Eur. Cytokine Netw., Vol. 17 n° 2, *June 2006*, 90-7

Cytokine profile in PFAPA syndrome suggests continuous inflammation and reduced anti-inflammatory response

Silvia Stojanov[1,*], Florian Hoffmann[1,*], Anja Kéry[1], Ellen D. Renner[1,2], Dominik Hartl[1], Peter Lohse[3], Kristina Huss[1], Peter Fraunberger[3], James D. Malley[4], Stephanie Zellerer[1], Michael H. Albert[1], Bernd H. Belohradsky[1]

[1] Department of Infectious Diseases and Immunology, Children's Hospital, University of Munich, Germany
[2] Department of Pediatrics, University of Washington & Children's Hospital, Seattle, USA
[3] Department of Clinical Chemistry - Grosshadern, University of Munich, Germany
[4] Center for Information Technology, National Institutes of Health, Bethesda, USA

Correspondence : S. Stojanov's present address: Genetics and Genomics Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Building 10, Room 9N210, 10 Center Drive, Bethesda, MD 20892
<stojanos@mail.nih.gov>

Accepted for publication March 8, 2006

ABSTRACT. PFAPA syndrome is characterized by periodic episodes of high fever, aphthous stomatitis, pharyngitis, and/or cervical adenitis. It is of unknown etiology and manifests usually before 5 years of age. We determined serum and intracellular cytokine levels in six PFAPA patients (4 males, 2 females, mean age 8 years (± 1.2 SEM), range 4-13) during the symptom-free period as well as 6-12 hours and 18-24 hours after fever onset. Values were compared to age-matched, healthy controls. Febrile PFAPA attacks led to a significant increase in IL-6 and IFN-γ serum concentrations compared to symptom-free periods and to controls, with IL-1β, TNF-α and IL-12p70 levels being significantly higher than in controls. Lymphocytic IFN-γ and CD8+ IL-2 production was consistently significantly elevated compared to healthy children. During the asymptomatic period, serum concentrations of IL-1β, IL-6, TNF-α and IL-12p70 were significantly increased compared to controls. Intracellular TNF-α synthesis was not elevated at any time point. Soluble TNFRp55 levels were even lower in between febrile episodes, reaching values comparable to controls during attacks, whereas soluble TNFRp75 levels increased during attacks compared to healthy children. Anti-inflammatory IL-4 in serum was at all times lower in PFAPA patients compared to controls with no difference in levels of intracellular IL-4 and IL-10 or serum IL-10. The observed increase of pro-inflammatory mediators, even between febrile attacks, suggests a dysregulation of the immune response in PFAPA syndrome, with continuous pro-inflammatory cytokine activation and a reduced anti-inflammatory response.

Keywords: PFAPA syndrome, periodic fever, cytokine, inflammation, IL-1β, IFN-γ

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.

Autoinflammatory diseases are defined by seemingly unprovoked attacks of multisystemic inflammation without underlying infection or autoantibody formation [1]. The periodic fever syndromes (PFS) encompass a group of diseases characterized by recurring bouts of fever. Subtypes include hereditary familial Mediterranean fever (FMF), hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) as well as the TNF receptor 1-associated and cryopyrin-associated periodic syndromes (TRAPS and CAPS, respectively) [2].

Most hereditary PFS are due to mutations in molecules of the pyrin and tumor necrosis factor receptor superfamilies, which are involved in innate immunity. The PFAPA (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis) syndrome, in contrast, is a PFS of unknown etiology that was first described in 1987 by Marshall *et al*. [3]. More than 200 patients have been reported worldwide. PFAPA is characterized by periodic episodes of high fever, lasting 3-6 days and recurring every 3-8 weeks, accompanied by aphthous stomatitis, pharyngitis, and/or cervical adenitis. Other symptoms that may be present during fever attacks include headache, nausea, vomiting, and abdominal pain [4-9]. Disease onset occurs mainly before the age of 5 years. Fever episodes are associated with leukocytosis (with a shift to the left), as well as elevated erythrocyte sedimentation rates (ESR) and C-reactive protein (CRP) levels. PFAPA is defined on clinical grounds, and the diagnosis is one of exclusion due to the lack of specific laboratory results.

Fever with an associated acute-phase reaction is the most prominent systemic manifestation of inflammation, and cytokines secreted by activated monocytes, T-lymphocytes and other cells are known to be involved in various inflammatory reactions. Evaluation of the cytokine network in

* Both authors contributed equally to this work.

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.

FMF [10-13], HIDS [14-17], TRAPS [18, 19] and CAPS [20-23] patients, points to differences in cytokine activation and to disordered interleukin 1 (IL-1) processing. The only information available with regard to cytokine levels in PFAPA is an increase in interferon-γ (IFN-γ), tumor necrosis factor α (TNF-α) and IL-6 concentrations during attacks [4]; however, detailed results, especially with regard to the course of cytokine expression, have not been published.

In PFAPA, each attack can be immediately resolved with one or two doses of prednisone. In about 30% of cases, treatment with cimetidine can lead to disease cessation [4, 24]. Due to immunomodulating properties of prednisone, a potent anti-inflammatory agent, and cimetidine, an H2 receptor antagonist, which inhibits suppressor T (CD8+) lymphocytes [25], we determined peripheral blood lymphocyte cytokine production in order to answer the question of whether disturbed cytokine synthesis plays a role in the PFAPA syndrome. We studied the *ex vivo* production of TNF-α, IFN-γ, IL-2, IL-10 and IL-4 in CD4+ and CD8+ lymphocytes by serial measurements. Simultaneously, we analysed the *ex vivo* synthesis of the inflammatory mediators C-reactive protein (CRP), TNF-α, IL-1β, IL-6, IL-12, IFN-γ and IL-2, and determined the concentration of the anti-inflammatory cytokines IL-4 and IL-10 and of the soluble TNF receptors p55 (sTNFRSF1A) and p75 (sTNFRSF1B).

