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Longitudinal analysis of serum interleukin-18 in patients with familial Mediterranean fever carrying MEFV mutations in exon 10

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Running title: Serum IL-18 in FMF

Abstract

Background: Familial Mediterranean fever (FMF) is an autoinflammatory disease caused by mutations in the *MEFV* gene. Mutations in exon 10 are associated with typical FMF phenotypes, and patients with exon 10 mutations have higher serum levels of interleukin (IL)-18 both during attacks and afebrile phases, compared to those without exon 10 mutations. However, longitudinal changes of serum IL-18 in FMF have not been fully characterized. *Methods:* We serially evaluated serum levels of pro-inflammatory cytokines, including IL-18, in 12 patients with FMF carrying exon 10 mutations, all of whom showed typical FMF attacks. *Results:* Markedly high concentrations of IL-18 were observed in all patients at diagnosis (5,099±6,084 pg/mL). Serum IL-18 levels declined progressively after colchicine treatment in 7 patients (group A), whereas 5 patients showed continued elevation of circulating IL-18, despite declines in IL-6 and neopterin (group B). The mean follow-up times in the two groups were 4.7±3.2 and 4.8±1.5 years, respectively. The mean serum IL-18 level at the last hospital visit in group B was 4,190±2,610 pg/mL. There were no differences in onset age, initial IL-18 levels, and colchicine doses between the groups. FMF attacks almost disappeared in both groups, but there were trends towards more frequent subtle symptoms such as abdominal discomfort in group B. *Conclusions:* Sustained elevation of serum IL-18 may suggest the presence of persistent subclinical inflammation. Therefore, longitudinal examination of serum IL-18 may contribute to better follow-up of FMF patients with exon 10 mutations.

Key words: autoinflammatory disease, familial Mediterranean fever, MEFV, IL-18

Abbreviations: FMF, familial Mediterranean fever; IL, interleukin; NLRP3, nucleotide-binding domain and leucine-rich repeat pyrin containing protein-3.

1. Introduction

Familial Mediterranean fever (FMF) is the prototype of inherited autoinflammatory diseases. It is characterized by recurrent episodes of fever with serositis, synovitis, or skin rash, and is caused by autosomal recessive mutations in the *MEFV* gene, which has 10 exons encoding a 781-amino acid protein called pyrin [1]. Pyrin is expressed in innate cells, including granulocytes, cytokine-activated monocytes, dendritic cells, and synovial and peritoneal fibroblasts, and appears to act as a pivotal regulator of inflammation and apoptosis. Most disease-associated mutations are missense substitutions in exon 10, such as M694V, V726A, M680I, and M694I [2]. Exons 2 and 3 include various missense changes that are benign polymorphisms or of unknown pathogenic significance. Approximately 30% of patients with a typical clinical presentation of FMF have only one demonstrable mutation, although FMF is an autosomal recessive disease [1].

Studies of serum cytokines from patients with FMF have found elevated concentrations of several pro-inflammatory cytokines, such as interleukin (IL)-6, IL-17 and IL-18, during attacks, and shown the utility of a combination of these cytokines as a diagnostic biomarker [3-7]. IL-18 was identified as an interferon-gamma-inducing factor that promotes a variety of innate immune processes associated with infection, inflammation, and autoimmunity [8]. In contrast to IL-6, serum IL-18 levels are also elevated during afebrile phases in FMF patients with typical phenotypes carrying exon 10 mutations [6]. However, the longitudinal course of circulating IL-18 after initiation of treatment with colchicine has not been fully characterized in these patients. To address

this issue, we performed serial analysis of serum cytokines from FMF patients, and identified two subgroups based on changes in serum IL-18 over time.

2. Materials and Methods

2.1. Patients

The subjects were 12 patients with FMF who had pathogenic mutations in exon 10 of the *MEFV* gene. Clinical and laboratory data for these patients are shown in Table 1. All patients were born to non-consanguineous Japanese parents, except for one FMF patient with an M694V mutation. A diagnosis of FMF was made on the basis of the Tel Hashomer criteria [9]. A typical FMF attack was defined as recurrent episodes lasting 12 hours to 3 days with fever of 38°C or higher. An attack was considered 'atypical' if it differed from the definition of a typical attack in only 1 or 2 of the following features: temperature less than 38°C, an attack duration longer or shorter than specified (but not shorter than 6 hours or longer than a week), no signs of peritonitis during an abdominal attack, localized abdominal attacks, or an unusual distribution of arthritis. We also evaluated serum cytokines from 11 patients with FMF carrying sequence variants in exon 2, 3 or 5 of *MEFV*, but not exon 10 mutations, as disease controls. Approval for the study was obtained from the Human Research Committee of Kanazawa University Graduate School of Medical Science, and informed consent was provided according to the Declaration of Helsinki.

