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Polyclonal, newly derived T cells with low expression of inhibitory molecule PD-1 in tonsils define the phenotype of lymphocytes in children with Periodic Fever, Aphtous Stomatitis, Pharyngitis and Adenitis (PFAPA) syndrome

Dytrych Petra[a], Krol Petra[b], Kotrova Michaela[c], Kuzilkova Daniela[c], Hubacek Petr[c,d], Krol Ladislav[c], Katra Rami[a], Hrusak Ondrej[c], Kabelka Zdenek[a], Dolezalova Pavla[b], Kalina Tomas[c,1], Fronkova Eva[c,*,1]

[a] Department of ENT, Charles University, 2nd Faculty of Medicine, Charles University Prague and University Hospital Motol, Czech Republic
[b] Pediatric Rheumatology Unit, Department of Pediatrics and Adolescent Medicine, Charles University Prague, and General University Hospital in Prague, 1st Faculty of Medicine, Czech Republic
[c] CLIP, Department of Paediatric Haematology/Oncology, 2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Czech Republic
[d] Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Czech Republic

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ABSTRACT

Purpose: PFAPA syndrome is a benign, recurrent inflammatory disease of childhood. Tonsillectomy is one of the therapeutic options with a yet unexplained biological mechanism. We tested whether specific lymphocyte subsets recruited from blood to human tonsils participate in PFAPA pathogenesis.
Methods: Paired tonsils/peripheral blood (PB) samples were investigated (a) from children with PFAPA that successfully resolved after tonsillectomy ($n = 10$) (b) from children with obstructive sleep apnoea syndrome as controls ($n = 10$). The lymphocyte profiles were analysed using 8-colour flow cytometry, immunoglobulin (IGH) and T-cell receptor (TCR) gene rearrangements via PCR and next generation sequencing; a TREC/KREC analysis was performed using qPCR.
Results: The PFAPA tonsils in the asymptomatic phase had a lower percentage of B-lymphocytes than controls; T-lymphocyte counts were significantly higher in PB. The percentages of cytotoxic CD8pos T-lymphocytes were approximately 2-fold higher in PFAPA tonsils; the transitional B cells and naïve stages of both the CD4pos and CD8pos T-lymphocytes with a low expression of PD-1 molecule and high numbers of TREC were also increased. With the exception of elevated plasmablasts, no other differences were significant in PB. The expression levels of CXCL10, CXCL9 and CCL19 genes were significantly higher in PFAPA tonsils. The IGH/TCR pattern showed no clonal/oligoclonal expansion. DNA from the Epstein-Barr virus, Human Herpervirus-6 or adenovirus was detected in 7 of 10 PFAPA tonsils but also in 7 of 9 controls.
Conclusions: Our findings suggest that the uninhibited, polyclonal response of newly derived lymphocytes participate in the pathogenesis of PFAPA. Because most of the observed changes were restricted to tonsils and were not present in PB, they partly explain the therapeutic success of tonsillectomy in PFAPA syndrome.

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1. Introduction

PFAPA syndrome in children ranks among the most common periodic autoinflammatory diseases. In 1987, PFAPA was first described by Marshall according to its most characteristic symptoms: periodic fever, pharyngitis, aphthous stomatitis and cervical lymphadenitis. The diagnosis is made partly by exclusion; the diagnostic criteria for PFAPA syndrome modified by Thomas et al. (Thomas et al., 1999) include the following: (1) onset of disease prior to 5 years of age, (2) the absence of upper respiratory tract infection with at least one of the following symptoms: aphthous stomatitis, cervical lymphadenitis, pharyngitis, (3) asymptomatic interval between episodes, (4) exclusion of cyclic neutropenia and

* Corresponding author. Tel.: +420 224436489.
E-mail address: eva.fronkova@lfmotol.cuni.cz (F. Eva).
[1] Contributed equally.

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(5) normal growth and development. Additional features such as vomiting, nausea, fatigue, abdominal pain and arthralgia may occur (Hofer et al., 2014). The aetiology and pathogenesis of the syndrome remains unknown; a dysregulation of the immune system or abnormal response to an unknown infection trigger is suspected. Laboratory findings in peripheral blood (PB) during fever flares revealed elevated white blood cell counts (WBC) that was caused mostly by the increased neutrophil and monocyte counts accompanied by decrease in lymphocytes and eosinophils. Elevation of erythrocyte sedimentation rate, of C-reactive protein and of serum amyloid A was recorded during PFAPA flares (Thomas et al., 1999; Brown et al., 2010; Feder and Salazar, 2010; Kolly et al., 2013; Stojanov et al., 2006; Stojanov et al., 2011). Investigation of classic proinflammatory cytokines in sera revealed elevated serum concentration of IL- 6, but unchanged levels of TNF-alpha and IL1β approximately 15 h after the onset of the fever (Brown et al., 2010). The levels of lymphocyte activator IL-7 and Th17-associated cytokine IL17 were decreased both in febrile and in afebrile sera compared to controls in the same study (Brown et al., 2010). Another study exploring cytokine production came to slightly different results, showing increased plasma concentration of IL-1β, IL-6, TNF-alpha and IL-12p70 after fever onset, but also in asymptomatic periods compared to controls (Stojanov et al., 2006). Increased percentages of $CD4^{+}$ and $CD8^{pos}$ T cells produced IFN-γ after stimulation compared to controls (Stojanov et al., 2006). Both studies concluded that the cytokine pattern is typical for an IFNγ-dependent (Th1) inflammatory response. A study by Stojanov et al. (Stojanov et al., 2011) was able to distinguish PFAPA flares from asymptomatic period samples, control samples and also from hereditary periodic fever (HPF) flares based solely on the gene expression profile. During PFAPA flares, complement, IL-1-related and IFN-induced genes were significantly overexpressed. The gene expression profile during asymptomatic periods was indistinguishable from that of healthy children. PFAPA flares were accompanied by increased protein levels of chemokines known as the T cell chemoattractants IP-10/CXCL10, MIG/CXCL9 and MIP-1β/CCL4. The most recent study suggested that dysregulated IL-1β monocyte production is linked to PFAPA syndrome (Kolly et al., 2013).