PATIENTS AND METHODS

Patients

Six patients (four boys and two girls) seen at the University Children's Hospital in Munich, Germany, were included in this study. The diagnosis of PFAPA was made according to the standard clinical criteria: 1) periodic fever (> 38.5°C) accompanied by aphthous stomatitis, and/or pharyngitis, and/or cervical lymphadenitis; 2) age of onset before 5 years; 3) exclusion of infection, rheumatic diseases, primary immunodeficiency and cyclic neutropenia; 4) completely asymptomatic intervals between episodes; 5) normal growth and development [4].

DNA was extracted from each patient's leukocytes, and sequence analysis of all exons of the *TNFRSF1A*, *MVK,* and *MEFV* genes and of exon 3 of the *CIAS1* gene was performed in order to exclude the hereditary PFS TRAPS, HIDS, FMF and CAPS, respectively.

Patients were instructed to present during a symptom-free period and within 12 hours of onset of an attack defined by febrile temperatures (> 38.5°C) and signs of active disease. A symptom-free period, in contrast, was the absence of any symptoms for at least 10 days. Physicians experienced in the diagnosis of PFAPA interviewed and examined each patient at each visit. Blood samples for measurement of acute-phase reactants and cytokines were drawn during the symptom-free period (SFP) as well as 6-12 hours (F1) and 18-24 hours (F2) after the onset of an attack. These time points were chosen in view of the half-life of the cytokines and the duration of the patients fever episodes, in order to detect early changes in the cytokine profile. Patients were not allowed to take steroids during the study period. Only non-steroidal anti-inflammatory drugs were given following the blood sampling during an attack. To exclude infections caused by common bacterial and viral antigens, microbiological analyses of throat swabs were performed, and antibody titres to viral antigens were determined during and in-between attacks.

The mean age of the six patients at the time of sampling was 8 years (±1.2 SEM; range 4–13). The mean interval between blood sampling during the symptom-free period and the previous febrile attack was 17 days (± 3.4 SEM; range 11-33). Febrile episodes lasted 2.5 to 6.5 days with intervals of the symptom-free period ranging from 3.5 to 6.0 weeks. Fever attacks were accompanied by at least adenitis and pharyngitis in all patients. A summary of the demographic data and main clinical features is presented in *table 1*.

The study was approved by the Ethics Committee of the Medical Faculty of the Ludwig-Maximilians-University Munich, and was performed according to the most recent version of the Declaration of Helsinki. Written informed consent was obtained from the parents of all patients and age-matched healthy controls prior to enrollment.

Processing of blood samples

Blood samples were drawn from a peripheral vein into an endotoxin-free tube containing sodium heparin and into a serum tube (Sarstedt, Nürnbrecht, Germany). To avoid spontaneous *ex vivo* cytokine production and release, tubes for determination of serum cytokine levels were

Table 1
Demographics and clinical data of the six PFAPA patients

Patient ID	Sex	Age at onset (years)	Duration of attacks (days)	Duration of symptom-free intervals (weeks)	Maximum temperature (°C)	Cervical adenitis	Pharyngitis	Aphthous stomatitis
1	F	2.0	4.0	3.5	40.0	+	+	-
2	M	0.9	2.5	3.5	40.1	+	+	-
3	M	0.7	4.0	6.0	41.0	+	+	-
4	F	4.1	3.5	6.0	40.9	+	+	+
5	M	3.7	6.5	6.0	40.9	+	+	-
6	M	2.0	4.0	6.0	40.6	+	+	-

Abbreviations: M, male; F, female; +, present; - absent.

centrifuged immediately (225 *g* for 10 min), and the serum was kept frozen (-70°C) until measured.

Determination of lymphocytic cytokine synthesis by flow cytometry

Heparinized (1:1000) venous blood was collected under sterile conditions and analysed within 4-6 hours after blood withdrawal. Control values were obtained from 20 age-matched healthy children (15 males, 5 females; mean age 7.4 years, ± 0.69 SEM; range 4-13) without signs of acute infection, a history of atopy or medication, who were due to undergo elective surgery.

For *ex vivo* cell stimulation, 4 x 100 µL of blood from each subject were diluted to 1:10 with RPMI 1640 culture medium containing 10% heat-inactivated fetal calf serum (FCS). The kinetics of cytokine production were investigated by analysing unstimulated cells and cells stimulated with phorbol 12-myristate 13-acetate (PMA; Sigma, Munich, Germany) plus ionomycin (Sigma) in the presence of monensin (Sigma) for 6 hours at 37°C in a 5% CO_2 atmosphere.

Intracellular antibody staining was performed according to Jung *et al*. [26] and Prussin *et al*. [27, 28] with slight modifications. Stimulated cells were washed twice in 3 mL of phosphate-buffered saline (PBS)/5% FCS, and red cells were lysed with 500 µL formaldehyde-fixing solution for 10 minutes at 4°C. After centrifugation (1250 rpm/min, 5 min, 4°C), the supernatant was decanted, and the cells washed twice with PBS/5% FCS. The next day, cells were washed once, resuspended in 1 mL permeabilisation buffer (PBS with 5% FCS and 5% saponin), incubated for 10 minutes at 4°C and washed once in permeabilisation buffer; 50-µL aliquots of unstimulated and stimulated cultures were incubated at 4°C for 25 minutes with the cell surface markers CD3 PerCP (Peridinin-chlorophyll-protein complex) (IgG2a) (Becton Dickinson, Heidelberg, Germany) and CD8 FITC (Fluorescein) (IgG1) (Coulter Immunotech, Krefeld, Germany) as well as the phycoerythrin (PE)-labelled anti-cytokine monoclonal antibodies TNF-α (IgG1), IFN-γ (IgG1), IL-2, IL-10 and IL-4 (IgG1) (Coulter Immunotech) and the respective isotype control antibodies (IgG1 + IgG2a, Becton Dickinson, Heidelberg, Germany). Stained cells were washed and resuspended in 250 µL fixing solution containing 4% paraformaldehyde.

