
T-cell activation in tonsils of patients with pustulosis palmaris et plantaris

Miki, Takahara ; Kan, Kishibe ; Hayabusa, Nozawa ; Yasuaki, Harabuchi
Title: T-cell activation in tonsils of patients with pustulosis palmaris et plantaris

Authors: Miki Takahara, M.D., Kan Kishibe, M.D., Ph.D., Hayabusa Nozawa, M.D.,
Yasuaki Harabuchi, M.D., Ph.D.

Departments of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College,
Asahikawa, Japan.

Presented orally at the 5th International Symposium on Tonsils and Mucosal Barriers of the
Upper Airways, 9-11 April 2003, Wakayama City, Japan.

Session Category : Free Paper Session

Session Title : symposium 10  focal infections and basic problems-PPP

Reference Number : S10-1

Mailing Address for all authors:

Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College,
Midorigaoka E 2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan.

Phone: +81-166-68-2554, Fax: +81-166-68-2559
E-mail address for authors

Yasuaki Harabuchi: hyasu@asahikawa-med.ac.jp

Mailing address for correspondence and reprints:

Yasuaki Harabuchi, Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College,

Midorigaoka E 2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan.

Phone: +81-166-68-2554, Fax: +81-166-68-2559
Abstracts

Pustulosis palmaris et plantaris (PPP) is a well-known skin disease closely related to tonsillar focal infections, and tonsillectomy is very effective for treatment of this condition. However, etiology of PPP was unclear. In this study, we investigated the characteristics of tonsils from PPP patients by clinical, immunohistochemical, immunological, and molecular-biological approach, and considered the etiology of PPP. For 47 Japanese patients with PPP who have tonsillectomy in Asahikawa Medical Collage, the skin lesion of PPP was improved in 87% of PPP patient at twelve month after tonsillectomy. In quantitative immunohistologic analysis by measurement of T-cell nodule areas on tonsillar sections from those patients, there was positive correlation between the enlargement and improvement of the skin lesion. These results suggested that the T-cell nodule expansion may be one of the important clues to clarify the pathogenesis of this tonsil related disease. In flow cytometric analysis, CD3+, CD4+ and CD4+CD25+ cells were significantly elevated in tonsillar lymphocyte from PPP patients. In RT-PCR and western blotting analyses of TGF-beta, IL-10, and Smad group, Smad7 mRNA and protein in tonsillar CD3+ cells from PPP patients were expressed at higher level than those from OSAS patients. These results suggested that helper T-cells may be frequently activated and proliferating in tonsils of PPP patients, and the activation and proliferation of helper T-cells may due to enhanced inhibition by Smad7 in intracellular signal-transduction of TGF-beta.
1. INTRODUCTION

Pustulosis palmaris et plantaris (PPP) is a well-known skin disease closely related to tonsillar focal infections, and tonsillectomy is very effective treatment of this condition. Improvement of skin lesion was reported in 90% of PPP patients after tonsillectomy [1]. However, etiology of PPP was unclear. Tonsils of PPP patients differ from those of other patients by histological features; e.g. expanded T cell nodules were detected in the tonsils of PPP patients compared to normal tonsils[2]. This result may reflect some difference of T-cell status between normal and PPP tonsils. In this study, we investigated T-cell status of tonsils from PPP patients by clinical, immunohistochemical, immunological, and molecular-biological approach, and considered the etiology of PPP.

2. METHODS.

The study group consisted of 47 Japanese patients with PPP who had tonsillectomy in Asahikawa Medical Collage. The degree of improvement was classified on a scale of one to ten by individual evaluation of each PPP patients at one, six, and twenty month after tonsillectomy. The skin condition evaluated as over five scale was regarded as improvement.

Quantitative immunohistologic analysis was performed in sections from tonsils of 31 patients with PPP and 13 patients with obstructive sleep apnea syndrome (OSAS) as normal control. The sections were stained by anti-CD20 mouse monoclonal antibody (L26:
Area of T-cell nodules was measured by using NIH image software (Fig. 1a). Percentage of the area occupied by the T-cell nodules was calculated. The relationship between this percentage and the degree of improvement were statistically examined.

Flow cytometric analysis was performed in tonsillar mononuclear cells from tonsils of 18 patients with PPP and 12 patients with OSAS. The antibodies were CD3-FITC, CD4-PE, and CD25-FITC (Becton Dickinson, San Jose, CA, USA). $10^6$ cells were stained with 20ul antibody, and analyzed on EPICS ELITE (Beckman and Coulter).

Reverse transcription-polymerase chain reaction (RT-PCR) analysis for TGF-beta, IL-10, and Smad 3, 4, and 7 was performed in CD3+ cells from tonsils of five patients with PPP and five patients with OSAS. CD3+ cells were separated by magnetic cell separation system (MACS, Miltenyi Biotec, Germany). cDNAs were synthesized from extracted RNAs by MMLV reverse transcriptase. PCR products were electrophoresed on agarose gel, and amplified DNA bands were measured using NIH image software.

