ORIGINAL ARTICLE

Potential Effects of Interleukins on the Pathogenesis of Systemic Onset Juvenile Idiopathic Arthritis

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Key Words
disease activity score 28;
IL-17;
IL-6;
Systemic onset juvenile idiopathic arthritis;
visual analogue scale

Background: To analyze the correlation of cytokines with clinical inflammatory indexes in systemic onset juvenile idiopathic arthritis (SOJIA).

Methods: A total of 30 active SOJIA, 30 remission SOJIA, and 20 normal controls were enrolled. The clinical inflammatory indexes such as tender joints counts, swelling joints counts, C-reactive protein, erythrocyte sedimentation rate, visual analogue scale (VAS), and disease activity score 28 (DAS28) were detected. The serum cytokines interleukin (IL)-17, IL-6, IL-21, interferon (IFN)-γ, and IL-4 levels were determined with enzyme-linked immunosorbent assay method. The correlation coefficients between these cytokines and two clinical indexes (VAS and DAS28) in the active SOJIA group were calculated with the Spearman’s method.

Results: The serum IL-17 and IL-6 levels in active SOJIA group were significantly increased compared with those in the remission SOJIA group and control group (p < 0.05), and the serum IL-21, IFN-γ, and IL-4 levels showed no obvious difference. In the active SOJIA group, the Spearman coefficients between IL-17 and DAS28, IL-17 and IL-6, IL-6 and DAS28, and between IL-17 and VAS were 0.686 (p = 0.000), 0.833 (p = 0.000), 0.633 (p = 0.000), and 0.524 (p = 0.003), respectively. There was no correlation between cytokines of IL-21, IFN-γ, and IL-4 and the clinical indexes of DAS28 and VAS. Furthermore, in the other two groups, none of the five cytokines exhibited an association with DAS28 or VAS.

Conclusion: IL-6 and IL-17 were significantly correlated with DAS28 and VAS, and they might be considered as therapeutic targets for the treatment of SOJIA.

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1. Introduction

Systemic onset juvenile idiopathic arthritis (SOJIA) is a rare inflammatory disorder disease and the symptoms of SOJIA may be accompanied by evanescent rashes, lymph node enlargement, hepatomegaly, splenomegaly, or serositis.\textsuperscript{1,2} SOJIA accounts for 5–10% of deaths from JIA, which is an important cause of short- and long-term disability.\textsuperscript{3,4} Moreover, macrophage activation syndrome is a potentially fatal complication of SOJIA.\textsuperscript{5} It is therefore important to study the pathogenesis of SOJIA further.

Cytokines play an important role in the pathogenesis of SOJIA. Pascual et al.\textsuperscript{6} have reported that interleukin (IL)-1 contributes to the development of SOJIA through the mediation of the inflammatory cascade. Other cytokines, such as IL-10, IL-1β, IL-6, IL-17, and tumor necrosis factor alpha (TNFα), participate and play crucial roles in the development of JIA, SOJIA, or rheumatoid arthritis (RA).\textsuperscript{7–11} IL-21, similar to IL-6, together with transforming growth factor β could induce the differentiation of Th17 cells, which secrete IL-17, IL-22, and IL-21.\textsuperscript{12} The Th17 cells belong to the CD4+ T cells that produce IL-17.\textsuperscript{13} Our previous findings that Th17 cell abundance was associated with the SOJIA activity confirmed the involvement of Th17 in the pathogenesis of SOJIA. High levels of IL-17 were commonly discovered in RA patients and it was confirmed that IL-17 played a significant role in the pathogenesis of RA via the contribution to inflammatory process.\textsuperscript{14,15} In addition, other cytokines which are secreted by the Th-cell subsets also play significant roles in the inflammation process. For instance, interferon (IFN)-γ and IL-4 participate in the development or differentiation of Th1 cells and Th2 cells, respectively, acting as regulators of immune responses and inflammatory diseases.\textsuperscript{16,17} Although the cytokines including IL-17, IL-6, IL-21, IFN-γ, and IL-4 are implicated in the development of arthritis or inflammatory diseases, studies rarely reported their roles in the development of SOJIA. Thus, our research explored the effects of these five cytokines on the pathogenesis of SOJIA, and examined whether these cytokines would exert a proinflammatory function during this regulation.