The intensity of fluorescence was analysed by flow cytometry using a FACScan flow cytometer and the CellQuest software (Becton Dickinson). For each analysis, a lymphocyte gate was defined using dot-plot graphs of forward-scatter versus side-scatter. Dot plot quadrant statistics were set on the basis of CD8+ (FL-1, FITC) or CD8-/CD4+ T-cells versus cytokine fluorescence (FL-2). This was done because PMA/ionomycin stimulation can cause down-regulation of CD4 expression, thereby skewing the results for CD4+ cells [29]. Cytokine-positive CD4+ or CD8+ T-cells were defined by setting regions with the lower limits for cytokine positivity determined from the unstimulated cultures. Data were expressed as the percentage of CD4+ or CD8+ cells within the lymphocyte gate positive for each cytokine.

Cytometric bead array (CBA) immunoassay for determination of circulating cytokines

The Th1/Th2- and Inflammatory Cytometric Bead Arrays (CBA, Becton Dickinson) were used for serum cytokine detection of IFN-γ, TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-10 and IL-12 as previously described [30]. The sensitivity of the arrays with 5-10 µl sample volumes was 15.6 pg/mL (IFN-γ), 3.7 pg/mL (TNF-α), 7.2 pg/mL (IL-1β), 6.6 pg/mL (IL-2), 6.5 pg/mL (IL-4), 2.5 pg/mL (IL-6), 3.3 pg/mL (IL-10) and 1.9 pg/mL (IL-12). Control values were obtained from 15 healthy children with no overt signs of inflammation or atopy (7 males, 8 females; mean age 7.9 years (± 0.6 SEM), range 4 to 12).

Determination of soluble TNF receptors

Soluble TNF receptors p55 and p75 were measured automatically on a CobasCore analyzer (Roche, Switzerland) with enzyme-linked immunobinding assays (Biosource Europe, Belgium). The receptor assay has a detection limit of 100 pg/mL, and is not affected by the presence of free or unbound TNF at a concentration below 10 ng/mL [31].

Determination of leukocytes, C-reactive protein, and erythrocyte sedimentation rate

Leukocyte counts between 5000 and 15500/µL were considered to be within the normal range. ESR measured by the Westergreen method was recorded within 1 hour. Rates of 0-15 mm/h were considered to be within the normal range. Serum levels of CRP, determined by an immunoturbidimetric assay from Roche, were considered to be normal below 0.5 mg/dL.

Statistical analysis

Paired and unpaired t-tests were applied where either was appropriate. When the unpaired t-test was used, an initial Levene test for equality of variances was done, followed by the Welch adjustment, as needed. Due to the small sample size (n = 6 paired or unpaired outcomes), the study results should be considered as exploratory, so no multiple testing adjustments were made (see *tables 2* and *3*). Programming was done using SPSS® 12.0 for Windows.

RESULTS

The febrile episodes experienced by the six PFAPA patients included in this study were characterized by a strong acute-phase reaction which normalized in between attacks (*table 2*). Leukocytes and ESR increased significantly 6-12 hours after fever onset compared to the symptom-free period, while CRP levels and ESR showed a significant rise 18-24 hours after the start of the febrile episode.

The results of the serum cytokine levels are shown in *table 3*. Febrile attacks were associated with a significant increase in IL-6 and IFN-γ plasma concentrations 6-12 hours after fever onset. IL-6 and INF-γ, as well as IL-1β, TNF-α and IL-12p70, were significantly increased when comparing the patient group during an attack with the control group. PMA-stimulated lymphocytic cytokine

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.

Table 2
Leukocytes and acute-phase reactant levels of the six PFAPA patients studied before and during a febrile attack

	N	Mean	SEM	p value	95% CI
Leukocytes (10^3/µl)					
SFP vs	5	6.3	± 0.9	0.019	-8.4; -1.3
F1	5	11.1	± 0.9		
SFP vs	5	6.6	± 0.8	0.052	-7.9; 0.06
F2	5	10.5	± 0.9		
F1 vs	4	11.7	± 0.9	0.368	-2.7; 5.4
F2	4	10.3	± 1.1		
CRP (mg/dL)	N	Mean	SEM	p value	95% CI
SFP vs	5	0.09	± 0.02	0.054	-5.6; -0.07
F1	5	2.8	± 1.0		
SFP vs	4	0.09	± 0.02	0.007	-12.8; -4.6
F2	4	8.8	± 1.3		
F1 vs	3	4.0	± 1.2	0.024	-6.2; -1.2
F2	3	7.7	± 0.8		
ESR (mm/h)	N	Mean	SEM	p value	95% CI
SFP vs	5	14.4	± 5.6	0.023	-20.2; -2.6
F1	5	25.8	± 3.6		
SFP vs	4	14.0	± 7.3	0.031	-29.6; -2.8
F2	4	30.2	± 3.3		
F1 vs	3	26.7	± 5.8	0.130	-13.6; 3.6
F2	3	31.7	± 4.2		

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SFP, symptom-free period; F1, 6-12 hours after fever onset; F2, 18-24 hours after fever onset; SEM, standard error of mean; CI, confidence interval of the difference.

