Effects of SOCS1 and SOCS3 in peripheral blood on CD4+T cell differentiation in children with Henoch–Schonlein purpura

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Objective: To study the effects of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in peripheral blood on CD4+T cell differentiation in children with Henoch-Schonlein purpura.

Methods: Children with Henoch-Schonlein purpura who were treated in Zigong Maternal and Child Health Hospital between June 2014 and February 2018 were selected as the HSP group of the study, and healthy children who received physical examination during the same period were selected as the control group of the study. Peripheral blood was collected to determine the expression of SOCS1 and SOCS3 as well as the contents of CD4+T cell subsets, and serum was collected to determine the contents of CD4+T cytokines.

Results: SOCS1 and SOCS3 mRNA expression levels as well as SOCS3/SOCS1 ratio in peripheral blood of HSP group were significantly higher than those of control group; Th1 and Treg contents in peripheral blood as well as IFN-γ and TGF-β1 contents in serum of HSP group were lower than those of control group whereas Th2 and Th17 contents in peripheral blood as well as IL-4, IL-5 and IL-17 contents in serum were higher than those of control group, and Th1 and Treg contents in peripheral blood as well as IFN-γ and TGF-β1 contents in serum of HSP children with high SOCS3/SOCS1 ratio were lower than those of HSP children with low SOCS3/SOCS1 ratio whereas Th2 and Th17 contents in peripheral blood as well as IL-4, IL-5 and IL-17 contents in serum were higher than those of HSP children with low SOCS3/SOCS1 ratio.

Conclusions: Changes in SOCS1 and SOCS3 expression in peripheral blood of children with Henoch-Schonlein purpura can affect the differentiation of CD4+T cells.

1. Introduction

Henoch-Schonlein purpura (HSP) is an allergic disease mainly involving the capillaries, and abnormal immune response is an important pathological link in the occurrence and development of HSP, and specifically involves the humoral immune response and cellular immune response disorders[1,2]. CD4+T cells are important cell groups involved in immune response regulation in vivo, which can be differentiated into different subsets such as Th1, Th2, Th17 and Treg to participate in the allergic reaction of capillaries in HSP pathogenesis[3]. Suppressors of cytokines signaling (SOCS) is a family of proteins generated by cytokines, which include a total of eight members, SOCS1-7 and cytokine inducible SH2-containing protein, and have significant negative regulatory effect on JAK-STAT signal pathway[4,5]. SOCS1 and SOCS3 is a pair of SOCSs family members that regulate CD4+T cell subset differentiation through the JAK-STAT pathway, it has been reported that the SOCS1 and SOCS3 expression are significantly abnormal in peripheral blood of children with HSP[6], but it is not yet clear whether SOCS1 and SOCS3 are involved in regulating CD4+T cell subset differentiation in the course of HSP. In the following studies, we specifically analyzed the effects of SOCS1 and SOCS3 on CD4+T cell differentiation in peripheral blood of children with Henoch-Schonlein purpura.
2. Materials and methods

2.1. Clinical case information

Children with Henoch-Schonlein purpura who were treated in Zigong Maternal and Child Health Hospital between June 2014 and February 2018 were chosen as the HSP group of the study, all children met the diagnostic criteria for HSP, were with the first onset and the course of disease < 3 d, and didn’t use corticosteroids or other immunosuppressive agents within 4 weeks before inclusion, and the children who were combined with asthma or other allergic diseases were eliminated. Healthy children who received physical examination during the same period were chosen as the control group of the study. There were 48 cases in HSP group, including 26 males and 22 females who were 5-12 years old; there were 60 cases in the control group, including 32 males and 28 females who were 5-11 years old. There was no significant difference in general information between the two groups (P>0.05).

2.2. Laboratory detection of peripheral blood SOCS1 and SOCS3 expression

A total of 1-2 mL of fasting peripheral venous blood was collected from the two groups of subjects, and the RNA in the venous blood was isolated according to the instructions of the whole blood RNA extraction kit; the SOCS1 and SOCS3 expression in RNA were determined according to the instructions of reverse transcription reaction and PCR reaction.

2.3. Laboratory detection of peripheral blood CD4+T cell subsets

0.5-1.0 mL of fasting peripheral venous blood was collected from the two groups of subjects and anti-coagulated with EDTA to incubate CD4, IFN-γ, IL-4, IL-17 and CD25 fluorescent antibody, and then flow cytometer was adopted to determine the contents of CD4+IFN-γ+Th1, CD4+IL4+Th2, CD4+IL17+Th17 and CD4+CD25+Treg.

2.4. Laboratory detection of serum CD4+T cell cytokines

3-5 mL of fasting peripheral venous blood was collected from the two groups of subjects, let stand for natural coagulation and centrifuged to separate serum, and the Elisa kit instructions were referred to determine IFN-γ, IL-4, IL-5, IL-17 and TGF-β1 contents in serum.