2.2. Mutation analysis and cytokine determination

DNA was extracted from blood samples using a standard method. Direct sequencing of the *MEFV* gene was performed as described previously [10]. Serum concentrations of cytokines were determined using enzyme-linked immunosorbent assay kits: neopterin (IBL, Hamburg, Germany); IL-6 (R&D Systems, Minneapolis, MN); and IL-18 (MBL, Nagoya, Japan) [11].

2.3. Statistical analysis

Analysis of differences among groups was performed by Student *t*-test for unpaired samples or by Fisher exact test. Differences with p-values less than 0.05 were considered significant.

3. Results

3.1. Patient characteristics

As shown in Table 1, a heterozygous M694I mutation in exon 10 was found in all patients except for one who carried a heterozygous M694V mutation. The E148Q variant in exon 2 was also found frequently (11/12). No patients were homozygous for exon 10 mutations or were compound heterozygotes carrying two different exon 10 mutations. Typical FMF attacks occurred in all 12 patients carrying exon 10 mutations. All patients were successfully treated with colchicine after molecular diagnosis of FMF, and were followed up for more than one year. Significantly higher concentrations of IL-18 at diagnosis were found in patients with exon 10 mutations (5,099±6,084 pg/mL) compared with disease controls without exon 10 mutations (334±181 pg/mL). No differences in

serum levels of IL-6 and neopterin at diagnosis were found between the patients and controls (data not shown).

3.2. Longitudinal examination of serum IL-18

Patients with exon 10 mutations were divided into two groups based on the serial analysis of serum IL-18 (Table 1, Fig 1A): serum IL-18 levels declined progressively after initiation of treatment with colchicine in 7 patients (group A), whereas 5 patients showed continued elevation of circulating IL-18 (more than 1,000 pg/mL; group B). Serum IL-18 levels at the last hospital visit in groups A and B were 686±281 and 4,190±2,610 pg/mL, respectively. In contrast to IL-18, there were no differences in the patterns of serum levels of IL-6 and neopterin in groups A and B (Fig 1B and data not shown). The mean follow-up time in groups A and B were 4.7±3.2 and 4.8±1.5 years, respectively. There were also no differences in onset age, delay in diagnosis, and disease duration between the two groups (Table 1), or in initial levels of serum IL-18 and colchicine doses. Typical FMF attacks disappeared in both groups, but patients in group B but not in group A occasionally suffered atypical attacks. In addition, there were trends for more frequent subtle symptoms such as abdominal discomfort in group B (3/5) compared to group A (2/7).

4. Discussion

Inflammasome activation results in production of mature IL-1β and IL-18, both of which are cleaved from precursor forms by caspase-1. Pyrin modulates nucleotide-binding domain and leucine-rich repeat pyrin containing protein-3 (NLRP3)

inflammasome-dependent IL-1β secretion, and mutated pyrin leads to aberrant production of IL-1β in FMF [12]. IL-1 inhibitors, such as anakinra and canakinumab, have been used successfully in FMF patients who are resistant to colchicine, a treatment of choice for FMF, which supports the view that FMF is an IL-1β activation disorder [13]. However, systemic circulating levels of IL-1β are undetectable in most patients with FMF, even during attacks [5-7]. No or little elevation of serum IL-1β has also been found in most patients with cryopyrin-associated periodic syndromes, which are also IL-1β activation disorders and are caused by gain-of-function mutations in *NLRP3* [12, 14]. These findings may be attributable to restricted production of IL-1β at local sites of inflammation, or rapid clearance of IL-1β in the circulation [7]. In contrast to IL-1β, and consistent with previous reports [6], serum levels of IL-18 were elevated in FMF patients with exon 10 mutations during an attack and in the clinically stable phase, whereas FMF patients without exon 10 mutations tended to exhibit an atypical FMF phenotype and had much less elevation of IL-18. These findings further support the importance of serum IL-18 as a diagnostic and disease activity marker for FMF [7].