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.

production during F2 demonstrated a significantly increased IFN-γ synthesis in CD4+ lymphocytes compared to F1 and controls (26.7% F2, 21.5% F1; $p = 0.041$; 15.9% controls; $p = 0.030$) *(figure 1A)*. Increased percentages of CD8+ T cells expressed IFN-γ (46.5% F2, 27.9% controls; $p = 0.029$) and IL-2 (4.8% F2, 1.5% controls; $p = 0.015$) during F2 compared to healthy children *(figure 1A, B)*.
We found no significant correlation between serum or intracellular cytokines and the length of the interval between the symptom-free period and the preceding attack in our PFAPA patients (data not shown). During their symptom-free periods, PFAPA patients had significantly elevated pro-inflammatory IL-1β, IL-6, TNF-α and IL-12p70 plasma levels in comparison to controls *(table 3)*. No spontaneous IFN-γ cytokine production was observed in between episodes, and the serum IFN-γ levels were significantly lower than the normal values, whereas lymphocytic INF-γ was significantly increased within CD4+ cells (SFP 22.7%, controls 15.9%; $p = 0.010$) *(figure 1A)*. No increase in lymphocytic *ex vivo* synthesis of TNF-α was demonstrable at any time point, for neither CD4+ nor CD8+ lymphocytes *(figure 1C)*.
Anti-inflammatory IL-10 and IL-4 serum levels *(table 3)* did not differ significantly between fever episodes and symptom-free periods, with IL-4 levels being significantly lower during the symptom-free periods and F2 compared to healthy controls. Intracellular IL-4 and IL-10 production by lymphocytes was not inducible during any disease state *(figure 1D, E)*. Soluble TNF receptors showed an increase during febrile episodes, which was significant for sTNFRp55 18-24 hours after fever onset compared to the symptom-free period, and for sTNFRp75 during attacks in comparison to controls *(table 3)*). However, sTNFRp55 secretion was significantly lower during the symptom-free period compared to healthy controls, reaching control values only during febrile episodes *(table 3)*.

DISCUSSION

We performed serial measurements of a broad range of serum cytokines, and determined their *ex vivo* production in peripheral blood lymphocytes from six children with the PFAPA syndrome. During febrile episodes, patients showed a marked increase in the acute-phase parameters CRP and ESR, as reported in previous studies [4, 5, 7]. Although the acute-phase reactants completely normalized between febrile episodes, serum levels of pro-inflammatory cytokines IL-1β, IL-6, TNF-α and IL-12 remained elevated in patients compared to healthy controls. This observation is important for the prognosis and patient management, as elevated acute-phase parameters between attacks are associated with an increased risk of amyloidosis in FMF and TRAPS patients [32]. Up to now, amyloidosis has not been described in PFAPA. Whether this is attributable to cessation of the disease in adulthood, thereby reducing the overall period of inflammation, or because the pro-inflammatory cytokine stimulus during the afebrile interval was not high enough to induce acute-phase parameters, needs further investigation.
A key player in the febrile response is IL-1β, leading to fever, neutrophilia as well as elevated acute-phase proteins and circulating IL-6 levels [33, 34]. Disordered IL-1 processing has recently been shown to be involved in the pathogenesis of hereditary PFS [1, 2, 34]. Our data suggest that a dysregulated production of IL-1β could play a criti-

Table 3
Circulating cytokine levels of 15 healthy controls (7 males, 8 females; mean age 7.9 years, ± 0.6 SEM, range 4-12) and of the six PFAPA patients during and in between febrile episodes

IL-6 (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	2.6	± 0.5	0.001	-38.3; -11.3
PFAPA_SFP	6	27.4	± 10.5		
Controls vs	15	2.6	± 0.5	< 0.001	-86; -69.2
PFAPA_F1	6	80.2	±15.9		
Controls vs	15	2.6	± 0.5	< 0.001	-177.4; -68.6
PFAPA_F2	5	125.6	± 47.6		
PFAPA_SFP vs	6	27.4	± 10.5	0.008	-84.6; -21
PFAPA_F1	6	80.2	± 6.5		
PFAPA_SFP vs	5	30.3	± 12.4	0.07	-203.3; 12.6
PFAPA_F2	5	125.6	± 47.6		
PFAPA_F1 vs	5	79.8	± 7.9	0.363	-169.4; 77.9
PFAPA_F2	5	125.6	± 47.6		

IL-2 (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	2.0	± 0.6	0.659	-4.2; 2.8
PFAPA_SFP	6	2.6	± 1.3		
Controls vs	15	2.0	± 0.6	0.374	-4.6; 1.9
PFAPA_F1	6	3.3	± 1.3		
Controls vs	15	2.0	± 0.6	0.004	0.7; 3.2
PFAPA_F2	5	0.0	± 0.0		
PFAPA_SFP vs	6	2.6	± 1.3	0.607	-3.6; 2.3
PFAPA_F1	6	3.3	± 1.3		
PFAPA_SFP vs	5	2.2	± 1.6	0.232	-2.1; 6.6
PFAPA_F2	5	0.0	± 0.0		
PFAPA_F1 vs	5	3.3	± 1.5	0.097	-0.9; 7.6
PFAPA_F2	5	0.0	± 0.0		

