Levels of interleukin-6 (IL-6) and its soluble receptor (sIL-6R) in familial Mediterranean fever (FMF) patients and their first degree relatives

S. Öktem[1], T.U. Yavuzsen[2], B. Sengül[2], H. Akhunlar[3], S. Akar[2], M. Tunca[2]

Department of Rheumatology[1], Ege University School of Medicine; and Departments of Internal Medicine[2] and Biochemistry[3], Dokuz Eylül University School of Medicine, Izmir, Turkey.

Please address correspondence to: Selda Öktem, MD, Ege University School of Medicine, Department of Rheumatology, Bornova 35100, Izmir, Turkey.
E-mail: seldaoktem@yahoo.com

Received on June 19, 2003; accepted in revised form on January 22, 2004.

Clin Exp Rheumatol 2004; 22 (Suppl. 34): S34-S36.

Key words: Familial Mediterranean fever (FMF), IL-6, sIL-6R, FMF carriers.

ABSTRACT

Objective. *Familial Mediterranean Fever (FMF) is a hereditary disease characterized by recurrent inflammatory attacks. A subclinical inflammation may persist in periods between the attacks and heterozygotes may have higher than normal levels of acute phase proteins. We investigated the levels of interleukin-6 (IL-6) and its soluble receptor (sIL-6R) in FMF patients and their obligatory carrier relatives.*

Methods. *Serum levels of IL-6 and sIL-6R were measured during acute attacks (n = 18) and in attack-free FMF patients (n = 26), obligatory carriers of FMF (n = 17) and normal controls (n = 11).*

Results. *The median levels of IL-6 were significantly higher (45.71 pg/mL, p = 0.001) during acute attacks of FMF only, and were normal (0.01 pg/mL) in the other groups studied. There was no statistically significant difference in the median sIL-6R values between any of the groups (p = 0.22).*

Conclusion. *IL-6 was extremely elevated during FMF attacks but could not detect hypothetical "subclinical" inflammation during attack-free intervals or in the heterozygote relatives of patients. Serum levels of sIL-6R were comparable in all four groups.*

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent inflammatory attacks of the serosal membranes (1). The disease is caused by mutations in a gene named MEFV, which encodes a protein called pyrin or marenostrin (2, 3). This protein may play an important role in inflammatory pathways and in the regulation of apoptosis of neutrophils (4). The acute attacks are often accompanied by increased plasma concentrations of a group of acute phase proteins, including C-reactive protein (CRP), serum amyloid A (SAA) and fibrinogen, and elevations of these proteins are supportive criteria for the diagnosis of FMF (5). The acute phase proteins may remain somewhat elevated in certain patients during the attack-free intervals and they may also be above normal in heterozygous carriers of the disease (6-8). IL-6 is the chief stimulator of the production of most acute phase proteins and may play a major role in acute attacks of FMF (9-11). The soluble receptor of IL-6 (sIL-6R) is present in the circulation and inflammatory body fluids; it may play an independent role while enhancing the activity of IL-6 (12-14). We measured the serum levels of IL-6 and its soluble receptor (sIL-6R) during acute attacks and attack-free periods in FMF patients and their obligatory carrier (heterozygote) relatives.

Materials and methods

All of the patients fulfilled the diagnostic criteria for FMF (5) and were on regular colchicine treatment. Informed consent approved by the ethical committee of our institute was obtained from each patient.

Serum levels of IL-6 and sIL-6R were studied in the venous blood samples taken from 18 adult FMF patients during 27 individual acute attacks, and from 26 attack-free FMF patients, 17 obligatory heterozygote relatives (parents and siblings) and 11 healthy controls. Four patients were seen during more than one attack (5 separate attacks in 1 patient, 4 separate attacks in one patient and 2 separate attacks in 2 patients). Blood samples from FMF patients suffering acute attack were taken during the first 4-6 hours of the attack period. All venous blood samples, which were obtained from the antecubital vein, were transported in an ice

Table I. Age and sex distribution of the patient groups.

