ORIGINAL

Differential cytokine profiles in pediatric patients with PFAPA syndrome and recurrent tonsillitis

Seiichi Nakano[a], Eiji Kondo[b], Hidetaka Iwasaki[b], Hironori Akizuki[c], Kazunori Matsuda[b], Takahiro Azuma[b], Go Sato[b], Yoshiaki Kitamura[b], Koji Abe[c], and Noriaki Takeda[b]

[a]Department of Otolaryngology, Kochi National Hospital, Kochi 780-8077, Japan, [b]Department of Otolaryngology, Tokushima University Graduate School of Biomedical Sciences, Tokushima 770-8503, Japan, [c]Department of Otolaryngology, Tokushima Red Cross Hospital, Tokushima 773-8502, Japan

Abstract : Objective : An attempt was made to identify characteristic cytokine profiles to distinguish periodic fever with aphthous stomatitis, pharyngitis and cervical adenitis syndrome (PFAPAS) from recurrent tonsillitis, of which clinical manifestations are similar to those of PFAPAS in children. Methods : Serum concentrations of IL-6, IL-4 and IFN-γ were measured during febrile episodes in pediatric patients. Results : The levels of IL-6 during febrile episodes were markedly increased above the upper limit of normal ranges in patients with both PFAPAS and recurrent tonsillitis, but there were no significant differences between groups. The levels of IL-4 during febrile episodes in PFAPAS patients were significantly lower than those in recurrent tonsillitis patients. The levels of IFN-γ during febrile episodes in PFAPAS patients were significantly higher than those in recurrent tonsillitis patients. Conclusion : In pediatric patients with PFAPAS, despite an increase of IL-6, IL-4 was suppressed with a marked increase of IFN-γ during febrile episodes. On the contrary, in febrile pediatric patients with recurrent tonsillitis, both IL-6 and IL-4, but not IFN-γ were increased. The characteristic cytokine profiles of IL-6, IL-4 and IFN-γ can be used for differential diagnosis of PFAPAS from recurrent tonsillitis in children in clinical ear, nose and throat (ENT) settings. J. Med. Invest. 68 : 38-41, February, 2021

Keywords **:** *PFAPA syndrome, recurrent tonsillitis, IFN- γ, IL-6, IL-4*

INTRODUCTION

Periodic fever with aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome was first described by Marshall in 1987 (1) and is characterized by periodic episodes of high fever lasting 4 to 6 days and regularly recurring every 3 to 5 weeks, which are accompanied with aphthous stomatitis, pharyngitis and cervical adenitis (2). PFAPA syndrome is an autoinflammatory disease that develops before age 5 in most patients, but the responsible gene has yet to be identified (3). Because PFAPA syndrome is diagnosed by exclusion for lack of specific biomarkers, cardinal signs and symptoms must be carefully observed for a differential diagnosis. But, otolaryngologists may not usually suspect PFAPA syndrome when treating children with a history of periodic fever. Especially, because patients with PFAPA syndrome show either tonsillar erythema or white spots on the tonsils with cervical adenitis in addition to elevated levels of white blood cell (WBC) and C-reactive protein (CRP) during febrile episodes (4, 5), these clinical findings lead to misdiagnosis of recurrent tonsillitis, of which clinical manifestations are similar to those of PFAPA syndrome in children. Consequently, the misdiagnosed patients would receive unnecessary antibacterial therapy and their diagnosis of PFAPA syndrome might be delayed.

Recently, it was reported that in patients with PFAPA syndrome, inflammatory cytokines such as IL-6, IFN-γ, IL-1β and TNF-α but not IL-4 were elevated during febrile episodes (6). However, it was also reported that levels of IL-1β and TNF-α reached a peak early in febrile episodes and quickly return to normal levels in patients with PFAPA syndrome before hospital visit (5, 7). Moreover, IL-6 is commonly elevated in many inflammatory diseases and may not confirm a diagnosis of PFAPA syndrome alone. In the present study, an attempt was made to identify characteristic cytokine profiles to distinguish PFAPA syndrome from recurrent tonsillitis in children in clinical ENT settings. For this purpose, we measured the serum levels of IL-6, IL-4 and IFN-γ in pediatric patients with PFAPA syndrome during an episode of high fever, and compared to those in pediatric patients with recurrent tonsillitis during high fever.