IL-1β (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	1.4	± 0.4	0.001	-109; -30.6
PFAPA_SFP	6	71.2	± 30.8		
Controls vs	15	1.4	± 0.4	< 0.001	-110.4; -52.6
PFAPA_F1	6	82.9	±22.7		
Controls vs	15	1.4	± 0.4	< 0.001	-118.2; -41.9
PFAPA_F2	5	81.5	± 33.4		
PFAPA_SFP vs	6	71.2	± 30.8	0.585	-63.2; 39.8
PFAPA_F1	6	82.9	± 22.7		
PFAPA_SFP vs	5	83.0	± 34.9	0.955	-69.9; 73.0
PFAPA_F2	5	81.5	± 33.4		
PFAPA_F1 vs	5	82.4	± 27.8	0.931	-25.4; 27.1
PFAPA_F2	5	81.5	± 33.4		

IL-4 (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	11.9	± 1.0	0.004	3.7; 14.0
PFAPA_SFP	6	3.1	± 2.0		
Controls vs	15	11.9	± 1.0	0.069	-0.5; 10.8
PFAPA_F1	6	6.8	± 2.2		
Controls vs	15	11.9	± 1.0	0.001	3.6; 10.8
PFAPA_F2	5	4.7	± 1.2		
PFAPA_SFP vs	6	3.1	± 2.0	0.299	-11.8; 4.5
PFAPA_F1	6	6.8	± 2.2		
PFAPA_SFP vs	5	3.7	± 2.3	0.594	-5.8; 3.8
PFAPA_F2	5	4.7	± 1.2		
PFAPA_F1 vs	5	6.1	± 2.5	0.58	-4.8; 7.5
PFAPA_F2	5	4.7	± 1.2		

TNF-α (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	3.7	± 0.4	0.030	-9.1; -0.7
PFAPA_SFP	6	8.6	± 1.6		
Controls vs	15	3.7	± 0.4	0.006	-7; -1.7
PFAPA_F1	6	8.1	±1.0		
Controls vs	15	3.7	± 0.4	0.001	-9.5; -2.7
PFAPA_F2	5	9.8	± 2.7		
PFAPA_SFP vs	6	8.6	± 1.6	0.741	-3.3; 4.3
PFAPA_F1	6	8.1	± 1.0		
PFAPA_SFP vs	5	9.2	± 1.9	0.636	-4.0; 2.7
PFAPA_F2	5	9.8	± 2.7		
PFAPA_F1 vs	5	7.9	± 1.3	0.383	-7.4; 3.5
PFAPA_F2	5	9.8	± 2.7		

IL-10 (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	13.7	± 2.8	0.272	-3.6; 12
PFAPA_SFP	6	9.5	± 2.4		
Controls vs	15	13.7	± 2.8	0.401	-15.8; 6.9
PFAPA_F1	6	18.1	± 4.2		
Controls vs	15	13.7	± 2.8	0.796	-10; 7.8
PFAPA_F2	5	14.8	± 3.0		
PFAPA_SFP vs	6	9.5	± 2.4	0.138	-21.3; 4.0
PFAPA_F1	6	18.1	± 4.2		
PFAPA_SFP vs	5	11.4	± 1.8	0.28	-10.9; 4.1
PFAPA_F2	5	14.8	± 3.0		
PFAPA_F1 vs	5	18.9	± 5.1	0.561	-14.1; 22.4
PFAPA_F2	5	14.8	± 3.0		

IL-12p70 (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	12.2	± 2.6	0.002	-107.4; -29.5
PFAPA_SFP	6	80.7	± 29.9		
Controls vs	15	12.2	± 2.6	0.006	-124.6; -24.2
PFAPA_F1	6	86.6	± 38.9		
Controls vs	15	12.2	± 2.6	0.024	-146.2; -11.6
PFAPA_F2	5	91.1	± 58.2		
PFAPA_SFP vs	6	80.7	± 29.9	0.722	-46.4; 34.5
PFAPA_F1	6	86.6	± 38.9		
PFAPA_SFP vs	5	84.3	± 36.3	0.82	-84.2; 70.7
PFAPA_F2	5	91.1	± 58.2		
PFAPA_F1 vs	5	85.8	± 47.6	0.658	-35.9; 25.4
PFAPA_F2	5	91.1	± 58.2		

sTNFRp55 (ng/mL)	N	Mean	SEM	p value	95% CI
Controls vs	13	2.0	± 0.1	0.036	0.04; 0.8
PFAPA_SFP	5	1.5	± 0.1		
Controls vs	13	2.0	± 0.1	0.517	-0.8; 0.5
PFAPA_F1	5	2.1	± 0.2		
Controls vs	13	2.0	± 0.1	0.169	-1.0; 0.2
PFAPA_F2	4	2.3	± 0.2		
PFAPA_SFP vs	4	1.5	± 0.2	0.11	-1.0; 0.2
PFAPA_F1	4	1.9	± 0.04		
PFAPA_SFP vs	3	1.6	± 0.2	0.014	-0.8; -0.3
PFAPA_F2	3	2.2	± 0.2		
PFAPA_F1 vs	4	2.2	± 0.3	0.546	-0.7; 0.5
PFAPA_F2	4	2.3	± 0.2		