Groups	N	Mean age	(range)	Male/female
FMF patients during an attack	18	30.37	(21-46)	12/6
Attack-free FMF patients	26	32.69	(18-53)	10/16
Heterozygotes	17	38.12	(18-57)	9/8
Healthy controls	11	31.64	(25-52)	5/6

box and immediately centrifuged. Centrifuged samples were stored at -70ºC and thawed before analysis. The serum levels of IL-6 and sIL-6R were measured by photometric enzyme immunoassay methods (human IL-6 ELISA kit 1534475 and soluble IL-6R ELISA kit 1699334, Boehringer Mannheim).

Statistics

The distribution of the IL-6 and sIL-6R concentrations were expressed as median levels, and non-parametric tests (Kruskal-Wallis test) were performed for the statistical analyses. Median serum levels of IL-6 and sIL-6R were tested using the Mann Whitney U test for comparisons between groups. Pvalues < 0.05 were considered significant. The Statistical Package for Social Sciences (SPSS) 8.0 for Windows was utilized to analyze the data.

Results

There was no statistically significant difference in the age and sex distribution of the groups (p = 0.20 and 0.34, respectively) (Table I). The median and mean levels of IL-6 and sIL-6R (ranges shown in parentheses) of the four groups studied are summarized in Table II and Table IIII, respectively.

A significant difference between IL-6 levels was found among the four groups. Comparison of the results between groups by means of the Kruskal-Wallis test showed that the levels were significantly higher in acute attack patients (p = 0.001). Results of the statistical evaluation of the comparison of other groups were not significantly different (p>0.05).

Since 4 of the patients had been seen during more than one attack, the total number of blood samples taken was 27. Two of these 4 patients had one attack each with IL-6 levels measuring 0.01 pg/mL, while IL-6 levels during their other attacks were higher. One woman patient had 5 attacks; 5 samples were taken and IL-6 levels were found to be very high in all of these samples. The IL-6 level in the blood sample taken during from this patient during an attack-free period measured 0.01 pg/mL. Similarly, IL-6 levels measured in the other 3 attack patients during the attack-free period were found to be low. The levels of sIL-6R were found to be comparable in all groups (p = 0.22).

Discussion

There is a dramatic rise in acute phase response parameters such as leukocytes, CRP, SAA and fibrinogen during acute attacks of FMF. The process resolves spontaneously in about 24-48 hours and these parameters return to normal levels.

However, several investigations have disclosed an ongoing “subclinical” inflammation in FMF patients and carriers of FMF gene mutations may also have significantly higher than normal levels of serum CRP values (6-8). Although one retrospective study found no evidence of an increased prevalence of ischemic heart disease in colchicine-treated FMF patients (15), even low levels of chronic inflammation may enhance the development of atherosclerosis (16) and IL-6 is generally regarded to be a sensitive sign of inflammation and the increased risk of atherosclerosis in various patient groups (17-20). It was determined that IL-6 was significantly higher during attacks of FMF (10, 11), and normal (21) or higher than controls (22) in attack-free patients. This is the first study to investigate levels of IL-6 and sIL-6R in FMF and in obligatory carriers of the disease.

Serum levels of IL-6 were increased several thousand-fold in our patients during acute attacks, this difference being highly significant (p = 0.001). However, the IL-6 value was 0.01 pg/mL in 4 of the 27 separate measurements, thus seriously lowering the sensitivity of the test. The systemic inflammatory response can be induced by other cytokines, even when IL-6 is absent (23) and sIL-6R can induce the acute phase response alone by itself (20). Interestingly, IL-6 was shown to be insufficiently sensitive in acute appendicitis, a disease frequently mimicking FMF in clinical practice (24).

