Correlation of STATs family expression in oral lichen planus tissue with peripheral blood PD-1 and PD-L1 expression as well as immune function

Hong Zhang 1,2, Ying Zhang 2

1. Introduction

Oral lichen planus (OLP) is a type of chronic noninfectious inflammatory disease that trends to occur in the oral mucosa, the pathogenesis is complex and abnormal immune response is considered to be closely related to the occurrence of OLP[1,2]. Although the relationship between the abnormal immune response and the onset of OLP has received more and more attention, the influence of the change in the systemic immune response on molecular expression in local tissue is unclear. STAT family is a family of proteins with transcription activity function, and after it is regulated by upstream cytokines, its configuration will change, and it accumulates in the nucleus and can target and combine DNA sequences and then regulate gene expression and exert corresponding biological effect. At present, it is not clear whether...
the STAT family plays a part in the pathogenesis of oral lichen planus. In the following study, the correlation of STATs family expression in oral lichen planus tissue with peripheral blood PD-1 and PD-L1 expression as well as immune function was analyzed.

2. Subjects and methods

2.1 Research subjects

A total of 47 patients diagnosed with oral lichen planus in our hospital between May 2015 and March 2016 were selected as the oral lichen planus group (OLP group) of the study, and all patients were diagnosed with oral cavity lichen planus by pathological examination, not complicated with systemic disease or autoimmune disease, and never received local or systemic immune stimulant treatment in last three months. 50 healthy volunteers receiving physical examination in our hospital during the same period were selected as the control group of the study, and all were without history of oral diseases. OLP group included 33 male cases and 14 female cases that were (48.4±6.3) years old; control group included 35 male cases and 15 female cases that were (47.6±6.7) years old. The two groups of subjects were not significantly different in general information (P>0.05).

2.2 Research methods

2.2.1 Sample collection methods

10 mL of peripheral blood was collected from OLP group and control group and divided into two. One peripheral blood sample was let stand for 30 min and then centrifuged to separate the upper serum and store it in a -80 °C refrigerator; the other peripheral blood sample was anticoagulated with EDTA and then added in lymphocyte separation medium, and after density gradient centrifugation, the middle suspended mononuclear cells were extracted, washed with PBS twice and then centrifuged to take cell precipitation and store it in a -80 °C refrigerator. Proper amount of lesion tissue and adjacent normal tissue were collected from OLP group, added in protein lysis buffer and fully homogenized, the homogenate was centrifuged, and then the upper supernatant liquid was collected and stored in a -80 °C refrigerator.

2.2.2 PD-1 and PD-L1 expression detection methods

Peripheral blood mononuclear cells were collected, Trizol lysis buffer was added to full break cells, to the kit instructions were followed to separate total RNA in the cells, and then cDNA synthesis kits were used to reverse-transcribe the RNA into cDNA; cDNA samples were collected for fluorescence quantitative PCR reaction, PD-1, PD-L1 and GAPDH were amplified respectively, and GAPDH was used as reference to calculate PD-1 and PD-L1 mRNA expression.

2.2.3 Immune cell surface marker expression detection methods

Peripheral blood mononuclear cells were taken to incubate CD3, CD4, CD8, CD19, CD16 and CD56 monoclonal antibody away from light for 20 min and then wash them with PBS twice, and then the mean fluorescence intensity of CD3+, CD4+, CD8+, CD19+ and CD16+CD56+ were measured from flow cytometer.

2.2.4 Th1 and Th2 cytokines as well as immunoglobulin content detection methods

Serum samples were taken, and enzyme-linked immunosorbent assay kits were used to detect interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-4, IL-10, immunoglobulin G (IgG), IgM and IgA content.

2.2.5 Detection methods of STATs family expression in lesion tissue

The protein homogenate samples of lesion tissues and normal tissues were taken, the kit instructions were followed to configure polyacrylamide gel, protein samples were added in the gel sample application holes, vertical electrophoresis and horizontal membrane transfer were conducted in turn, the NC membrane was closed in 5% skim milk for 2 h, then the corresponding NC membrane was cut according to the molecular weight of proteins, the first antibody of STAT1, STAT2, STAT3, STAT4, STAT5a and STAT5b as well as p-STAT1, p-STAT2, p-STAT3, p-STAT4, p-STAT5a and p-STAT5b were incubated overnight at 4 °C, the NC membrane was taken out the next day, the second antibody were incubated, finally the chemical developer was used for development, protein bands were obtained, and then STAT1, STAT2, STAT3, STAT4, STAT5a and STAT5b were used as reference to calculate p-STAT1, p-STAT2, p-STAT3, p-STAT4, p-STAT5a and p-STAT5b protein expression.

2.3 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data analysis between two groups was by t test, correlation analysis was by Pearson test and P<0.05 indicated statistical significance in differences.
3. Results

3.1 PD-1 and PD-L1 expression in peripheral blood mononuclear cells

Analysis of PD-1 and PD-L1 expression in peripheral blood mononuclear cells between OLP group and control group was as follows: PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of OLP group were significantly higher than those of control group. Differences in PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells were statistically significant between the two groups (P<0.05).