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Address correspondence and reprint requests to Seiichi Nakano, M.D., Department of Otolaryngology, Kochi National Hospital, Kochi 1-2-25, Kochi 780-8077, Japan and Fax : +81-88-843-6385.

SUBJECTS AND METHODS

Subjects

Three patients with PFAPA (One male and 2 females, mean age : 5.2 ± 0.2 years old) who met the diagnosis criteria proposed by Thomas *et al*. (8), Padeh *et al*. (9) and Gattorno *et al*. (10), and 4 patients with recurrent tonsillitis (One male and 3 females, mean age : 11.5 ± 7.6 years old) which was diagnosed by otolaryngologists were included in the present study.

This study was approved by the Committees for Medical Ethics of Tokushima University Hospital and National Kochi Hospital. Written informed consent was obtained from a parent of each child with recurrent tonsillitis prior to blood sampling. The retrospective chart review of patients with PFAPA syndrome was also performed.

Methods

Serum samples were collected during febrile episodes from

each patients and serum concentrations of IL-6, IL-4 and IFN-γ were measured by SRL Co. Ltd., Japan. White blood cell counts and CRP in the serum were also measured at the hospital laboratory. One to 4 days after the onset of a typical febrile episode, blood samples were taken from patients with PFAPA, and 2 to 3 days after the onset of high fever, blood samples were also taken from patients with recurrent tonsillitis. Neither systemic steroid nor non-steroid anti-inflammatory drug were given to the patients in both groups before blood samples had been taken at hospital visit.

Statistical analysis

Comparisons between groups were analyzed by Welch's t-test. P-values of < 0.05 were considered statistically significant, and all statistical analyses were performed using Statcel 3 (OMS Publishing Inc, Saitama, Japan).

RESULTS

The clinical characteristics of the pediatric patients with PFAPA and recurrent tonsillitis are shown in Table 1. There were no significant differences in gender, age, sampling day after the onset of fever, maximal temperature, WBC counts or levels of CRP between groups. The mean serum levels of IL-6 during febrile episodes in patients with both PFAPA syndrome (33.8 ± 5.6 pg/ml) and recurrent tonsillitis (46.4 ± 18.8 pg/ml) were markedly increased above the upper limit of normal ranges, but there were no significant differences between groups (Fig. 1). The mean serum levels of IL-4 during febrile episodes in patients with recurrent tonsillitis (7.8 ± 1.5 pg/ml) were significantly higher than those in patients with PFAPA syndrome (3.0 ± 0.4 pg/ml) (Fig. 2). The mean serum levels of IFN-γ during febrile episodes were markedly increased above the upper limit of normal ranges in patients with PFAPA syndrome (319.0 ± 18.3 IU/ml) and significantly higher than those in patients with recurrent tonsillitis (1.02 ± 0.72 IU/ml) (Fig. 3).

Table 1. Clinical characteristics of pediatric patients with PFAPA and recurrent tonsillitis

	PFAPA syndrome (n = 3)	Recurrent tonsillitis (n = 4)
Gender (Male : Female)	1 : 2	1 : 3
Age (years old)	5.2 ± 0.2	11.5 ± 7.6
Sampling day after the onset of fever	2.3 (1-4)	2.5 (2-3)
Maximal temperature (℃)	39.4	39.5
WBC at sampling (counts/mm^3)	10977	13015
CRP at sampling (mg/dl)	3.13	4.48

WBC : white blood cell, CRP : C-reactive protein.