In our study, IL-6 and sIL-6R levels were found to be normal in attack-free FMF patients. One of our patients who was seen during separate attacks had extremely high IL-6 values on all occasions but it was normal during the attack-free interval. Only 2 attack-free patients had elevated IL-6 levels, but these were not statistically significant. Probably IL-6 is not sensitive in detecting “subclinical” inflammation, which itself has not yet resolved conclusively. In one study, IL-6 values were higher than normal initially but normalized with colchicine treatment at the end of 2 months (25). All of our patients had been on regular colchicine therapy for several years.

That MEFV carriers have an ongoing and relatively high acute phase response is an interesting and debatable issue. There are at least two contradictory observations in the medical literature (6, 26). We did not find any report on IL-6 and sIL-6R measurements in heterozygous carriers of MEFV. None of these two parameters of the acute

Table II. Median and mean levels of IL-6 (pg/mL) (p = 0.001).

Groups	Median	Mean ± SD	Min. – Max.
FMF patients during attack	45.71	89.94 ± 147.85	0.01 – 718.09
Attack-free FMF patients	0.01	0.55 ± 2.43	0.01 – 12.38
Heterozygotes	0.01	0.20 ± 0.60	0.01 – 2.38
Healthy controls	0.01	0.01 ± 0.00	0.01 – 0.01

Table III. Median and mean levels of sIL-6R (pg/mL) (p = 0.22).

Groups	Median	Mean ± SD	Min. – Max.
FMF patients during attacks	17.31	17.72 ± 6.27	6.78 / 29.83
Attack-free FMF patients	16.91	16.83 ± 6.04	6.47 / 30.16
Heterozygotes	19.60	20.26 ± 5.21	11.06 / 29.97
Healthy controls	19.84	20.48 ± 7.30	10.91 / 33.51

phase response could detect "subclinical" inflammation in our MEFV carriers. Whether this was due to the low sensitivity of IL-6 and its soluble receptor or not is highly speculative.

Why did the levels of sIL-6R remain normal during the acute attacks of FMF while IL-6 values were generally very high? One possible explanation would be that high values could not be detected because serum levels of sIL-6R increase and normalize quite rapidly (27). On the other hand, sIL-6R can be locally produced and act as a paracrine mediator (11,28). This aspect of sIL-6R activity has not been thoroughly investigated. If sIL-6R is preferentially expressed in serosal membranes, coupled with IL-6, it may play a crucial role in the evolution of the clinical picture of FMF.

In conclusion, IL-6 was frequently but not consistently elevated during FMF attacks and could not detect "subclinical" inflammation during the attack-free intervals. In addition to its relatively high cost, this cytokine is not a very sensitive parameter. The clinical implications of the normal serum levels of sIL-6R in all four groups need further investigation.