The optimal treatment method for PFAPA syndrome remains a matter of debate. The administration of antibiotics and non-steroidal anti-inflammatory agents has been considered ineffective (Thomas et al., 1999). A single dose of prednisone aborts PFAPA flares but does not prevent their recurrence (Feder and Salazar, 2010; Berlucchi et al., 2003; Wurster et al., 2011). The administration of the recombinant IL-1R antagonist anakinra was successful in all five patients (based on the findings of above mentioned study) (Stojanov et al., 2011). A positive effect of tonsillectomy has been documented in several studies (Feder and Salazar, 2010; Stojanov et al., 2011; Garavello et al., 2009; Krol et al., 2013; Peridis et al., 2010a), although the pathogenetic background for this therapeutic intervention is unknown. Because tonsils are primarily a lymphoid tissue and because the gene expression profiles revealed the involvement of adaptive immunity, we decided to evaluate the perturbation of lymphocyte composition and function within tonsils removed as a treatment for PFAPA treatment. As anticipated in a previous study describing elevated levels of T cell chemoattractants in peripheral blood (Stojanov et al., 2011), we show that T cells, especially early developmental stages of $CD8^{pos}$ cytotoxic and $CD4^{pos}$ helper T cells with low expressions of inhibitory molecule PD-1, are more abundant in the tonsils of patients with PFAPA. The activation of the immune system by a virus could be a tempting explanation of tonsillar pathology; we found the DNA of common viruses in most PFAPA tonsils. However, the same viruses were also present in control tonsils. Interestingly, most of the observed differences in our study were limited to tonsils and were not present in the blood.

2. Patients and methods

2.1. Ethics statement

This study was approved by the Institutional Review Board of 2nd Faculty of Medicine and Motol University Hospital in Prague, Czech Republic. The parents of the patients were informed about the aim of the study and gave their written consent. All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. PFAPA and control samples

Ten children with PFAPA syndrome (5 boys, 5 girls) and 10 children with obstructive sleep apnoea syndrome (control group) underwent elective tonsillectomy with or without adenoidectomy at the ENT Department of the 2nd Faculty of Medicine, Charles University in Prague, between October 2010 and May 2013. The identification of PFAPA patients, diagnosis and follow up of the children with PFAPA syndrome were performed in cooperation with the Periodic Fever Clinic of the Pediatric Rheumatology Unit, General University Hospital in Prague. The children with tonsillectomy due to PFAPA syndrome consisted of three groups: (1) the patients whose parents opted for tonsillectomy as a first line treatment (3 patients); (2) the patients treated with episodic prednisone whose disease duration and episode frequency were an unacceptable burden for the family (4 patients) and (3) the patients treated with prednisone with increasingly frequent episodes (3 patients). Tonsillectomy was performed during symptom-free intervals. The cold knife method for tonsillectomy was employed, and adenoidectomy was performed if needed. The patients were followed for a minimum of 6 months after tonsillectomy: initially during common postoperative ENT follow up then via telephone contact. A sample of the peripheral blood was obtained during the tonsillectomy procedure.

2.3. Diagnostic algorithm

In order to exclude other causes of recurrent fevers all patients underwent general pediatric and ENT assessments.

The case history inquired was:

- age at the first manifestation of the symptoms
- age at diagnosis
- duration of fever episodes
- interval between fever episodes
- clinical findings during fever episodes: pharyngitis, cervical lymphadenopathy, aphthous stomatitis, arthralgia, abdominal pain and vomiting.
- clinical status during afebrile intervals

Laboratory evaluation:

- erythrocyte sedimentation rate, full blood count with differential, liver and kidney function tests, anti streptolysin O.
- nose and throat culture
- serum immunoglobulin (Ig) levels (namely IgG, IgA, IgD), herpetic virus serology and mevalonic acid in urine collected during the febrile episode (Krol et al., 2013).

Finally, clinical diagnosis of PFAPA syndrome was confirmed by a pediatric rheumatologist in the Periodic Fever Clinic.

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