In our study, the clinical inflammatory indexes, such as tender joints counts (TJC), swelling joints counts (SJC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), visual analogue scale (VAS), and disease activity score 28 (DAS28) were detected, and the expression levels of serum IL-17, IL-6, IL-21, IFN-γ, and IL-4 in patients with SOJIA were determined in order to analyze the correlations among them. We also analyzed the correlation between the five cytokines and the clinical inflammatory indexes.

2. Methods

2.1. Patients

This study was approved by the ethics committee of Renji Hospital, School of Medicine, Shanghai Jiaotong University. All patients were diagnosed with SOJIA based on the International League of Associations for Rheumatology classification.\textsuperscript{18,19} SOJIA patients enrolled in this study had had no infectious diseases for at least 2 weeks prior to the diagnosis. Patients fulfilling the following criteria were excluded from this study: (1) the patients or their immediate family had a history of psoriasis; (2) the patients were boys over 6 years old with HLA-B27 arthritis; (3) the patients or their immediate family were or had been diagnosed with ankylosing spondylitis, infection arthritis related to attachment points, acute anterior uveitis, inflammatory bowel disease, or Reiter’s syndrome; and (4) the patients tested positive for rheumatoid factors twice within a period of 3 months.

SOJIA patients were divided into two groups: the active SOJIA group and the remission SOJIA group. Active SOJIA patients were diagnosed as follows: (1) suffering from fevers more than twice in one day and arthritis occurring in more than two joints; (2) serum CRP concentration ≥ 30 mg/L; and (3) serum glucocorticoids dosage ≥ 1 mg/kg/d. Remission SOJIA patients were diagnosed as follows: (1) no fever and the arthritis occurring on one or no joints; (2) serum CRP concentration < 30 mg/L; and (3) serum glucocorticoids dosage < 1 mg/kg/d. Thus, 60 SOJIA patients (n = 30 for each group) were enrolled from January 2012 to December 2013.

Normal healthy children were enrolled as the control group (n = 20). All 60 SOJIA patients accepted the conventional treatments in our hospital during their illness. Fasting vein blood (2 mL) was obtained before breakfast. Written informed consent was obtained from every participant’s parents or other guardians.

2.2. SOJIA disease assessment

The SOJIA clinical inflammatory indexes (TJC, SJC, CRP, ESR, VAS, and DAS28) were constructed based on the American College of Rheumatology and European League of Associations for Rheumatology guideline.\textsuperscript{20} Pain index was evaluated with VAS from 0 (no pain) to 10 (unbearable pain).\textsuperscript{21} DAS28 is a well-established evaluation system to define remission in RA.\textsuperscript{22} To assess the criteria for remission based on DAS28 and to compare disease activity and improvement, DAS28 was calculated based on the formula described in Fransen’s study.\textsuperscript{23} Based on DAS28 score, SOJIA disease activity was classified into five grades: remission (DAS28 ≤ 2.6), low activity (2.6 < DAS28 ≤ 3.2), moderate activity (3.2 < DAS28 ≤ 5.1), and high activity (DAS28 > 5.1).

2.3. Evaluation of the serum IL-17, IL-6, IL-21, IFN-γ, and IL-4 levels

Two milliliters of venous blood was obtained from all patients and centrifuged at 1500 r/min for 10 minutes. The supernatant was used to measure the expression levels of serum IL-17, IL-6, IL-21, IFN-γ, and IL-4 using enzyme-linked immunosorbent assay kits (Shanghai Suntech Biotech Co Ltd.) following the manufacturers’ instructions.