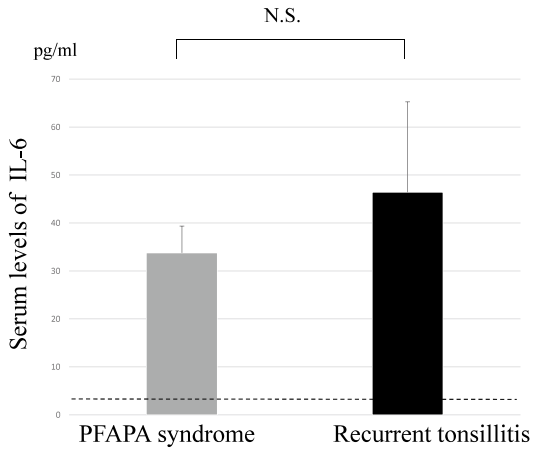

Fig. 1. Levels of IL-6 in the blood during febrile episodes in pediatric patients with PFAPA syndrome and recurrent tonsillitis. Columns and bars represent means ± S.E. PFAPA syndrome : n = 3, recurrent tonsillitis : n = 4. N.S. : not significant. Dashed bar means the upper limit of normal ranges (4.0 pg/ml).

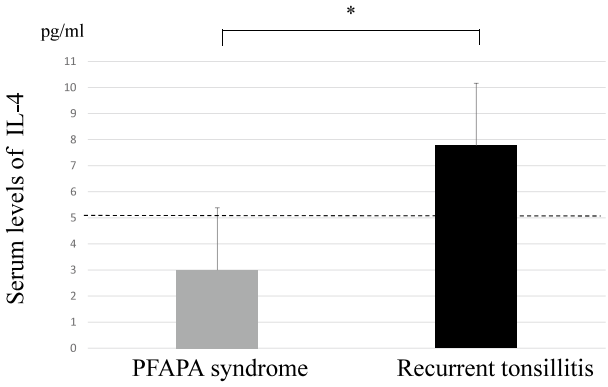

Fig. 2. Levels of IL-4 in the blood during febrile episodes in pediatric patients with PFAPA syndrome and recurrent tonsillitis. Columns and bars represent means ± S.E. PFAPA syndrome : n = 3, recurrent tonsillitis : n = 4. *p < 0.05. Dashed bar means the upper limit of normal ranges (5.0 pg/ml).

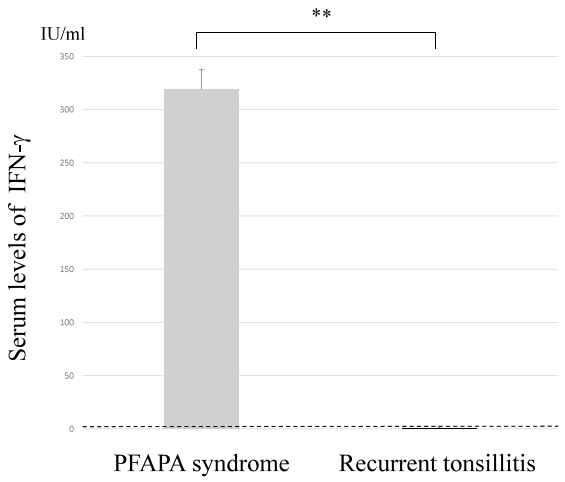

Fig. 3. Levels of IFN-γ in the blood during febrile episodes in pediatric patients with PFAPA syndrome and recurrent tonsillitis. Columns and bars represent means ± S.E. PFAPA syndrome : n = 3, recurrent tonsillitis : n = 4. **p < 0.01. Dashed bar means the upper limit of normal ranges (0.1 IU/ml).

DISCUSSION

PFAPA syndrome is an autoinflammatory disease, which is caused by dysfunction of inflammasome (3). Inflammasome is a cytosolic protein complex of the innate immune system responsible for the activation of inflammatory responses in the macrophages and dendritic cells. Inflammasome activation is initiated by pattern recognition receptors that respond to foreign pathogen-related molecules, and the activated caspase-1 finally causes the production of inflammatory cytokines (11). Because it is suggested that PFAPA syndrome is a complex genetic disorder of proteins related to inflammasome, PFAPA syndrome is characterized by a cytokine dysfunction, including the production of pro-inflammatory cytokines (12). In the present study, the serum levels of IL-6, a pro-inflammatory cytokine were increased during febrile episodes in pediatric patients with PFAPA syndrome. It was also reported that the serum levels of IL-6 were elevated in patients with PFAPA syndrome during febrile episodes, compared to those in remission or to controls (6, 7). However, in the present study, the serum levels of IL-6 were also increased in febrile patients with recurrent tonsillitis, because of production of IL-6 during acute infection in response to bacterial or viral infections (13). Chen *et al*. also reported that in children with hematological disorders, blood levels of IL-6 were elevated by bacterial infection (14). Therefore, it is suggested that the increased levels of IL-6 in the blood during a high fever are not helpful in the differential diagnosis of PFAPA syndrome from acute infection of recurrent tonsillitis in children.