Table 1.
Comparison of PD-1 and PD-L1 expression in peripheral blood mononuclear cells between two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>PD-1</th>
<th>PD-L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLP</td>
<td>47</td>
<td>2.28±0.37</td>
<td>1.96±0.26</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>1.07±0.14</td>
<td>1.03±0.14</td>
</tr>
<tr>
<td>T</td>
<td>12.782</td>
<td>9.592</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Immune cell surface marker expression in peripheral blood mononuclear cells

Analysis of immune cell surface markers CD3, CD4, CD8, CD19, CD16 and CD56 expression in peripheral blood mononuclear cells between OLP group and control group was as follows: the mean fluorescence intensity of CD3+, CD4+, CD8+ and CD16+CD56+ in peripheral blood mononuclear cells of OLP group were significantly lower than those of control group while the mean fluorescence intensity of CD19+ was significantly higher than that of control group. Differences in mean fluorescence intensity of immune cell surface markers CD3+, CD4+, CD8+, CD19+ and CD16+CD56+ in peripheral blood mononuclear cells were statistically significant between the two groups (P<0.05).

Table 2.
Comparison of immune cell surface marker expression in peripheral blood mononuclear cells between two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>CD3+</th>
<th>CD4+</th>
<th>CD8+</th>
<th>CD19+</th>
<th>CD16+CD56+</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLP</td>
<td>47</td>
<td>3.65±0.51</td>
<td>1.77±0.24</td>
<td>1.02±0.13</td>
<td>1.89±0.25</td>
<td>0.77±0.08</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>7.95±0.89</td>
<td>3.27±0.52</td>
<td>2.85±0.41</td>
<td>1.03±0.14</td>
<td>1.78±0.24</td>
</tr>
<tr>
<td>T</td>
<td>11.038</td>
<td>8.957</td>
<td>16.582</td>
<td>9.315</td>
<td>13.583</td>
<td></td>
</tr>
<tr>
<td>P</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>
<td></td>
</tr>
</tbody>
</table>

3.3 Serum Th1 and Th2 cytokine as well as immunoglobulin content

Analysis of serum Th1 cytokines IFN-γ and IL-2, Th2 cytokines IL-4 and IL-10 as well as immunoglobulin IgG, IgM and IgA between OLP group and control group was as follows: serum IFN-γ and IL-2 content of OLP group were significantly lower than those of control group while IL-4, IL-10, IgG, IgM and IgA content were significantly higher than those of control group. Differences in serum IFN-γ, IL-2, IL-4, IL-10, IgG, IgM and IgA content were statistically significant between the two groups (P<0.05).

Table 3.
Comparison of serum Th1 and Th2 cytokine as well as immunoglobulin content between two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Immunoglobulin (g/L)</th>
<th>Th1 cytokines (ng/mL)</th>
<th>Th2 cytokines (ng/mL)</th>
<th>cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
<td>IgA</td>
<td>IgM</td>
<td>IFN-γ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IL-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IL-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IL-10</td>
</tr>
<tr>
<td>OLP</td>
<td>47</td>
<td>13.8±1.8</td>
<td>2.4±0.4</td>
<td>1.6±0.2</td>
<td>11.6±1.8</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>11.3±1.5</td>
<td>2.1±0.3</td>
<td>1.1±0.1</td>
<td>18.5±2.4</td>
</tr>
<tr>
<td>T</td>
<td>6.276</td>
<td>6.883</td>
<td>8.147</td>
<td>8.938</td>
<td>9.582</td>
</tr>
<tr>
<td>P</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>

3.4 STATs family expression in lesion tissue

Analysis of STATs family p-STAT1, p-STAT2, p-STAT3, p-STAT4, p-STAT5a and p-STAT5b expression in lesion tissue and adjacent normal tissue was as follows: p-STAT1, p-STAT3 and p-STAT5a expression in lesion tissue were significantly higher than those in normal tissue while p-STAT2, p-STAT4 and p-STAT5b expression...
were not significantly different from those in normal tissue. Differences in p-STAT1, p-STAT3 and p-STAT5a expression in lesion tissue and adjacent normal tissue were statistically significant (P<0.05).

4. Discussion

Oral lichen planus (OLP) is a kind of chronic noninfectious inflammatory disease closely associated with autoimmune response disorder, and it mainly involves the oral mucosa[3]. In the occurrence and development process of OLP, the cellular immune response and the humoral immune response significantly change, and the synthesis and secretion of a variety of cytokines and immune globulins are abnormal[4,5]. PD-1 and PD-L1 is a pair of negative costimulatory molecules regulating the immune response, PD-1 is mainly expressed in the activated B lymphocytes and T lymphocytes, and PD-L1 is mainly expressed in macrophages, B lymphocyte and other antigen-presenting cells[6,7]. PD-1 and PD-L1 combination can negatively regulate T lymphocyte, especially CD4+T cell maturation and differentiation as well as the corresponding cytokine synthesis and secretion[8]. In the study, the analysis of PD-1 and PD-L1 expression in peripheral blood of OLP patients showed that PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of OLP group were significantly higher than those of control group. It indicates that the expression of negative costimulatory molecules PD-1/PD-L1 that are with immune regulating function significantly increase in peripheral blood of OLP patients, and the immune cells regulated by PD-1/PD-L1 may also change correspondingly.

