
A study of costimulatory factors in tonsils of pustulosis palmaris et plantaris

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Abstract
Pustulosis palmaris et plantaris (PPP) is known to be closely related to tonsillar focal infections and tonsillectomy is quite effective. It was reported that hyper-immune response to alpha streptococci in patients with PPP. On the other hand, costimulatory factors were studied on autoimmune disease. CD28 and cytotoxic T lymphocyte-associated 4 (CTLA4) participate in these factors. CD28 take part in T cell activation, while CTLA4 take part in T cell suppression. Therefore, we investigated CD28 and CTLA4 mRNA levels by reverse transcription-polymerase chain reaction (RT-PCR) in tonsillar tissues and CD3 positive lymphocytes from patients of PPP, recurrent tonsillitis, and obstructive sleep apnea syndrome. And then, we also investigated the expression levels of CD28 and CTLA4 by flow cytometry analysis in tonsillar mononuclear cells stimulated with alpha-streptococci in these patients. It was revealed that the CTLA4 mRNA in tonsillar CD3 positive lymphocytes from PPP patients was expressed at lower level than non-PPP patients. Then, the expression levels of CTLA4, comparison before and after stimulation, were lower level in patients with PPP patients than non-PPP patients. These results suggest that the lower expression level of CTLA4 in PPP may cause hyper-immune response to alpha streptococci and abnormal regulation of T cell activation.

Key Words: pustulosis palmaris et plantaris, CTLA-4, tonsil focal infection, costimulatory factor, CD28

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Background

PPP is known to closely relate to tonsillar focal infections and tonsillectomy is quite effective. The molecular mechanisms involved in PPP are still poorly understood. It was reported that hyper-immune response to alpha streptococci in patients with PPP. On the other hand, co stimulatory factors were studied on autoimmune disease. It was reported that these factors related to abnormal regulation of T cell activation. CD28 and CTLA4 participate in these factors. T cell receptor engagement and the B7-CD28/CTLA4 signaling pathways play critical roles in T cell activation and regulation. CD28 engagement results in T cell activation, while CTLA4 signals block IL-2 production, and cell cycle progression. Since there may be abnormal expression of costimulatory molecules in tonsils of PPP, we investigated the expression of CD28 and CTLA4 in tonsils.

Methods

Fifteen patients undergoing tonsillectomy were enrolled in this study. The 3 patients groups were composed, respectively of 7 patients with PPP and 5 patients with recurrent tonsillitis (RT) and 2 patients with obstructive sleep apnea syndrome (OSAS). The PPP group comprised 1 male and 6 females, aged 22-36 years, the RT group comprised 3 males and 2 females, aged 19-26 and the OSAS group comprised 2 males, aged 19-31.
Outline of this study are shown in Figure 1.

We investigated CD28 and CTLA4 mRNA levels by reverse transcription-polymerase chain reaction (RT-PCR) in CD3 positive lymphocytes from patients of PPP, recurrent tonsillitis (RT), and obstructive sleep apnea syndrome (OSAS). Primer design and cycle times of PCR are shown in Table 1. And then, we also investigated the expression levels of CD28 and CTLA4 by flow cytometry analysis in tonsillar mononuclear cells stimulated with alpha-streptococcus antigens for three days in these patients.

**Results**

1. mRNA expression of co stimulatory factors in tonsil CD3+ cells

mRNA expression of CD28 was not difference between PPP and the others. On the other hand, mRNA expression of CTLA4 was lower in PPP than in non-PPP. (Figure 2)

A semi-quantitative measurement of RT-PCR products revealed that the CTLA4 mRNA in tonsillar CD3+ lymphocytes from PPP patients was expressed at lower level than non-PPP patients. (Figure 3)

2. Costimulatory factors compare with and without stimulus

The expression of CD28 was not difference between PPP and non-PPP with and without stimulus. (Figure 4: rate of CD28+ cells in CD3+ cells before and after stimulus) When compared with and without the stimulus by S. mitis, the expression of CTLA4 was
up-regulated with the stimulus in non-PPP. On the other hand, the expression of CTLA4 was not up-regulated with the stimulus by S. mitis in PPP. (Figure 5: rate of CTLA4+ cells in CD3+ cells with and without stimulus)

**Conclusion**

The lower expression level of CTLA4 in PPP may cause hyper-immune response to alpha streptococci and abnormal regulation of T cell activation.
<table>
<thead>
<tr>
<th>Targets</th>
<th>Sense primer</th>
<th>Antisense primer</th>
<th>PCR product size</th>
<th>cycles</th>
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<tbody>
<tr>
<td>CD28</td>
<td>ATGCTTGAGCGGTACGACAA</td>
<td>CCACTGTACTTAGCAAGCTATAGC</td>
<td>436</td>
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</tr>
<tr>
<td>CTLA4</td>
<td>ATGGCTTGCCCTTGGATTTCAG</td>
<td>TTCTGGATCAATTACATAAATCTGG</td>
<td>465</td>
<td>32</td>
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<tr>
<td>β-actin</td>
<td>ATGGGTCAGAAGGATTCCTATGT</td>
<td>TCAGGAGGAGCAATGATCTTGA</td>
<td>863</td>
<td>25</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Outline of this sturdy
Figure 2. CD28 and CTLA4 mRNA expression in tonsil CD3+ cells
Figure 3. Semi-quantitative expression analysis of co stimulatory factor genes
Figure 4. Two color analysis with anti-CD3-FITC and CD28-PE in tonsillar lymphocyte with and without stimulation
Figure 5. Two color analysis with anti-CD3-FITC and CTLA4-PE in tonsillar lymphocyte with and without stimulation
Tonsil tissue

CD3+ cell separation by MACS (Magnetic cell sorting)

- Total RNA isolation
- Treated with DNase
- Reverse transcription
- PCR
- Electrophoresis
- Densitometer

Mononuclear cell separation

- Without stimulation
- With stimulation
  - S. mitis (NCTC3256)
  - S. sanguis (ATCC10556)
  - S. salivarius (ATCC7073)
  - PHA: phytohemagglutinin

3 days

Analysis of CD28 and CTLA4 expression by two-color flow cytometry
(CD3/CD28, CD3/CTLA-4)
RT: recurrent tonsillitis, OSAS: obstructive sleep apnea syndrome, PPP: Pustulosis palmaris et plantaris
RT: recurrent tonsillitis
OSAS: obstructive sleep apnea syndrome
PPP: Pustulosis palmaris et plantaris

Relative gene expression

CD28

CTLA4
S. mitis
S. salivarius
S. sanguis

Non-PPP

PPP

%CD28/CD3+

Without stimulus
Without stimulus
Without stimulus
Without stimulus

%CD28/CD3+

Without stimulus
Without stimulus
Without stimulus
Without stimulus

NS

NS

NS

NS

NS

NS