Serum interleukin 17 and interleukin 18 levels in familial Mediterranean fever

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ABSTRACT

Objective. *Familial Mediterranean fever (FMF) attacks are characterized by serosal inflammation rich in PMNL leukocytes and activation of a definite cytokine network. Moreover, there is sustained inflammation in attack-free FMF patients. Interleukin (IL)-17 and IL-18 are recently described proinflammatory cytokines, which can modulate certain neutrophil functions. In this study we measured serum levels of IL-17 and IL-18 in FMF patients.*

Methods. *The study groups comprised of 18 FMF patients in attack-free period (mean age: 30.2 ± 9.5 years; male/female: 10/8), and 18 patients with an acute FMF attack (mean age: 25.4 ± 4.9 years; male/female: 10/8). Twenty age-matched healthy subjects were included as a control group (male/female: 10/10). Levels of IL-17 and IL-18 were determined by commercial ELISA kits (Biosource International, USA).*

Results. *Serum IL-17 levels were 42.8 ± 3.7, 42.7 ± 3.2, and 39.9 ± 2.3 pg/mL for FMF patients in attack-free period, FMF patients with acute attack, and healthy controls, respectively. Serum IL-18 levels were 878.8 ± 315.0, 854.2 ± 261.4, and 314.6 ± 80.8 pg/mL for FMF patients in an attack-free period, FMF patients with acute attack, and healthy controls, respectively. Levels of both IL-17 and IL-18 were significantly higher in FMF patients with and without acute attack compared to control group ($p < 0.05$). Concentrations of those cytokines were comparable in FMF patients with acute attack and in attack-free period ($p > 0.05$).*

Conclusion. *Our data suggest that IL-17 and IL-18 contribute to the cytokine network in the inflammatory cascade of FMF. However, their roles for the initiation of FMF attacks remain to be established.*

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent acute attacks of fever and inflammatory reactions of serosal membranes. Several non-specific immunological abnormalities and elevations in acute phase reactant levels were observed during the FMF attacks. Neutrophils play a key role in the inflammatory processes during the attacks of FMF. Moreover, there is data demonstrating the presence of sustained inflammation in attack-free FMF patients as evidenced by elevated levels of certain proinflammatory cytokines (1-4). Interleukin (IL)-17 and IL-18 are recently described proinflammatory cytokines, which can modulate certain neutrophil functions (5-12). We conducted this study to measure serum levels of IL-17 and IL-18 in FMF patients with or without acute attacks in comparison to healthy controls.

Patients and methods

Patients

The study groups were comprised of 18 FMF patients in attack-free period (mean age: 30.2 ± 9.5 years; male/ female: 10/8), and 18 patients with acute FMF attack (mean age: 25.4 ± 4.9 years; male/female: 10/8). The diagnosis of FMF was established according to the Tell-Hashomer criteria. The disease durations were 13.2 ± 8.8 years and 8.7 ± 6.7 years for patients in attack-free periods and patients with acute FMF attack, respectively. Age and sex distributions and disease durations in both groups were similar. All patients were receiving colchicine during blood sampling. None of the patients was receiving any other drug that could influence cytokine levels. Twenty age-matched healthy subjects were included as a control group (male/ female: 10/10).

Table I. Serum interleukin (IL)-17 and IL-18 levels in attack-free familial Mediterranean fever (FMF) patients, in patients with acute FMF attack, and in healthy controls. Results are expressed as mean ± SD.

	Attack-free FMF patients	FMF patients with acute attack	Healthy controls
Sex (male/female)	10/8	10/8	10/10
Disease duration (years)	13.2 ± 8.8	8.7 ± 6.7	-
ESR (mm/hr) [1]	10.8 ± 6.4	60.7 ± 33.9	-
C-reactive protein (mg/L) [1]	0.4 ± 0.4	25.2 ± 41.9	-
Fibrinogen (mg/dL) [1]	312.4 ± 65.9	654.4 ± 153.0	-
IL-17 (pg/mL) [2,3]	42.8 ± 3.7	42.7 ± 3.2	39.9 ± 2.3
IL-18 (pg/mL) [2,3]	878.8 ± 315.0	854.2 ± 261.4	314.6 ± 80.8

[1] $p < 0.05$ for FMF patients with acute attack vs attack-free FMF patients
[2] $p < 0.05$ for FMF patients with acute attack vs healthy controls, and for attack-free FMF patients vs healthy controls
[3] $p > 0.05$ for FMF patients with acute attack vs attack-free FMF patients.