In the present study, the mean serum levels of IL-4 during febrile episodes in pediatric patients with recurrent tonsillitis were significantly higher than those in patients with PFAPA syndrome. It was reported that IL-4 in the blood is increased in anti-inflammatory responses to the activation of pro-inflammatory cytokine such as IL-6 during bacterial or viral infection (15, 16). In fact, Yusa *et al*. reported that both IL-6 and IL-4 levels were elevated in children with bacterial infection (17). On the contrary, Stojanov *et al*. reported that the serum levels of IL-4 did not increase during a febrile episode in PFAPA syndrome patients, compared with controls (6). It was assumed that a continuous pro-inflammatory cytokine activation with a reduced anti-inflammatory cytokine response is due to the dysregulation of immune response in PFAPA syndrome (6). Therefore, it is suggested that the suppressed blood levels of IL-4 despite an increase of IL-6 can differentiate PFAPA syndrome from acute infection of recurrent tonsillitis in children.

The present study showed a marked increase in serum levels of IFN-γ during high fever in pediatric patients with PFAPA syndrome, but not in pediatric patients with recurrent tonsillitis. In fact, in patients with PFAPA syndrome, the increased levels of IFN-γ in the blood was also reported during a febrile episode, compared to remission period or to controls (6, 7). Because IFN-γ suppresses the production of IL-4 (18, 19), its increase in patients with PFAPA syndrome is thought to be responsible for the suppressed levels of IL-4 in these patients. Although febrile attacks are followed by anti-inflammatory cytokine response to avoid exacerbating inflammation under physiological conditions, overproduction of IFN-γ due to dysfunction of inflammasome (20) suppressed the increase of anti-inflammatory cytokines such as IL-4, leading to persistent inflammation in patients with PFAPA syndrome. Therefore, it is suggested that IFN-γ plays a key role in continuing pro-inflammatory cytokine activation with its suppressing anti-inflammatory response in patients with PFAPA syndrome. On the contrary, as shown in patients with recurrent tonsillitis of the present study, levels of IFN-γ in the blood were around the upper limit of normal ranges during febrile episode. It was also reported that in children with hematological disorders, IFN-γ in the blood was not elevated by bacterial infection (11). All these present findings suggested that the characteristic cytokine profiles in the blood during high fever that despite an increase of IL-6, a suppression of IL-4 with a marked increase of IFN-γ in patients with PFAPA are helpful for differential diagnosis from recurrent tonsillitis in children.

Patients with PFAPA syndrome show elevated values of WBC and CRP during typical episodes of fever (5). But, leukocytosis is also found in most febrile episodes in children, like in acute tonsillitis. CRP is an acute phase protein and its high levels during febrile episodes are also observed in bacterial infection or other inflammatory disease in children. In the present study, elevated values of WBC and CRP during febrile episodes are observed in children with both PFAPA syndrome and recurrent tonsillitis.

In conclusion, the present findings showed that in patients with PFAPA syndrome, despite an increase of IL-6, IL-4 was suppressed under upper limit of normal ranges with a marked increase of IFN-γ in the blood during a febrile episode. On the contrary, in patients with recurrent tonsillitis, both IL-6 and IL-4, but not IFN-γ in the blood were increased during febrile episode. The characteristic cytokine profiles of continuous pro-inflammatory cytokine activation with a reduced anti-inflammatory cytokine response leads to sustained inflammation in patients with PFAPA syndrome. Taken together, the findings of the present study suggest that the characteristic cytokine profiles of IL-6, IL-4 and IFN-γ can be the differential features between PFAPA syndrome and recurrent tonsillitis in children in clinical ENT settings.

CONFLICT OF INTEREST

The authors state no conflicts of interest.

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