IFN-γ (pg/mL)	N	Mean	SEM	p value	95% CI
Controls vs	15	13.1	± 1.2	< 0.001	9.2; 17.0
PFAPA_SFP	6	0.0	± 0.0		
Controls vs	15	13.1	± 1.2	< 0.001	-211.6; -78.6
PFAPA_F1	6	158.2	± 52.2		
Controls vs	15	13.1	± 1.2	< 0.001	-158.2; -56.5
PFAPA_F2	5	120.5	± 44.3		
PFAPA_SFP vs	6	0.0	± 0.0	0.029	-292.5; -23.9
PFAPA_F1	6	158.2	± 52.2		
PFAPA_SFP vs	5	0.0	± 0.0	0.053	-243.5; 2.6
PFAPA_F2	5	120.5	± 44.3		
PFAPA_F1 vs	5	130.2	± 54.0	0.887	-169.4; 188.9
PFAPA_F2	5	120.5	± 44.3		

sTNFRp75 (ng/mL)	N	Mean	SEM	p value	95% CI
Controls vs	13	3.9	± 0.2	0.342	-1.1; 0.4
PFAPA_SFP	5	4.3	± 0.2		
Controls vs	13	3.9	± 0.2	0.012	-3.6; -0.5
PFAPA_F1	5	6.0	± 1.1		
Controls vs	13	3.9	± 0.2	< 0.001	-4.0; -1.4
PFAPA_F2	4	6.6	± 0.8		
PFAPA_SFP vs	3	4.4	± 0.4	0.428	-4.5; 2.8
PFAPA_F1	3	5.2	± 1.1		
PFAPA_SFP vs	3	4.4	± 0.4	0.096	-4.4; 0.8
PFAPA_F2	3	6.2	± 1.0		
PFAPA_F1 vs	4	6.0	± 1.1	0.193	-1.9; 0.6
PFAPA_F2	4	6.6	± 0.8		

Abbreviations: PFAPA_SFP, symptom-free period of PFAPA patients; PFAPA_F1, 6-12 hours after fever onset; PFAPA F2, 18-24 hours after fever onset; SEM, standard error of mean; CI, confidence interval of the difference.

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.

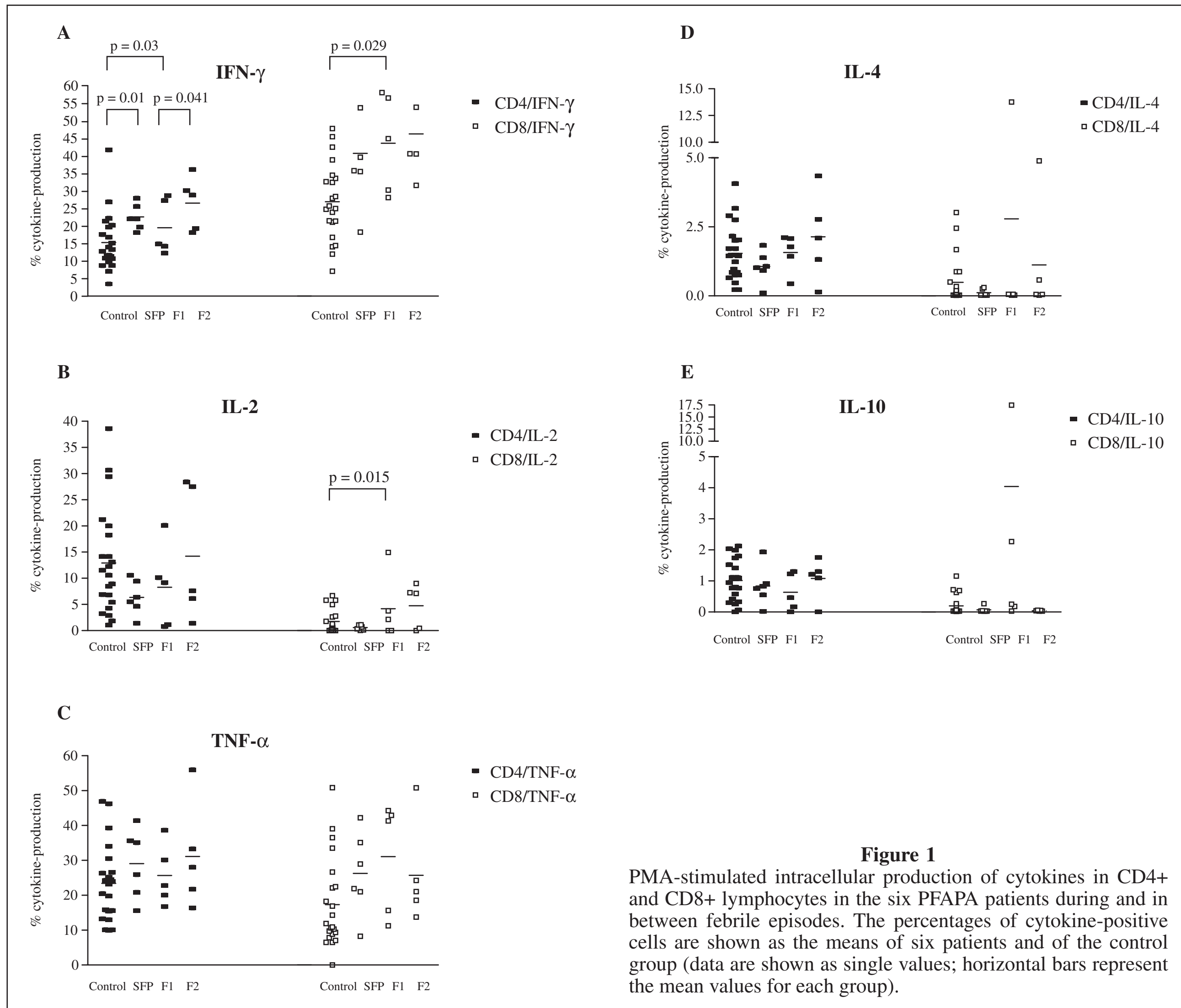

Figure 1

PMA-stimulated intracellular production of cytokines in CD4+ and CD8+ lymphocytes in the six PFAPA patients during and in between febrile episodes. The percentages of cytokine-positive cells are shown as the means of six patients and of the control group (data are shown as single values; horizontal bars represent the mean values for each group).