In this study, we investigated the longitudinal course of circulating IL-18 after initiation of treatment with colchicine in FMF patients with exon 10 mutations. These patients could be divided into two groups based on changes in serum levels of IL-18 over time. Serum IL-18 declined progressively after colchicine treatment in 7 patients (group A), whereas 5 patients showed continued elevation of circulating IL-18 (group B). There were no differences in onset age, initial IL-18 levels, and colchicine doses between the two groups, indicating difficulty in prediction of the course at diagnosis. The reasons underlying these findings are unclear. FMF attacks almost disappeared, and patterns of

serum levels of acute-phase reactants, including IL-6, did not differ in the two groups. However, there were trends towards more frequent subtle symptoms such as abdominal discomfort in group B.

These results may suggest that sustained elevation of serum IL-18 reflects the presence of persistent subclinical inflammation due to dysregulated inflammasome activation. If this is the case, determination of serum IL-18 may allow discrimination of real remission from apparent improvement, and consequently allow provision of appropriate treatment based on monitoring of disease activity, which may lead to a significant clinical benefit for FMF patients with exon 10 mutations. However, this study cohort is small, and larger studies will be necessary to confirm our observations. Further investigations will be also required to assess the longitudinal course of serum IL-18 in FMF patients with different ethnic backgrounds, and those homozygous for exon 10 mutations or compound heterozygotes carrying two different exon 10 mutations.

In summary, our results support the importance of serum IL-18 as a disease activity marker and show that longitudinal examination of serum IL-18 may contribute to better follow-up of FMF patients with exon 10 mutations.

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Table 1. Patient characteristics.

	FMF patients				
	With exon 10 mutations				With exon 2, 3 or 5, but not exon 10 mutations
	Total	Group A	Group B	*p*-value	
	n = 12	n = 7	n = 5		n = 11
Sex (male/female)	5/7	3/4	2/3		
Age at onset (y)	15.4 ± 11.5	15.7 ± 15.3	15.0 ± 3.6	n.s.	26.5 ± 12.5
Age at diagnosis (y)	30.1 ± 16.6	33.4 ± 19.9	25.4 ± 10.7	n.s.	29.3 ± 14.0
Delay in diagnosis (y)	14.7 ± 9.3	17.7 ± 9.7	10.4 ± 7.6	n.s.	2.8 ± 3.8
Age at last visit (y)	34.3 ± 17.7	37.0 ± 21.7	30.6 ± 11.2	n.s.	31.5 ±13.2
Disease duration (y)	18.9 ± 10.7	21.3 ± 12.0	15.6 ± 8.8	n.s.	5.0 ± 3.4
Typical FMF attacks	12/12	7/7	5/5	n.s.*	2/11
Duration of fever attack (d)	1.7 ± 0.7	1.8 ± 0.7	1.6 ± 0.7	n.s.	3.3 ± 2.1
Colchicine treatment	12/12	7/7	5/5	n.s.*	6/11
Mean colchicine dose (mg/d)	1.0 ± 0.3	0.9 ± 0.3	1.1 ± 0.5	n.s.	0.9 ± 0.4
Typical attacks after treatment	0/12	0/7	0/5	n.s.*	1/2
Amyloidosis	2/12	1/7	1/5	n.s.*	0/11
Serum cytokines at diagnosis					
IL-6 (pg/mL)	3.5 ± 12.1	< 3	8.4 ±18.8	n.s.	< 3
IL-18 (pg/mL)	5,099 ± 6,084	5,547 ± 7,805	4,472 ± 3,091	n.s.	334 ± 181
Neopterin (nmol/L)	8.1 ± 9.7	7.2 ± 8.0	9.4 ± 12.7	n.s.	6.0 ± 3.6
MEFV mutations					
M694I/E148Q	7/12	3/7	4/5		
M694I/L110P/E148Q	4/12	3/7	1/5		
M694V/wild-type	1/12	1/7	0/5		
L110P/E148Q					4/11
L110P/E148Q/S503C					1/11
E148Q/R202Q					1/11
E148Q/P369S					1/11
E148Q/P369S/R408Q					1/11
R202Q/wild-type					1/11
P369S/ R408Q					1/11
S503C/wild-type					1/11

