

INVESTIGATIVE REPORTS

Restricted Usage of the T-cell Receptor V β Repertoire in Tonsillitis in Association with Palmoplantar Pustulosis

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Focal infections such as chronic tonsillitis or dental caries occasionally play a role in the induction or exacerbation of palmoplantar pustulosis (PPP). Arthro-osteitis is sometimes a complication in severe cases of PPP. To study the effects of bacterial infection on the exacerbation of cutaneous lesions and arthralgia, we investigated the T-cell receptor V β repertoire in peripheral blood mononuclear cells (PBMC) and tonsil tissue after tonsillectomy in 4 cases, who had chronic tonsillitis and a history of exacerbation of cutaneous lesions following a sore throat. First, serum levels of interleukin-6 (IL-6) and IL-8 were measured before and after tonsillectomy by enzyme-linked immunosorbent assay (ELISA). Second, ^3H -TdR incorporation was used to examine the effects of the culture supernatant on the PBMC of the autologous patients, other PPP patients without tonsillitis and normal controls. T-cell receptor V β repertoire was examined by the reverse transcriptase-polymerase chain reaction method. Results showed that IL-8 was significantly high in the serum and abundantly released from tonsillar lymphocytes, which may play a role in the accumulation of neutrophils in lesional skin. T-cell receptors V β 6 and 12 were preferentially expressed on tonsillar lymphocytes, and V β 4, 7, 9, 17 and 18 were detected relatively frequently. These data suggest that restricted usage of T-cell receptor V β subsets may play a crucial role in the induction of tonsillitis associated with PPP. **Key words:** tonsillectomy; focal infection; cytokine; IL-6; IL-8.

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Palmoplantar pustulosis (PPP) usually affects the palms and soles with recurrent sterile pustules. In severe cases, bone and joint symptoms are involved, which is defined as arthro-osteitis (1, 2). Although the pathogenesis of PPP still remains unclear, a number of proinflammatory and/or inflammatory cytokines have been implicated to be involved. Foci such as tonsillitis, dental caries, periodontal abscess, or appendicitis have been suggested to be a cause, since PPP occasionally improves following the treatment of these focal infections. We have often experienced cases showing improvement not only of cutaneous manifestations but also of joint pain after treatment of such focal infections. Especially, tonsillitis is supposed to play an important role as a triggering or exacerbating factor in patients with PPP presenting severe chronic tonsillitis with recurrent bacterial infections, and tonsillectomy occasionally brings about dramatic effects in such patients.

Staphylococcal enterotoxin is a prototypic bacterial superantigen, which binds to MHC class II molecules on antigen-presenting cells and stimulates a large population of T-cells

bearing appropriate T-cell receptor (TCR) V β gene segments (3–5). Superantigens may cause fever, diarrhoea, erythema or arthralgia (6). In this study, we assessed the mitogenic activity of culture supernatants of the clinical isolates of *Staphylococcus aureus* (*S. aureus*) to determine whether the mechanism by which these culture supernatants activate T-cells has the characteristics of superantigens. TCR V β repertoire on tonsillar lymphocytes of PPP patients with chronic tonsillitis was examined using the reverse transcriptase-polymerase chain reaction (RT-PCR) method.

MATERIAL AND METHODS

Patients

Six patients with severe PPP were enrolled, all of whom had chronic tonsillitis. They consisted of 2 males and 4 females, aged 27 to 48 years (mean: 38.3 years). Arthro-osteitis was present in 4 cases. Both the cutaneous lesions and the joint pain dramatically improved after tonsillectomy within several months in all cases.

Serum samples and isolation of peripheral blood mononuclear cells (PBMC)

Serum was obtained from 6 patients with PPP by veno-puncture before and after tonsillectomy and stored at -20°C until use.

PBMC were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Uppsala, Sweden). PBMC were suspended in RPMI 1640 supplemented with 7% fetal calf serum (FCS).