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.

cal role in PFAPA, as we detected increased IL-1β and IL-6 serum levels in between and during febrile episodes compared to healthy controls.

Given the slightly, albeit significant, increased serum levels of TNF-α, another major endogenous pyrogen, showing no differences in intracellular levels during attacks in comparison to the control population, it is remarkable that concentrations of its soluble receptor sTNFRp55 were even lower during the symptom-free period, reaching levels comparable to controls only during attacks. Although lymphocytes are not the major source of TNF-α, which could explain the lack of production in these cells, based on our serum findings, we hypothesize that TNF-α might not be a key player in the pathogenesis of PFAPA.

Even 6 to 12 hours after fever onset, patients showed a sharp rise in circulating IFN-γ, accompanied by increased lymphocytic IFN-γ and CD8+ IL-2 production 18-24 hours after the start of an attack, suggesting a Th1 activation. Between attacks however, the serum IFN-γ concentrations of our patients were significantly lower compared to healthy controls, while the intracellular production capacity of their CD4+ lymphocytes for IFN-γ was increased. During the course of our study, Aypar *et al.* [35] published a report on intracellular IFN-γ and IL-4 measurements in lymphocytes of FMF patients, indicating a Th1 polarization due to an increased percentage of IFN-γ-positive T-cells compared to healthy controls. However, there were no differences with regard to the IL-4 production of T-cells between FMF patients and healthy controls. Increased serum levels of IFN-γ have also been described for HIDS [15, 16], thus suggesting a common IFN-γ activation in these PFS.

Under physiological conditions, febrile attacks are followed by a rise in anti-inflammatory molecules to counteract an exaggerated inflammation. As IFN-γ is also known to suppress the synthesis of IL-4 and IL-10, the failure of these anti-inflammatory cytokines to increase in our PFAPA patients could indicate a critical role of the highly elevated intracellular and circulating IFN-γ levels during fever episodes in PFAPA. Nevertheless, there is a slight chance that this result could be due to the small number of patients investigated. However, our assumption that IFN-γ is a potential key player in PFAPA is supported by the fact that cimetidine, which has a suppressive effect on CD8+ cells (another source of IFN-γ), can influence the disease course by inducing complete remission.

The cytokine changes observed in our patients which are characterized by a continuous, pro-inflammatory cytokine

activation and a reduced anti-inflammatory response, suggest a dysregulated innate and adaptive immunity in PFAPA. These results may help to elucidate the pathophysiological basis of this, still obscure, syndrome.

Acknowledgements. *This study was supported by a research grant (FöFoLe) from the Ludwig-Maximilians-University of Munich, Germany to S.S.. We are grateful to all patients and their parents, who participated in this study. We thank Genoveva Kim, Sabine Maier, Franz Jansen and Dieter Kunz for excellent technical and Barbara Asam for secretarial assistance. The authors state that there are no commercial associations to disclose.*

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.