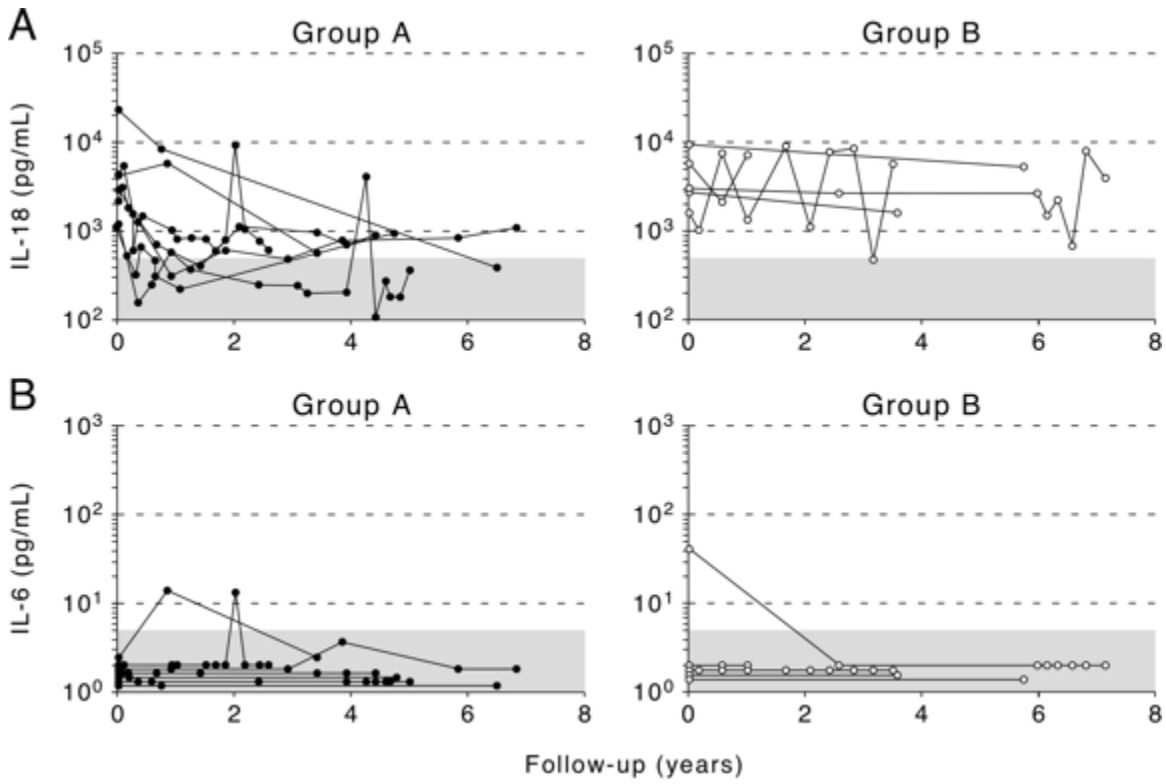
FMF, familial Mediterranean fever; IL, interleukin; n.s., not significant.
Data are presented as the mean ± SD for continuous variables. *P* values were obtained using an unpaired t test or *Fisher exact text.
Normal ranges of serum IL-6, IL-18, and neopterin are <5 pg/mL, <500 pg/mL, and 2-8 nmol/L, respectively.

Figure Legend

Figure 1. Longitudinal examination of interleukin (IL)-18 and IL-6 levels in patients with familial Mediterranean fever (FMF).

Serum concentrations of IL-18 (A) and IL-6 (B) were measured over time in FMF patients with exon 10 mutations after diagnosis. Patients showed progressive decline in serum IL-18 (group A), or continued elevation of serum IL-18 at >1,000 pg/mL (group B). Shaded areas represent the ranges of normal values.

Fig 1


A
Group A
Group B
IL-18 (pg/mL)
10^5
10^4
10^3
10^2
0
2
4
6
8
B
Group A
Group B
IL-6 (pg/mL)
10^3
10^2
10^1
10^0
Follow-up (years)