Cytokine assay of serum and the culture supernatant

Serum IL-6 and IL-8 concentration was measured using an enzyme-linked immunosorbent assay (ELISA) kit (IL-6; R&D Systems, Minneapolis, MN, IL-8; Toray-Fuji Co. Ltd., Tokyo, Japan). In 2 cases who were revealed sterile by bacterial culture, resected tonsil tissues were minced with scissors and then cultured in RPMI 1640 supplemented by 7% FCS with hyaluronidase for 24 h. The tonsillar lymphocytes were collected by nylon wool filtration. After further culture of 24 h, spontaneous release of IL-1 β (R&D), IL-6, IL-8 and tumor necrosis factor- α (TNF- α) (R&D) in the culture supernatants was measured by ELISA.

Preparation of *S. aureus*

S. aureus was isolated from the tonsils of 2 patients. Organisms (10^5 /colony) were grown in 1 l of Broth liquid medium (Heart Infusion Broth, Gifco Labo., USA) and incubated at 37°C for 24 h with gentle shaking. Culture supernatants were collected by centrifugation, and after filtration through a $0.22\text{-}\mu\text{m}$ filter these fractions were assayed for mitogenic activity on human PBMC.

Assessment of PBMC proliferation

PBMC (5×10^4) were cultured in the presence or absence of the culture supernatants of *S. aureus* for 5 days (decided in preliminary experiment) and then pulsed with $0.2\text{ }\mu\text{Ci}$ /well of ^3H -TdR. Cells were harvested 9 h later and uptake of radioactivity was determined in a liquid scintillation counter. PBMC were also incubated with staphylococcal enterotoxin B (SEB) (Sigma Chemical Co. Ltd., St. Louis, MO) ($1\text{ }\mu\text{g/ml}$; decided in preliminary experiment) for 5 days.

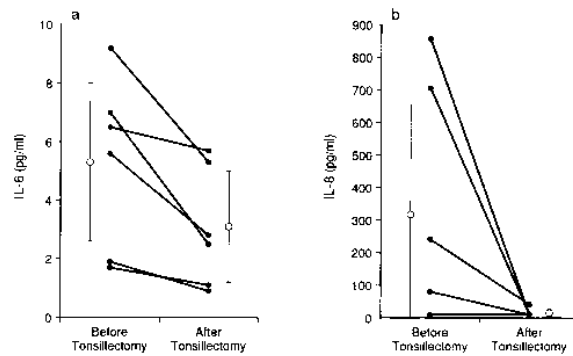


Fig. 1. Serum IL-6 (a) and IL-8 (b) level before and after tonsillectomy. Open circle with error bars represents mean $\pm$ SD.

Analysis of TCR V β expression

Total RNA was isolated from fifty 5- μ m frozen biopsied tissue specimens of each tonsil and paired peripheral blood lymphocytes (PBL) in 4 patients using RNA zol (Biotex, TX, USA) and then reversely transcribed to cDNA by RAV-2 reverse transcriptase (Takara Co. Ltd., Kyoto, Japan). PCR analysis of the TCR V β repertoire was accomplished by using oligonucleotide primers to amplify specific V β gene segments paired with a consensus β -chain constant region (C β) primer (7). As internal controls, a pair of 5' sense C α -specific primers and 3' antisense C α -specific primers were also amplified, as described previously by Choi et al. (8). PCR amplification was started with an initial denaturation step at 94°C, followed by a 35-cycle profile consisting of denaturation at 94°C (1 min), annealing at 57°C (1 min) and extension at 72°C (1 min and 5 s), and completed with a final extension step of 7 min at 72°C according to the method of Dunn et al. (7). Ten microliters of PCR products were electrophoresed in 1.7% agarose gel containing 1% ethidium bromide and visualized under ultraviolet light. The intensity of each band was measured with a densitometer (EPA-3000, Chemiway, Tokyo, Japan). As a control, two tonsillar tissues obtained at surgical operation of 2 male patients (23 and 24 years old) with atopic dermatitis were also examined.

Statistical analysis

Results were expressed as mean $\pm$ SD. Statistical analysis was performed using Student's *t*-test. A *p* value <0.05 was considered as significant.

RESULTS

Serum IL-6 and IL-8 before and after tonsillectomy

Serum IL-6 and IL-8 were significantly elevated before tonsillectomy and declined in all cases (Fig. 1). The IL-6 level (5.31 ± 2.7 pg/ml) was reduced (3.06 ± 1.9 pg/ml), but without any significance. IL-8 (317 ± 339 pg/ml) was significantly

reduced (15.4 ± 11.4 pg/ml) ($p < 0.005$), and in 4 cases it was reduced to normal levels.