2.4. Statistical analysis

Quantitative data were calculated by mean ± standard error of the mean. Statistical analysis was carried out using
Table 1  The basic characteristics and clinical data of patients in active systemic onset juvenile idiopathic arthritis patients group, remission systemic onset juvenile idiopathic arthritis group, and normal controls.

<table>
<thead>
<tr>
<th>Item</th>
<th>Active</th>
<th>Remission</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>30</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>19:11</td>
<td>16:14</td>
<td>10:10</td>
</tr>
<tr>
<td>Age (yr), (mean, range)</td>
<td>7.9 ± 4.2 (4.5–16.2)</td>
<td>6.2 ± 3.8 (3.0–14.8)</td>
<td>5.8 ± 2.9 (3.8–16.0)</td>
</tr>
<tr>
<td>Duration (mo), (mean, range)</td>
<td>28.2 ± 18.5 (5–64)</td>
<td>34.0 ± 20.1 (8–84)</td>
<td>–</td>
</tr>
<tr>
<td>TJC (mean, range)</td>
<td>3 ± 0.8 (0–6)</td>
<td>0.5 ± 0.3 (0–2)</td>
<td>–</td>
</tr>
<tr>
<td>SJC (mean, range)</td>
<td>3 ± 1.9 (0–6)</td>
<td>0.5 ± 0.4 (0–2)</td>
<td>–</td>
</tr>
<tr>
<td>VAS (mean, range)</td>
<td>40 ± 15.2 (0–80)*</td>
<td>10 ± 3.6 (0–20)</td>
<td>–</td>
</tr>
<tr>
<td>CRP (mean, range)</td>
<td>58.3 ± 19.5 (30–220)*</td>
<td>6.4 ± 3.1 (3.16–22.5)</td>
<td>5.59 ± 2.0 (3.16–10)</td>
</tr>
<tr>
<td>ESR (mean, range)</td>
<td>53.1 ± 23.1 (10–120)</td>
<td>12.3 ± 5.4 (2–30)</td>
<td>8.95 ± 4.5 (0–20)</td>
</tr>
<tr>
<td>DAS28 (mean, range)</td>
<td>3.46 ± 0.7 (2.6–4.5)</td>
<td>1.83 ± 0.2 (1.5–2.9)</td>
<td>–</td>
</tr>
</tbody>
</table>

Active = active systemic onset juvenile idiopathic arthritis patients; Control = normal control group; CRP = C-reactive protein; DAS28 = disease activity score 28; ESR = erythrocyte sedimentation rate; SJC = swelling joints counts; Remission = Remission systemic onset juvenile idiopathic arthritis patients; TJC = tender joints counts; VAS = visual analogue scale.

* Indicates significant differences among groups.

SPSS 20.0 software (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) test was compared among groups. Pearson’s correlation coefficient was used to analyze the correlation. Spearman’s correlation coefficient was used for comparing categorical variables. A p value <0.05 was chosen as cut-off criterion.

3. Results

3.1. Characteristics of the patients and controls

The basic characteristics of these patients or controls are shown in Table 1. The sex ratios (male:female) in the active SOJIA group, remission SOJIA group, and controls were 19:11, 16:14, and 10:10, respectively. The average ages of the three groups were 7.9 ± 4.2 years (range: 4.5–16.2 years), 6.2 ± 3.8 years (range: 3.0–14.8 years), and 5.8 ± 2.9 years (range: 3.8–16.0 years), respectively. The duration of SOJIA disease in the active SOJIA group and the remission SOJIA group were 28.2 ± 18.5 months (range: 5–64 months) and 34.0 ± 20.1 months (range: 8–84 months), respectively (Table 1).