2.5. Statistical methods

Software SPSS 21.0 was used to input data, the differences in measurement data between two groups were analyzed by t test and P<0.05 showed that the difference was statistically significant.

3. Results

3.1. Changes in peripheral blood SOCS1 and SOCS3 expression and ratio

SOCS1 and SOCS3 mRNA expression levels as well as SOCS3/SOCS1 ratio in peripheral blood of HSP group were (1.77±0.25), (2.98±0.42) and (1.68±0.26) respectively; SOCS1 and SOCS3 mRNA expression levels as well as SOCS3/SOCS1 ratio in peripheral blood of control group were (1.03±0.16), (1.01±0.14) and (0.98±0.13) respectively. After t test, SOCS1 and SOCS3 mRNA expression levels as well as SOCS3/SOCS1 ratio in peripheral blood of HSP group were significantly higher than those of control group.

3.2. Peripheral blood CD4+T cell subset contents

Th1 and Treg contents in peripheral blood of HSP group were lower than those of control group whereas Th2 and Th17 contents were higher than those of control group (P<0.05) (Table 1). Th1 and Treg contents in peripheral blood of HSP children with high SOCS3/SOCS1 ratio were lower than those of HSP children with low SOCS3/SOCS1 ratio whereas Th2 and Th17 contents were higher than those of HSP children with low SOCS3/SOCS1 ratio (P<0.05) (Table 2).

<table>
<thead>
<tr>
<th>SOCS3/SOCS1</th>
<th>n</th>
<th>Th1</th>
<th>Th2</th>
<th>Th17</th>
<th>Treg</th>
</tr>
</thead>
<tbody>
<tr>
<td>High ratio</td>
<td>24</td>
<td>6.56±0.79</td>
<td>4.92±0.75</td>
<td>2.21±0.37</td>
<td>1.33±0.17</td>
</tr>
<tr>
<td>Low ratio</td>
<td>24</td>
<td>10.13±1.38</td>
<td>3.28±0.47</td>
<td>1.29±0.17</td>
<td>2.52±0.37</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>14.832</td>
<td></td>
<td></td>
<td>12.375</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2: Comparison of peripheral blood CD4+T cell subsets between HSP children with different SOCS3/SOCS1 ratio.
3.3. Serum CD4+T cytokine contents

IFN-γ and TGF-β1 contents in serum of HSP group were lower than those of control group whereas IL-4, IL-5 and IL-17 contents were higher than those of control group (p<0.05) (Table 3). IFN-γ and TGF-β1 contents in serum of HSP children with high SOCS3/SOCS1 ratio were lower than those of HSP children with low SOCS3/SOCS1 ratio whereas IL-4, IL-5 and IL-17 contents were higher than those of HSP children with low SOCS3/SOCS1 ratio (p<0.05) (Table 4).

Table 3
Comparison of serum CD4+T cytokines between the two groups of children (pg/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IFN-γ (pg/mL)</th>
<th>IL-4 (pg/mL)</th>
<th>IL-5 (pg/mL)</th>
<th>IL-17 (pg/mL)</th>
<th>TGF-β1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP group</td>
<td>48</td>
<td>20.31±3.48</td>
<td>34.18±5.28</td>
<td>19.33±2.32</td>
<td>27.58±3.76</td>
<td>1.04±0.16</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>29.61±4.28</td>
<td>16.22±1.89</td>
<td>11.45±1.37</td>
<td>18.29±2.25</td>
<td>2.33±0.37</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 4
Comparison of serum CD4+T cytokines between HSP children with different SOCS3/SOCS1 ratio (pg/mL).

<table>
<thead>
<tr>
<th>SOCS3/SOCS1</th>
<th>n</th>
<th>IFN-γ (pg/mL)</th>
<th>IL-4 (pg/mL)</th>
<th>IL-5 (pg/mL)</th>
<th>IL-17 (pg/mL)</th>
<th>TGF-β1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High ratio</td>
<td>24</td>
<td>16.02±2.03</td>
<td>45.92±6.86</td>
<td>25.09±3.27</td>
<td>33.11±4.28</td>
<td>0.69±0.09</td>
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<tr>
<td>Low ratio</td>
<td>24</td>
<td>24.75±3.95</td>
<td>23.12±3.86</td>
<td>13.75±1.76</td>
<td>22.37±2.93</td>
<td>1.45±0.20</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