Methods

Serum samples were stored at -80°C until assayed. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fibrinogen levels were measured with standard methods. Levels of IL-17 and IL-18 were determined by commercial ELISA kits (Biosource International, USA).

Statistical analysis

Data were analyzed using a statistical software package (SPSS for Windows version 10.0). One-way analysis of variance (ANOVA) was used to compare the differences between serum IL-17 and IL-18 levels for the groups. Statistically significant differences obtained from one-way ANOVA analysis were further tested by Tukey test for post hoc pairwise comparisons. Correlation analyses were performed by Pearson's correlation analysis. A p value below 0.05 was considered as statistically significant.

Results

Acute phase reactants

Results were expressed as mean ± SD. Serum ESR were 10.8 ± 6.4 and 60.7 ± 3.9 mm/hr for FMF patients in attack-free period and FMF patients with acute attack ($p < 0.05$). Serum CRP levels were 0.4 ± 0.4 and 25.2 ± 4 1.9 mg/L for FMF patients in attack-free period and FMF patients with acute attack (normal range for CRP: 0-6) ($p < 0.05$). Serum fibrinogen levels were 312.4 ± 65.9 and 654.4 ± 153.0 mg/dL for FMF patients in attack-free period and FMF patients with acute attack (normal range for fibrinogen: 180-350) ($p < 0.05$) (Table I).

IL-17 levels

Results were expressed as mean±SD. Serum IL-17 levels were 42.8 ± 3.7, 42.7 ± 3.2, and 39.9 ± 2.3 pg/mL for FMF patients in attack-free period, FMF patients with acute attack, and healthy controls, respectively. IL-17 levels were significantly higher in FMF patients with and without acute attack compared to healthy controls ($p < 0.05$). Concentrations of those cytokines were comparable in FMF patients with acute attack and in attack-free period ($p > 0.05$) (Table I).

IL-18 levels

Results were expressed as mean ±SD. Serum IL-18 levels were 878.8 ± 315.0, 854.2 ± 261.4, and 314.6 ± 80.8 pg/mL for FMF patients in attack-free period, FMF patients with acute attack, and healthy controls, respectively. IL-18 levels were significantly higher in both subgroups of FMF patients compared to control group ($p < 0.05$), while IL-18 concentrations were comparable in FMF patients with acute attack and in attack-free FMF patients ($p > 0.05$) (Table I).

Correlation analyses

There was a significant correlation between IL-17 and IL-18 levels ($p < 0.01$, $r = 0.405$) (Fig. 1). However there was no correlation between IL-17 or IL-18 levels and ESR, CRP levels, or fibrinogen levels.

Discussion

In this study we demonstrated that FMF patients, both in attack-free periods and with acute attacks, had higher serum IL-17 and IL-18 levels than

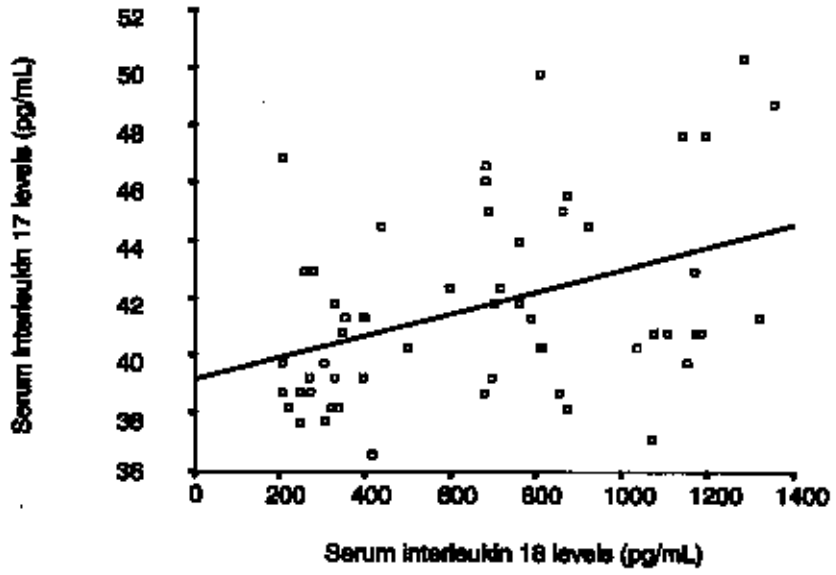

Fig. 1. Serum interleukin (IL)-17 levels plotted against serum IL-18 levels demonstrating a positive correlation between them.

healthy controls. The levels of those cytokines were comparable in FMF patients with acute attack and in attack-free period. Moreover, there was a positive correlation between serum IL-17 and IL-18 levels.

