESR</i> erythrocyte sedimentation rate, <i>PCT</i> procalcitonin, <i>MCP-1</i> monocyte chemoattractant protein-1, <i>VEGF</i> vascular endothelial growth factor		

4. Discussion

IL-23, a recent member of the IL-12 family, is supposed as a key cytokine in inflammatory and autoimmunity diseases. The aim of this study was to determine the role of IL-23 in the pathogenesis of KD and investigate the correlation between IL-23 and CALs in KD patients. Our study established that (1) serum IL-23 levels increased in KD patients, compared with the normal control group and the febrile control group, and even more (2) serum IL-23 levels increased in the KD with CALs group compared with the KD without CALs group. Moreover, (3) IL-23 was positively correlated with WBC, CRP, IL-6, IL-17A, IL-10, MCP-1, and VEGF in KD patients.

IL-23 is produced mainly by activated dendritic cells and macrophages, which is supposed as a key cytokine in inflammatory and autoimmunity diseases such as atherosclerosis, asthma, and rheumatoid arthritis (RA)[8–11]. However, few studies have examined the role of IL-23 in the pathogenesis of KD. Our research shows that serum IL-23 levels in KD patients were higher than those in normal children and febrile infectious patients, indicating that IL-23 may involve vasculitis in the acute phase of KD. Moreover,

serum IL-23 levels increased in the KD patients with CALs compared with the KD patients without CALs, which suggested that IL-23 may have an effect on the development of CALs in KD.

T-helper type 17 (Th17) cells have been reported to regulate inflammation in the acute phase of KD[17, 18]. Th17 cells can produce inflammatory cytokines, including IL-17, IL-6, and TNF- α , and lead to autoimmunity and tissue damage. IL-17 has pro-inflammatory properties to regulate tissue inflammation and acts on a broad range of cell types to induce the expression of various cytokines (such as IL-6, TNF- α , and IL-8), metalloproteinases, and chemokines [19, 20]. Many previous studies have shown that IL-17 levels were significantly elevated in children with acute Kawasaki disease[17, 21]. Recently, some studies have demonstrated that IL-23 can promote Th17 cell differentiation and activation to produce IL-17, IL-6, IL-22, TNF- α , and GM-CSF[12]. We got very similar results in this research where IL-23 levels were positively correlated with IL-17 and IL-6 levels. These results indicated that the IL-23/ IL-17 axis might be implicated in the pathogenesis of KD.

IL-10 is an immunomodulatory cytokine with anti-inflammatory and immunosuppressive properties, which inhibits the production of inflammatory cytokines (such as IL-1 β , IL-6, and TNF- α) and is implicated in the pathogenesis of inflammatory and autoimmune diseases[22–24]. IL-10 has been reported to be involved in the pathogenesis of KD. The adeno-associated virus (AAV) -mediated induction of IL-10 prevents vascular inflammation, fibrosis, and lethality in a murine model of KD[25, 26]. Some research shows that IL-23 can enhance IL-10 production, consistent with our results that IL-23 levels were positively correlated with IL-10 levels[27, 28]. Therefore, we speculate that IL-23 may have an effect on inducing the expression of IL-10 in the acute phase of KD.

This research found that serum IL-23 levels were positively associated with WBC, CRP, MCP-1, and VEGF levels in KD patients. MCP-1 is a new member of the CC-chemokine family that activates and attracts the monocytes, which has been confirmed to be positively associated with the development of KD[29–31]. There is almost no research on the relationship between IL-23 and MCP-1. Our study showed that serum IL-23 levels positively correlated with MCP-1 in KD patients so that IL-23 may promote the expression of MCP-1 and may promote vascular inflammation during the acute phase of KD can be speculated. Inflammation predictors of inflammation include WBC and CRP. High levels of CRP serve as an independent risk factor for predicting giant aneurysms in KD[32]. Furthermore, VEGF, a potent pro-angiogenic protein, can increase vascular permeability and endothelial cell proliferation, which is considered a predictor of CAL in acute KD [33–36]. Moreover, IL-23 could promote VEGF production in mammary cancer and human colorectal carcinoma [37, 38]. Our study showed that serum IL-23 levels were positively correlated with CRP, WBC, and VEGF. Therefore, it can be speculated that IL-23 may promote the production of VEGF and the formation of the CAL by promoting the pro-inflammatory process in KD patients.

Intravenous immunoglobulin (IVIG) combined with aspirin as first-line therapy for the treatment of KD can reduce inflammation and reduce the incidence of coronary artery disease. In recent years, adjuvant treatments such as corticosteroids, infliximab, cyclosporine A, anakinra, and plasma exchange have

emerged, providing more options for the treatment of Kawasaki disease[39]. Risankizumab is a humanized monoclonal antibody that binds to the p19 subunit of IL-23 and is effective in treating psoriasis and ulcerative colitis[40, 41]. Given IL-23 may be involved in the pro-inflammatory process and the pathogenesis of CALs in KD patients, it may be feasible to use its monoclonal antibodies to treat Kawasaki disease. Anti-IL-23 agents may become a potential therapeutic option for CALs in KD.

In conclusion, our study demonstrated that serum IL-23 levels increased in the KD patients, notably KD patients with CALs. Moreover, serum IL-17A, IL-10, IL-6, MCP-1, and VEGF levels were positively correlated with IL-23 in the acute phase of KD. The results of this study indicate that IL-23 might play a pro-inflammatory role in the response of vasculitis in KD. Additional research is needed to further clarify the mechanism of IL-23 in the pathogenesis of KD vasculitis.

Declarations

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Compliance with ethical standards

Conflict of interest All authors have no actual or potential conflicts of interest with other people or organizations with 3 years of initiating the work presented here.

Ethical approval The study protocol was approved by the Ethics Committee of Children's Hospital of Chongqing Medicine University, and written informed consent forms were obtained from the parents of all subjects.

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Figures

Figure 1

Comparisons of serum IL-23 levels among KD, FC and NC groups

Figure 2

Comparisons of serum IL-23 levels between the KD with CALs group and KD without CALs group