3.2. SOJIA disease assessment

The SOJIA disease assessment indicators (TJC, SJC, CRP, ESR, VAS, and DAS28) were evaluated (Table 1). The levels of TJC and SJC were very similar, and significant difference was found between the active SOJIA group and the remission SOJIA group (p < 0.05). VAS and DAS28 in the active SOJIA group were significantly higher than those in the remission SOJIA group (p < 0.05). CRP and ESR in the active SOJIA group were significantly higher than those in the remission SOJIA group and the control group (p < 0.05; Table 1).

3.3. Evaluation of the serum IL-17, IL-6, IL-21, IFN-γ, and IL-4 levels

The serum IL-17, IL-6, IL-21, IFN-γ, and IL-4 expression levels were determined using enzyme-linked immunosorbent assay (Table 2). One-way ANOVA indicated that pronounced differences of the IL-17 expression levels in three groups were observed (F = 33.88, p = 0.000). Furthermore, the comparison between any two groups revealed that the IL-17 level in the active SOJIA group was significantly higher than the other groups (p < 0.05), while significant differences were not detected between the remission SOJIA group and control group (p > 0.05).

Likewise, the IL-6 levels in the three groups were also significantly different (F = 7.33, p = 0.001). By the comparison between any two groups, the active SOJIA group showed a markedly higher IL-6 level than the other two groups, and notably the remission SOJIA group

Table 2  Evaluation of the expression levels of interleukin (IL)-17, IL-6, IL-21, interferon-γ, and IL-4 in the active systemic onset juvenile idiopathic arthritis group, remission systemic onset juvenile idiopathic arthritis group, and normal control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-17 (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>IL-21 (pg/mL)</th>
<th>IFN-γ (pg/mL)</th>
<th>IL-4 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>131.07 ± 56.28***</td>
<td>1567.23 ± 1284.19***</td>
<td>116.70 ± 72.49*</td>
<td>33.32 ± 28.06</td>
<td>65.03 ± 23.67</td>
</tr>
<tr>
<td>Remission</td>
<td>58.40 ± 29.82</td>
<td>1125.26 ± 504.93*</td>
<td>98.63 ± 67.79</td>
<td>20.57 ± 13.02</td>
<td>61.81 ± 28.22</td>
</tr>
<tr>
<td>Control</td>
<td>50.01 ± 18.36</td>
<td>159.33 ± 35.62</td>
<td>78.30 ± 51.58</td>
<td>17.24 ± 25.48</td>
<td>59.12 ± 25.48</td>
</tr>
<tr>
<td>p</td>
<td>F = 33.88</td>
<td>F = 7.33</td>
<td>F = 2.04</td>
<td>F = 2.54</td>
<td>F = 0.27</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.001</td>
<td>0.136</td>
<td>0.089</td>
<td>0.761</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with normal control group.

*** p < 0.05 compared with remission SOJIA group.
also presented a higher IL-6 level than control group ($p < 0.05$).

One-way ANOVA revealed no significantly different IL-21 levels among the three groups ($F = 2.04, p = 0.136$). However, comparison between any two groups showed that IL-21 in the active SOJIA group was slightly higher than in the controls ($p < 0.05$; Table 2).

No significantly different levels of IFN-$\gamma$ and IL-4 were observed among the three groups (IFN-$\gamma$: $F = 2.54, p = 0.089$; IL-4: $F = 0.27, p = 0.761$; Table 2).

3.4. Correlation analysis of cytokines and clinical index in active SOJIA group

In the active SOJIA group, Spearman coefficients suggested a significant positive correlation between the expression level of IL-17 and DAS28 ($r = 0.686, p = 0.000$; Figure 1A); the IL-17 level was also positively associated with IL-6 level with significance ($r = 0.833, p = 0.000$; Figure 1B); and significant positive association between IL-6 level and DAS28, was detected ($r = 0.633, p = 0.000$, Figure 1C). In addition, IL-17 level was positively correlated with VAS with significance ($r = 0.524, p = 0.003$, Figure 1D). However, the remaining three cytokines did not show any correlations with either DAS28 or VAS in this group.