T lymphocytes, B lymphocytes and NK cells are the important immune cells mediating immune response, CD3, CD4 and CD8 are the marker molecules on T lymphocyte surface, CD19 is the marker molecule on B lymphocyte surface, and CD16 and CD56 are the marker molecules on NK cell surface[9,10]. In order to define the effect of PD-1/PD-L1 expression change in OLP patients on immune cell maturation, immune cell surface marker molecule expression in peripheral blood mononuclear cells of patients with OLP were analyzed in the study, and the results showed that the mean fluorescence intensity of CD3+, CD4+, CD8+ and CD16+CD56+ in peripheral blood mononuclear cells of OLP group were significantly lower than those of control group while the mean fluorescence intensity of CD19+ was significantly higher than that of control group. It means that the highly expressed negative costimulatory molecules PD-1/PD-L1 in patients with OLP will inhibit the differentiation and maturation of CD3+CD4+T cells, CD4+CD8+T cells and CD16+CD56+NK cells, and promote the differentiation and maturation of B lymphocytes. CD3+CD4+T cell number change will affect the synthesis and secretion of a variety of cytokines, the B lymphocyte number change will affect the synthesis and secretion of immunoglobulin, and the changes in T lymphocyte-related cytokines and the changes in B lymphocyte-related immune globulins are involved in the OLP changes.

The main functions of B lymphocytes are to differentiate into plasma cells and secrete immunoglobulin, the B lymphocyte number change in OLP patients can cause humoral immune response disorder through abnormal immunoglobulin content, and the analysis of the immunoglobulin content in the study showed that serum IgG, IgM and IgA content of OLP group were significantly higher than those of control group. CD4+T lymphocytes can be further divided into Th1 and Th2, and Th1 cells mainly secrete IFN-γ, TNF-α, IL-2 and other pro-inflammatory factors, and can mediate the inflammation in tissue mucosa and form lesions[11,12]; Th2 cells mainly secrete IL-4, IL-10 and other inhibitory cytokines, and they can inhibit the Th1 differentiation and maturation, and promote the B cell maturity and the immunoglobulin secretion[13,14]. PD-1/PD-L1 have significant effect on CD4+T lymphocyte maturity, and can also affect the cell differentiation to Th1 and Th2, which is mainly characterized by inhibiting Th1 cell maturation and promoting Th2 cell maturation. In the study, the analysis of the content of Th1 and Th2 cytokines showed that serum IFN-γ and IL-2 content of OLP group were significantly lower than those of control group while IL-4 and IL-10 content were significantly higher than those of control group. It means that highly expressed negative costimulatory molecules PD-1/PD-L1 in patients with OLP could suppress the Th1 cell maturation and promote the Th2 cell maturation, which cause the formation of lichen planus lesions through the changes in the content of corresponding cytokines.

OLP Lesions mainly involve oral mucosa, and the abnormal secretion of cytokines and immunoglobulin caused by the abnormal systemic immune response can cause gene expression change in the oral mucosa, thus generating corresponding biological effects. STATs family are a group of proteins with DNA domain, SH2/SH3 domain and transcription activity function domain, and they will be regulated by upstream cytokines and then change in configuration and form dimers, which gather in nuclear, are combined in DNA promoter regions, start the gene transcription and generate corresponding biological effect[15]. STATs family include STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and multiple other members, phosphorylation modification is the main regulation way, and STATs family members activated by upstream cytokines will be phosphorylated and then regulate the expression of target genes[16,17]. In the study, analysis of the expression of above STATs molecules in OLP lesions showed that p-STAT1, p-STAT3 and p-STAT5a expression in lesion tissue were significantly higher than those in normal tissue while p-STAT2, p-STAT4 and p-STAT5b expression were not significantly different from those in normal
It means that the STAT1, STAT3 and STAT5a express changes are closely related to the occurrence of OLP. Further analysis of the correlation between systemic immune response and STAT expression in the lesions showed that STAT1, STAT3 and STAT5a expression were positively correlated with PD-1, PD-L1 and CD19+ expression as well as IL-4, IL-10, IgG, IgM and IgA content, and negatively correlated with CD3+, CD4+, CD8+ and CD16+CD56+ expression as well as IFN-γ and IL-2 content.

In conclusion, p-STAT1, p-STAT3 and p-STAT5a are abnormally highly expressed in oral lichen planus tissues, and the changes in above STAT family molecule expression are closely related to Th1/Th2 cellular immunity and humoral immunity disorder mediated by PD-1/PD-L1.

References