Mitogenic activity of culture supernatant

Culture supernatants from isolates of *S. aureus* from 2 patients both strongly stimulated autologous and/or allogenic PBMC. ^3H -TdR uptake by stimulation of culture supernatants derived from 2 patients showed a significantly greater response ($54,147 \pm 7,267$ dpm in Patient 1 and $49,053 \pm 4,283$ dpm in Patient 2, $p < 0.05$ in both cases as compared with control subjects) (Table I). ^3H -TdR uptake of the other PPP patients without tonsillitis ($n = 3$) showed $36,233 \pm 3,143$ dpm against culture supernatant from Patient 1 and $34,270 \pm 5,481$ dpm against that from Patient 2. This mitogenic effect of culture supernatant was still found even if 10-fold diluted ($43,962 \pm 5,151$ dpm in Patient 1 and $41,216 \pm 3,974$ dpm in Patient 2). Furthermore, this growth effect persisted even 6 months after tonsillectomy (data not shown).

Results of the mean level of spontaneous release of tonsillar lymphocytes demonstrated that IL-6 and IL-8 were markedly elevated (IL-1 β ; 9.07 pg/ml, normal <15.6 pg/ml, IL-6; 57.8 pg/ml, normal <2.94 pg/ml, IL-8; 353 pg/ml, normal <10 pg/ml, TNF- α ; 9.2 pg/ml, normal <7.5 pg/ml).

RT-PCR

A PCR-based method was adapted to compare the relative expression of TCR V β gene in the tonsillar tissue and peripheral blood. Visualization with ethidium bromide revealed that V β 6 and 12 were preferentially expressed in tonsillar tissue compared with paired PBL in 4 cases. TCR V β 4, 7, 9, 17 and 18 were detected relatively often. Results of densitometric analysis are shown in Fig. 2. Control tonsillar tissues from atopic patients showed that diverse usage of TCR was shown such as V β 1, 2, 4, 5-1, 5-2.3, 6, 7, 9, 11, 12, 16 and 18 in Case 1 and V β 2, 4, 5-1, 6, 7, 8, 10, 14, 16, 17 and 18 in Case 2.

DISCUSSION

PPP occasionally exacerbates following throat pain, and arthralgia is often present in severe cases. Staphylococcal enterotoxin can cause fever, gastrointestinal symptoms and arthralgias (6). It may be supposed that superantigen plays a role in the induction of cutaneous and joint manifestations in PPP.

Staphylococcal superantigens have recently been proposed as a possible antigen for psoriasis (9). In guttate psoriasis, which is occasionally induced following tonsillar infection, streptococci isolated from the pharynx produced superantigenic toxin. It is

Table I. ^3H -thymidine incorporation of PBMC of patients with PPP and normal subjects (N) in the stimulation of culture supernatant (Cul. sup) and SEB

Patients	^3H -TdR (dpm)			
	–	Cul. sup (Patient 1)	Cul. sup (Patient 2)	SEB (1 $\mu\text{g/ml}$)
N ($n = 6$)	$1,871 \pm 662$	$25,296 \pm 8,601$	$27,549 \pm 6,733$	$28,568 \pm 6,679$
PPP ($n = 3$)	$1,554 \pm 565$	$36,233 \pm 3,143$	$34,270 \pm 5,481$	$33,128 \pm 5,433$
Autologous				
Patient 1	$2,197 \pm 194$	$54,147 \pm 7,267^*$	–	$39,745 \pm 2,259$
Patient 2	$2,027 \pm 245$	–	$49,053 \pm 4,283^*$	$37,299 \pm 3,215$

* $p < 0.05$.