In the other two groups, none of the five cytokines exhibited an association with DAS28 or VAS.

4. Discussion

In this study, the expression levels of serum IL-17 and IL-6 in the active SOJIA group were significantly increased compared with those in the remission SOJIA group and control group, while no obviously different levels of serum IL-21, IFN-$\gamma$, and IL-4 were discovered among the three groups. Moreover, the expression level of IL-21 in the active...
SOJIA group was also significantly higher than in the control group. These results implied that IL-17 and IL-6 might contribute to the pathogenesis of SOJIA.

IL-6 is a pleiotropic cytokine with a wide range of biological activities in the regulation of immunity, inflammation, and oncogenesis, and the high level IL-6 has been detected in some inflammatory diseases such as JIA and RA, suggesting that the overexpression of IL-6 contributes greatly to the pathogenesis of these diseases.8–9,24–25 IL-6 is indispensable for the induction of Th17, which is involved in the pathogenesis of these inflammatory diseases, via the Janus family tyrosine kinase—signal transducer and activator of transcription and the Ras-mitogen-activated protein kinase pathways.25–28 However, a humanized anti-IL-6R (IL-6 receptor) antibody, which decreased the production of IL-6R, has been considered to be a new therapeutic approach for RA and systemic JIA.25 Furthermore, IL-6 is proposed as a therapeutic target in SOJIA and other intractable diseases, possibly through prevention of progression to macrophage activation syndrome.28

In the present study, the expression levels serum IL-17 and IL-6 in the active SOJIA group were significantly higher than those in the remission SOJIA group and the control group. In the active SOJIA group, significant positive associations between two cytokines of IL-17 and IL-6 and the clinical index DAS28 were detected. Additionally, IL-17 was positively correlated with IL-6 with statistical significance in this group. DAS28 is usually used to define remission RA or evaluate SOJIA disease activity.22,23 The close relationships between the two cytokines and DAS28 indicated that IL-17 and IL-6 could serve as two indicators of SOJIA disease activity. Moreover, IL-17 is secreted by Th17 cells which influence the pathogenesis of autoimmune diseases,12,30 and it contributes to the pathogenesis of RA by enhancing the production of IL-6 and IL-8.31,32 In addition, Fischer et al33 reported that combined inhibition of TNFα and IL-17 had a synergistic effect in suppression of human mesenchymal cells and that bispecific anti-TNFα/IL-17 antibodies could be used for the treatment of arthritis.33 These results collectively implied that both IL-17 and IL-6 might contribute to the development of SOJIA, and they might be considered as the therapeutic targets for the treatment of SOJIA.

Ma et al34 demonstrated that IL-17 might contribute to the balance of Th1/Th2 cells through the detection of IFN-γ and IL-4 expression, secreted by Th1 cells and Th2 cells, respectively.34 Furthermore, IL-21, similar to IL-6, together with transforming growth factor-β could induce the differentiation of Th17 cells, independent of the effect of IL-6.35 IL-6 could indirectly induce the production of IL-21 by activating CD4 cells.35 The blockade of IL-6R was accompanied by the decrease of IL-21 production.34 However, the expression of IL-21, IFN-γ, and IL-4 showed nonsignificant higher levels in the active SOJIA group compared with those in the other groups.

One limitation to our research was the lack of a statistical study on the changes of the expression levels of these cytokines during the illness treatments. This might be the reason for the conclusion that there were no correlations between cytokines and the clinical indexes of DAS28 or VAS. Based on these results, we speculated that IL-21, IFN-γ, and IL-4 might not be involved in the development of SOJIA. Therefore, further verification experiments are needed not only to confirm the speculations above, but also to ascertain the treatment for SOJIA.

5. Conclusion

Cytokines IL-17 and IL-6 were closely associated with the clinical inflammatory indexes and they might be used as therapeutic targets for the treatment of SOJIA.

Conflicts of interest

None.

Acknowledgments

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References