REFERENCES

1. Galon J, Aksentijevich I, McDermott MF, O'Shea JJ, Kastner DL. *TNFRSF1A* mutations and autoinflammatory syndromes. *Curr Opin Immunol* 2000; 12: 479.
2. Stojanov S, Kastner DL. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. *Curr Opin Rheumatol* 2005; 17: 586.
3. Marshall GS, Edwards KM, Butler J, Lawton AR. Syndrome of periodic fever, pharyngitis, and aphthous stomatitis. *J Pediatr* 1987; 110: 43.
4. Thomas KT, Feder HM, Lawton AR, Edwards KM. Periodic fever syndrome in children. *J Pediatr* 1999; 135: 15.
5. Padeh S, Brezniak N, Zemer D, Pras E, Livneh A, Langevitz P, Migdal A, Pras M, Passwell JH. Periodic fever, aphthous stomatitis, pharyngitis, and adenopathy syndrome: clinical characteristics and outcome. *J Pediatr* 1999; 135: 98.
6. Long SS. Syndrome of periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) – what it isn't. What is it? *J Pediatr* 1999; 135: 1.
7. Ovetchkine P, Bry ML, Reinert P. Marshall syndrome: results of a retrospective national survey. *Arch Pediatr* 2000; 7(Suppl 3): 578s.
8. Dahn KA, Glode MP, Chan KH. Periodic fever and pharyngitis in young children: a new disease for the otolaryngologist? *Arch Otolaryngol Head Neck Surg* 2000; 126: 1146.
9. Tasher D, Somekh E, Dalal J, PFAPA. syndrom - new clinical aspects revealed. *Arch Dis Child* 2006; [e-pub ahead of print].
10. Gang N, Drenth J, Langevitz P, Zemer D, Brezniak N, Pras M, van der Meer JW, Livneh A. Activation of the cytokine network in familial Mediterranean fever. *J Rheumatol* 1999; 26: 890.
11. Direskeneli H, Ozdogan H, Korkmaz C, Akoglu T, Yazici HG. Serum soluble intercellular adhesion molecule 1 and interleukin 8 levels in familial Mediterranean fever. *J Rheumatol* 1999; 26: 1983.
12. Baykal Y, Saglam K, Yilmaz MI, Taslipinar A, Akinci SB, Inal A. Serum sIL-2r, IL-6, IL-10 and TNF-α level in familial Mediterranean fever patients. *Clin Rheumatol* 2003; 22: 99.
13. Bagci S, Toy B, Tuzun A, Ates Y, Aslan M, Inal A, Gulsen M, Karaeren N, Dagalp K. Continuity of cytokine activation in patients with familial Mediterranean fever. *Clin Rheumatol* 2004; 23: 333.
14. Drenth JPH, van Deuren M, van der Ven-Jongekrijg J, Schalkwijk CG, van der Meer JWM. Cytokine activation during attacks of the hyperimmunoglobulinemia D and periodic fever syndrome. *Blood* 1995; 85: 3586.
15. Drenth JPH, Powell RJ, Brown NS, van der Meer JWM. Interferon-γ and urine neopterin in attacks of the hyperimmunoglobulinemia D and periodic fever syndrome. *Eur J Clin Invest* 1995; 25: 683.
16. Drenth JPH, van der Meer JWM, Kushner I. Unstimulated peripheral blood mononuclear cells from patients with the hyper-IgD syndrome produce cytokines capable of potent induction of C-reactive protein and serum amyloid A in Hep3B cells. *J Immunol* 1996; 157: 400.
17. Frenkel J, Rijkers GT, Mandey SH, Buurman SW, Houten SM, Wanders RJ, Waterham HR, Kuis W. Lack of isoprenoid products raises ex vivo interleukin-1β secretion in hyperimmunoglobulinemia D and periodic fever syndrome. *Arthritis Rheum* 2002; 46: 2794.
18. McDermott E, Powell R. Circulating cytokine concentrations in familial Hibernian fever. In: Sohar E, Gafni J, Pras M, eds. Familial Mediterranean fever, 1st International Conference; 1997; Jerusalem, Israel. Tel Aviv: Freund Publishing House, 1997: 189.
19. Nowlan ML, Drewe E, Bulsara H, Esposito N, Robins RA, Tighe PJ, Powell RJ, Todd I. Systemic cytokine levels and the effects of etanercept in TNF-receptor associated periodic syndrome (TRAPS) involving a C33Y mutation in TNFRSF1A. *Rheumatology* 2006; 45: 31.
20. Aksentijevich I, Nowak M, Mallah M, Chae JJ, Watford WT, Hofmann SR, Stein L, Russo R, Goldsmith D, Dent P, Rosenberg HF, Austin F, Remmers EF, Balow Jr. JE, Rosenzweig S, Komarow H, Shoham NG, Wood G, Jones J, Mangra N, Carrero H, Adams BS, Moore TL, Schikler K, Hoffman H, Lovell DJ, Lipnick R, Barron K, O'Shea JJ, Kastner DL, *et al.* De novo *CIAS1* mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. *Arthritis Rheum* 2002; 46: 3340.
21. Huttenlocher A, Frieden IJ, Emery H. Neonatal onset multisystem inflammatory disease. *J Rheumatol* 1995; 22: 1171.
22. Stojanov S, Weiss M, Lohse P, Belohradsky BH. A novel *CIAS1* mutation and plasma/cerebrospinal fluid cytokine profile in a German patient with neonatal-onset multisystem inflammatory disease responsive to methotrexate therapy. *Pediatrics* 2004; 114: e124.
23. Gerbig AW, Dahinden CA, Mullis P, Hunziker T. Circadian elevation of IL-6 levels in Muckle-Wells syndrome: a disorder of the neuro-immune axis? *QJM* 1998; 91: 489.
24. Feder Jr. HM. Cimetidine treatment for periodic fever associated with aphthous stomatitis, pharyngitis and cervical adenitis. *Pediatr Infect Dis J* 1992; 11: 318.
25. White WB, Ballow M. Modulation of suppressor-cell activity by cimetidine in patients with common variable hypogammaglobulinemia. *N Engl J Med* 1985; 312: 198.
26. Jung T, Schauer U, Heusser C, Neumann C, Rieger CJ. Detection of intracellular cytokines by flow cytometry. *J Immunol Methods* 1993; 159: 197.
27. Prussin C, Metcalfe DD. Detection of intracytoplasmatic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods* 1995; 188: 117.
28. Prussin C. Cytokine flow cytometry: understanding cytokine biology at the single-cell level. *J Clin Immunol* 1997; 17: 195.
29. Pelchen-Matthews A, Parsons IJ, Marsh M. Phorbol ester-induced downregulation of CD4 is a multistep process involving dissociation from p56lck, increased association with clathrin-coated pits, and altered endosomal sorting. *J Exp Med* 1993; 178: 1209.
30. Chen R, Lowe L, Wilson JD, Crowther E, Tzeggai K, Bishop JE, Varro R. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clin Chem* 1999; 45: 1693.
31. Kern P, Hemmer CJ, Gallati H, Neifer S, Kremsner P, Dietrich M, Porzsolt F. Soluble tumor necrosis factor receptors correlate with parasitemia and disease severity in human malaria. *J Infect Dis* 1992; 166: 930.

32. Grateau G, Jeru I, Rouaghe S, Cazeneuve C, Ravet N, Duquesnoy P, Cuisset L, Dode C, Delpech M, Amselem S. Amyloidosis and auto-inflammatory syndromes. *Curr Drug Targets Inflamm Allergy* 2005; 4: 57.

33. Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J Endotoxin Res* 2004; 10: 201.

34. Dinarello CA. Blocking IL-1 in systemic inflammation. *J Exp Med* 2005; 201: 1355.

35. Aypar E, Ozen S, Okur H, Kutluk T, Besbas N, Bakkaloglu A. Th1 polarization in familial Mediterranean fever. *J Rheumatol* 2003; 30: 2011.

Copyright © 2017 John Libbey Eurotext. Téléchargé par un robot venant de 54.191.40.80 le 10/07/2017.