Neutrophils are the major cell population involved in acute inflammation of FMF, and FMF attacks are characterized by serosal inflammation rich in PMNL leukocytes. On the other hand, there is available data demonstrating the activation of a definite cytokine network in FMF (1-3). Moreover, attack-free periods of FMF is complicated by increased levels of certain inflammatory mediators, including tumor necrosis factor alpha (TNF-α, IL-6, IL-8, ICAM-1, and interferon gamma (IFN-γ). Therefore, a sustained inflammation in attack-free FMF patients is evident (1-4).

IL-17 and IL-18 are newly diagnosed cytokines implicated in the inflammatory responses. Both molecules have certain proinflammatory properties. IL-17 can stimulate a wide variety of cell types and induce secretion of other inflammatory effectors including TNF-α, IL-6, IL-8, IFN-γ, and chemokines. A prominent feature of IL-17 is its ability to cooperate either additively or synergistically with various inflammatory cytokines or agonists to enhance inflammation (5,6). Therefore, a primary function of IL-17 may be to amplify ongoing inflammatory responses. On the other hand, IL-18 has direct proinflammatory properties. It can stimulate activation of nuclear factor kappa B, and can induce production of proinflammatory mediators such as TNF-α, IL-8, and ICAM-1 (7-9). Moreover, both IL-17 and IL-18 can play important roles in neutrophil activation. IL-17, acting either directly or indirectly, stimulates neutrophil maturation, migration, and function. Overexpression of IL-17 results in massive peripheral neutrophilia associated with increased levels of granulocyte colony-stimulating factor (G-CSF) and enhanced granulopoiesis (10). IL-17 can recruit neutrophils into the peritoneal cavity by releasing neutrophil-specific chemokines from the peritoneal mesothelium (11). IL-18 also can promote neutrophil adhesion and migration, cytokine and chemokine production, granule release, and respiratory burst. IL-18 administration induced peritoneal neutrophil recruitment (12). In this study, we demonstrated that serum levels of both IL-17 and IL-18 were elevated in FMF patients, with a positive correlation between them. Our data suggest that IL-17 and IL-18 contribute to the ongoing inflammatory cascade of FMF. Their contribution to the pathogenesis of FMF might be by the induction of the synthesis of other proinflammatory cytokines, and/or modulation of certain PNL functions. However, the levels of both IL-17 and IL-18 were comparable in FMF patients with or without acute attacks, making their possible involvement in the initiation of acute attacks unlikely.

There was no correlation between IL-17 or IL-18 levels and acute phase reactants (*i.e.* ESR, CRP, and fibrinogen) in our study. FMF attacks are characterized by significantly higher concentrations of those acute phase reactants than in attack free periods (1-3). Likewise, ESR, CRP and fibrinogen levels were significantly higher in FMF patients with acute attack than FMF patients in attack-free period in our study. However, since IL-17 and IL-18 levels were comparable in patients with or without acute FMF attacks, a correlation between levels of those cytokines and acute phase reactants should not be expected.

Recent data indicate a Th1 mediated immune response in FMF patients. Levels of IFN-γ, a Th1 type cytokine, are elevated both in FMF patients in the attack-free periods and in FMF patients with acute attacks. Moreover levels of IFN-γ were higher during acute attacks compared to attack-free periods (4). IFN-γ production by lymphocytes was significantly increased in patients who carried MEFV mutations (13). Those observations suggest Th1 predominance in FMF. Although IL-17 does not obviously polarize to either the Th1 or Th2 lineages, results with these T cell lines and clones derived from RA synovium allowed the classification of IL-17 as a Th1 cytokine (14). On the other hand, IL-18 acts as a costimulant for production of IFN-γ and other Th1 cytokines in synergy with IL-12. Hence, there is a unique synergism between IL-18 and IL-12 in the induction of IFN-γ and the Th1 response, and IL-18 functions primarily as a costimulant for Th1 cytokine production (15, 16). Taken together, our study represents further evidence for the presence of Th1 mediated inflammatory response in FMF.

In conclusion, our data suggest that both IL-17 and IL-18 are involved in the cytokine network of the inflammatory cascade of FMF. However, due to the limitations of a cross-sectional study to highlight the overall dynamic process, further investigations with prospective follow-up of patients are needed to confirm our observations. Moreover, exact mechanisms by which IL-17 and IL-18 contribute to disease pathogenesis of FMF, and their roles for the initiation of FMF attacks remain to be established.

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