4. Discussion

The capillary allergic inflammatory change caused by immune response disorder is the most prominent pathological feature in the course of HSP[7], but the mechanism of immune disorders is still unknown. CD4+T cells have played an important role in regulating humoral immune and cellular immune responses, and the subset can regulate the synthesis of immunoglobulin, the secretion of pro-inflammatory and anti-inflammatory cytokines as well as other process after it differentiates into Th1, Th2, Th17, Treg and other subsets[8]. SOCSs is a family of proteins that negatively regulate JAK/STAT pathway to influence the differentiation and activation of immune cells and inflammatory cells, the SOCS1 and SOCS3 is a pair of SOCSs family members that influence each other in the function and effect, the former is mainly expressed in the Th2 cells and has inhibitory effect on cell differentiation, and the latter is mainly expressed in Th1 cells and has inhibitory effect on cell differentiation[9,10]. It has been reported that the SOCS1 and SOCS3 expression are significantly abnormal in peripheral blood of children with HSP, the changes of SOCS1 and SOCS3 in peripheral blood of children with HSP were also analyzed in the study, and the results showed that SOCS1 and SOCS3 mRNA expression levels as well as SOCS3/SOCS1 ratio in peripheral blood of HSP group were significantly higher than those of control group. This means that SOCS1 and SOCS3 are highly expressed in the course of HSP, the increase of SOCS3 expression is more significant than that of SOCS1, and the inhibitory effect of SOCS3 on Th1 differentiation exceeds the inhibitory effect of SOCS1 on Th2 differentiation, which may cause Th1/Th2 balance to be broken.

Th1 and Th2 are the earliest discovered CD4+T cell subsets, the former differentiates and matures under the action of transcription factor T-bet and and secretes IFN-γ, IFN-β and other cytokines, and it promotes inflammation and mediates cellular immune response[11]; the former differentiates and matures under the action of transcription factor GATA-3 and secretes IL-4, IL-5 and other cytokines, and it inhibits inflammation and mediates the humoral immune response, and can significantly enhance the B cell activity, increase the synthesis of immunoglobulin and cause autoimmune change[11]. Analysis of the changes in the number of Th1 and Th2 cells in peripheral blood of children with HSP showed that Th1 content in peripheral blood of HSP group was lower than that of control group whereas Th2 content was higher than that of control group. This indicates that the shifting of Th1/Th2 balance to Th2 is closely related to the occurrence of HSP. Further analysis of the change in corresponding Th1 and Th2 cytokine contents to verify the change in the Th1 and Th2 function indicated that IFN-γ content in serum of HSP group was lower than that of control group whereas IL-4 and IL-5 contents were higher than those of control group. This indicates that the decreased secretion of Th1 cytokines and the increased secretion of Th2 cytokines are closely related to the occurrence of HSP. We also further analyzed the effects of SOCS1 and SOCS3 on Th1 and Th2 differentiation in children with HSP, and the results showed that Th1 content in peripheral blood and IFN-γ content in serum decreased whereas Th2 content in peripheral blood as well as IL-4 and IL-5 contents in serum increased in HSP children with high SOCS3/SOCS1 ratio. It means that the changes of SOCS1 and SOCS3 balance in the course of HSP can inhibit the differentiation CD4+T cell subset Th1, promote the differentiation of Th2 and then induce the capillary allergic inflammatory change through the biological effect of Th2.

Th17 and Treg are newly discovered CD4+T cell subsets in recent years, the former differentiates and matures under the function of transcription factor RORγt and specifically secretes IL-17, and it has significant pro-inflammatory activity and chemotactic activity,
and can mediate inflammatory cell infiltration within the capillaries and enhance allergic inflammation[12,13]; the latter differentiates and maturates under the action of Foxp3 and secretes TGF-β 1, and it can inhibit the immune response and avoid the excessive activation of allergy through the intercellular direct contact inhibition and the biological effect of TGF-β 1 [14–16]. Analysis the changes in the number of Th17 and Treg cells in peripheral blood of children with HSP showed that Treg content in peripheral blood of HSP group was lower than that of control group whereas Th17 content was higher than that of control group. This indicates that the shifting of Th17/Treg balance to Th17 is closely related to the occurrence of HSP. Further analysis of the changes in corresponding Th17 and Treg cytokine contents to verify the change of Th17 and Treg function indicated that IL-17 content in serum of HSP group was higher than that of control group whereas TGF-β 1 content was lower than that of control group. This indicates that the increased secretion of Th17 cytokines and the decreased secretion of Treg cytokines are closely related to the occurrence of HSP. We further analyzed the effect of SOCS1 and SOCS3 on Th17 and Treg differentiation in children with HSP, and the results showed that Th17 content in peripheral blood and IL-17 content in serum significantly increased whereas Treg content in peripheral blood and TGF-β 1 content in serum significantly decreased in HSP children with high SOCS3/SOCS1 ratio. It means that the changes of SOCS1 and SOCS3 balance in the course of HSP can not only affect the differentiation of CD4+ T cell subsets Th1 and Th2, but can also promote the differentiation of Th17, inhibit the differentiation of Treg, and then enhance the capillary allergic inflammatory change in the course of HSP through the biological effects of Th17.

Based on the discussion about above molecule expression, cell number and cytokine levels, it can be concluded that the SOCS1 and SOCS3 expression are significantly abnormal in peripheral blood of children with Henoch-Schönlein purpura, and the rising trend of SOCS3 expression is more significant than that of SOCS1; the abnormally expressed SOCS1 and SOCS3 can affect the differentiation of CD4+T cells.

References