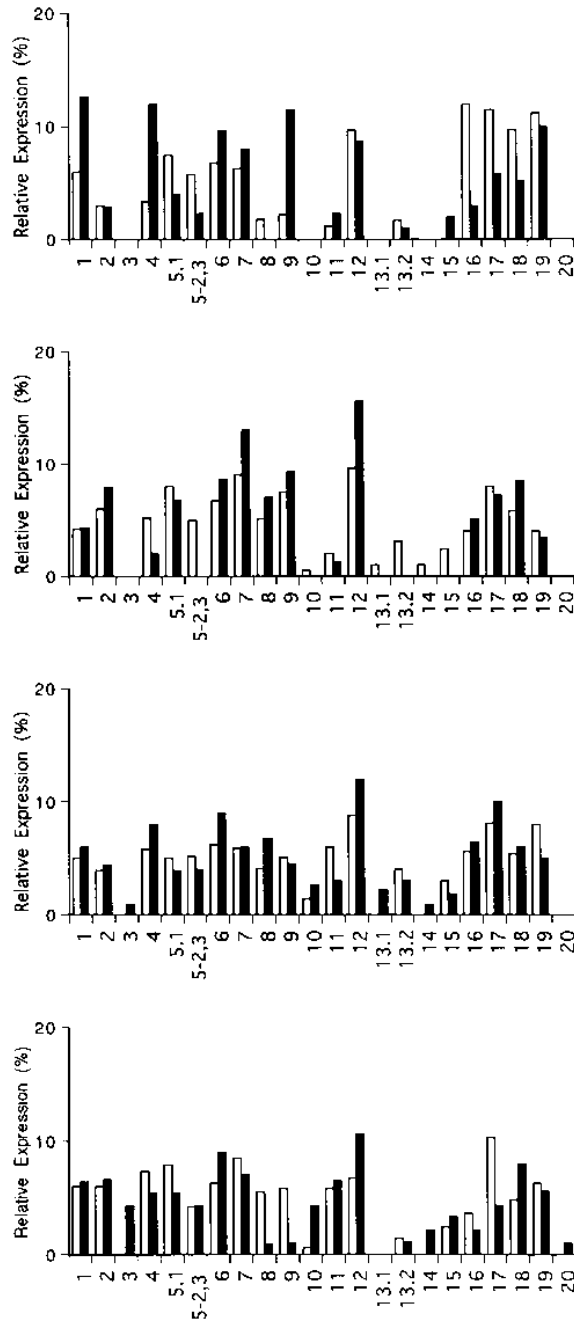


Fig. 2. Expression of TCR V β genes in PBLs (open bar) and tonsillar tissues (solid bar) in 4 patients with PPP. The relative amount of TCR V β gene transcripts was described as the ratio (%) of each V β to total V β .

supposed that bacterial superantigens initiate guttate psoriasis lesions by systemic lymphocyte activation, by systemic cytokine release priming local endothelial cell activation, and by intracutaneous lymphocyte activation by superantigen-released cytokines and/or directly by superantigens (10). Staphylococcal and/or streptococcal infection-induced arthritis is reported in

an experimental mouse model (11, 12). Our current observation demonstrates that PBMC of patients with psoriasis arthropathy showing severe lumbago respond significantly strongly against SEB. *S. aureus* can produce superantigens, and bacterial superantigens have been demonstrated to induce IL-1 and TNF- α from macrophages by binding to MHC class II molecules (13). Our results reveal that lymphocytes derived from resected tonsils release increased levels of IL-6, IL-8 and TNF- α . Especially, IL-8, which is a strong chemoattractant for neutrophils, was markedly elevated. Serum IL-8 was also significantly elevated but markedly decreased following successful treatment; it returned to normal in 4 cases.

To determine whether expansion of T-cells expressing particular V β gene segments was highly induced in tonsillar tissues, we used a PCR method and specific primers to analyze expression of 22 different V β families. Results showed that V β 6 and 12 were preferentially expressed, and V β 4, 7, 9, 17 and 18 were detected relatively often.

In conclusion, *S. aureus* infection stimulates a large population of T-cells in patients with PPP, which may cause superantigenic effects such as arthralgia by way of several inflammatory cytokines. IL-8 was supposed to be crucial for the accumulation of neutrophils, leading to the formation of pustules. TCR V β 6 and 12 may play an important role in the development of tonsillitis associated with PPP; however, further studies are necessary to clarify the role of these lymphocyte subsets in the induction of PPP.

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