Western blotting analysis for Smad3 and Smad7 was performed on aliquots of the CD3+ cells tested by RT-PCR analysis. The extracted protein was electrophoresed in 4-12% bis-tris gels (NuPAGE, Invitrogen Corp., Carlsbad, CA, USA) and transferred to PVDF membrane. Membranes were incubated with primary and secondary antibodies. Primary antibodies were rabbit polyclonal anti-human Smad3 and 7 (Santa Cruz Biotechnology, Inc. Santa Cruz, California, U.S.A.), and as secondary antibody we used Horseradish-Peroxidase-conjugated sheepanti-rabbit-immunoglobulin (Amersham,
Arlington Heights, IL). Immnoreactivity was visualized by chemilluminescent method using an ECL kit (Amersham) and protein bands were measured using NIH image software.

3. RESULTS

The percentage of patients with improved skin lesion increased as following period dependent manner by 12 months: of 47 patients with PPP, 24 (51%), 38 (81%), and 41 (87%) showed the improvement of the skin at the time of 1, 6, and 12 months after tonsillectomy, respectively.

In 31 tonsillar tissues of patients with PPP, T-cell nodule area was ranged from 10% to 44.1% with a median of 29.5%. In 12 tonsillar tissues of patients with OSAS, this area was found from 12.1% to 45.4% with a median of 21.1%. T-cell nodule area of tonsils from PPP patients was significantly larger than that from PPP patients (p=0.015). There was a significant positive correlation between T-cell nodule area and degree of skin improvement at 6 months after tonsillectomy (r=0.422, p=0.021; Fig. 1b).

In tonsillar lymphocytes from 18 patients of PPP, the median (25-75 percentlie) values of CD3-positive, CD4 positive, and CD4 CD25 double positive cells were 47.6% (25.5-63.8%), 37.2% (23.4-53.5%), and 3.9% (1.3-13.4%), respectively. In 12 patients of OSAS, these were 37.9% (27.0-55.8%) in CD3+cells, 29.6% (20.6-49.6%) in CD4+cells, and 1.8% (0.6-6.6%) in CD4+CD25+cells. The percentage of CD3-positive cells, CD4 -positive cells, and CD4 CD25 double-positive cells in PPP patients were significantly higher
than those in OSAS patients (p=0.02, p=0.03, p=0.01, respectively).

CD3-positive tonsillar cells between PPP and OSAS patients expressed similar level of TGF-beta and IL-10 mRNA. Smad7 mRNA was highly expressed in PPP patients (Fig.2a). Semiquantitative analysis using NIH image software showed that Smad7 mRNA expression was significantly higher in PPP patients than in non-PPP patients (p=0.031).

As similar to the results of RT-PCR analysis, Smad7 protein was highly expressed in PPP patients (Fig.2b). Semiquantitative analysis showed that Smad7 protein was expressed significantly higher in CD3 positive tonsillar cells from PPP patients than in those from OSAS patients (p=0.02).

4. DISCUSSION

It is reported that the skin lesion of PPP was improved in 90% of tonsillectomized patients [1]. In this clinical study, we also found that 87% of the patients improved by 12 months after tonsillectomy. Immunohistologic study showed that T-cell nodules of tonsils with PPP were expanded. Moreover, skin improvement correlated with enlargement of T-cell nodules, suggesting the T-cell nodule expansion may be one of the important clues to clarify the pathogenesis of this tonsil related disease. Flow cytometric analysis showed that CD4 CD25 double positive-cells, i.e., activated and proliferating helper T-cells, increased in PPP tonsils. Therefore, it is likely the activated and proliferating helper T-cells may prompt the enlargement of T-cell nodules in tonsillar tissues of PPP patients. RT-PCR and Western
blotting analysis showed no difference in expression of inhibitory cytokine such as TGF-beta and IL-10 between PPP and non-PPP, but expression of Smad7 that is intracellular signal-transducing inhibitor for TGF-beta increased in tonsil from PPP patients. Therefore, it is likely that the activation and proliferation of helper T-cells may due to enhanced inhibition by Smad7 in intracellular signal-transduction of TGF-beta, as already found in mucosal T-cells from mucosal autoimmune disease inflammatory bowel disease (IBD) [3].

REFERENCE


Figure legend

Figure 1

Quantitative immunohistologic analysis.

a: B- and T-cell nodules were bordered with black lines on tonsillar section stained by anti CD20 antibody. b: There was a positive correlation between T-cell nodule area and degree of improvement at six month after tonsillectomy ($r=0.422$, $p=0.021$).

Figure 2

a: RT-PCR analysis

The comparison between TGF-beta, IL-10 and Smad group mRNA expression of tonsillar CD3+ lymphocytes from PPP patients and those from OSAS patients. Smad7 mRNA from PPP patients was expressed at higher level than those from OSAS patients.

b: Western blotting analysis.

The comparison between Smad3 and 7 protein expression of tonsillar CD3+ lymphocytes from PPP patients and those from OSAS patients. Smad7 protein from PPP patients was expressed at higher level than those from OSAS patients.
Figure 1

(a) Image description

(b) Scatter plot diagram

- Degree of improvement
- Percentage of T-cell nodules
- $r = 0.422$
- $p = 0.021$

B : B-cell nodule  T : T-cell nodule

Figure 2

(a) Gene expression analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>PPP</th>
<th>OSAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smad 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smad 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smad 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>beta-actin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Western Blot analysis

- Smad7
- Smad3
- Alpha-tublin

(PPP : Pustulosis Palmaris et Plantaris  OSAS : Obstructive Sleep Apnea Syndrome)