References

1. LIVNEH A, LANGEVITZ P: Diagnostic and treatment concerns in familial Mediterranean fever. *Baillière's Best Pract Res Clin Rheumatol* 2000; 14: 477-98.
2. INTERNATIONAL FMF CONSORTIUM: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* 1997; 90: 797-807.
3. FRENCH FMF CONSORTIUM: A candidate gene for familial Mediterranean fever. *Nature Genet* 1997; 17: 25-31.
4. GUMUCHIO DL, DIAZ A, SCHANER P *et al*.: Fire and ICE: The role of pyrin domain-containing proteins in inflammation and apoptosis. *Clin Exp Rheumatol* 2002; 20 (Suppl. 26): S45-S53
5. LIVNEH A, LANGEVITZ P, ZEMER D *et al*.: Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum* 1997; 40: 1879-85.
6. TUNCA M, KIRKALI G, SOYTÜRK M, AKAR S, PEPYS MB, HAWKINS PN: Acute phase response and evolution of familial Mediterranean fever. *Lancet* 1999; 352: 1415
7. KORKMAZ C, ÖZDOGAN H, KASAPÇOPUR Ö, YAZICI H: Acute phase response in familial Mediterranean fever. *Ann Rheum Dis* 2002; 61: 79-81.
8. NOTARNICOLA C, DIDELOT MN, SEGURET F, DEMAILLE J, TOUITOU I: Enhanced cytokine mRNAlevels in attack-free patients with familial Mediterranean fever. *Genes Immun* 2002; 3: 43-5.
9. HIRANO T: Interleukin 6 and its receptor: ten years later. *Int Rev Immunol* 1998; 16: 249-84.
10. GANG N, DRENTH JP, LANGEVITZ P *et al*.: Activation of the cytokine network in familial Mediterranean fever. *J Rheumatol* 1999; 26: 890-7.
11. BAYKAL Y, SAGLAM K, YILMAZ MI, TASLIPINAR A, AKINCI SB, INAL A: Serum sIL-2r, IL-6, IL-10 and TNF-alpha level in familial Mediterranean fever patients. *Clin Rheumatol* 2003; 22: 99-101.
12. JONES SA, HORIUCHI S, TOPLEY N, YAMAMOTO N, FULLER GM: The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB J* 2001; 15: 43-58.
13. GABAY C, SILACCI P, GENIN B, MENTHA G, LE COULTRE C, GUERNA PA: Soluble IL-6 receptor strongly increases the production of acute phase protein by hepatoma cells but exerts minimal changes on human primary hepatocytes. *Eur J Immunol* 1995; 25: 2378-83.
14. ROMANO M, SIRONI M, TONIATI C *et al*.: Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* 1997; 6: 315-25.
15. LANGEVITZ P, LIVNEH A, NEUMANN L *et al*.: Prevalence of ischemic heart disease in patients with familial Mediterranean fever. *Isr Med Assoc J* 2001; 3: 9-12.
16. LIBBY P, RIDKER PM, MASERI A: Inflammation and atherosclerosis. *Circulation* 2002; 105: 1135-43.
17. IKEDA U, ITO T, SHIMADA K: Interleukin-6 and acute coronary syndrome. *Clin Cardiol* 2001; 24: 701-4.
18. BERMUDEZ EA, RIFAI N, BURING J, MANSON JE, RIDKER PM: Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. *Arterioscler Thromb Vasc Biol* 2002; 22: 1668-73.
19. STENVINKEL P, HEIMBURGER O, JOGESTRAND T: Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dialysis patients: association with Chlamydia pneumoniae seropositivity. *Am J Kidney Dis* 2002; 39: 274-82.
20. SCHÖBITZ B, PEZESHKI G, POHL T *et al*.: Soluble interleukin-6 receptor augments central effects of IL-6 *in vivo*. *FASEB J* 1995; 9: 659-64.
21. MEGE JL, DILSEN N, SANGUEDOLCE V *et al*.: Overproduction of monocyte derived tumor necrosis factor α, IL-6, IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. *J Rheumatol* 1993; 20: 1544-9.
22. DRENTH JP, VAN DER MEER JW, 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: 400-4.
23. FATTORI E, CAPPELTTI M, COSTA P *et al*.: Defective inflammatory response in interleukin-6 deficient mice. *J Exp Med* 1994; 180: 1243-50.
24. GÜRLEYIK G, GÜRLEYIK E, ÇETINKAYA F, ÜNALMISER S: Serum interleukin-6 measurement in the diagnosis of acute appendicitis. *ANZ J Surg* 2002; 72: 665-7.
25. KIRAZ S, ERTENLI I, ARICI M *et al*.: Effects of colchicine on inflammatory cytokines and selectins in familial Mediterranean fever. *Clin Exp Rheumatol* 1998; 16: 721-4.
26. POLAND DC, DRENTH JP, RABINOVITZ E *et al*.: Specific glycosylation of alpha(1)-acid glycoprotein characterises patients with familial Mediterranean fever and obligatory carriers of MEFV. *Ann Rheum Dis* 2001; 60: 777-80.
27. GEISTERFER M, RICHARDS C, BAUMANN M, FEY G, GYWNNE D, GAULDIE J: Regulation of the interleukin-6 and the hepatic interleukin-6 receptor in acute inflammation *in vivo*. *Cytokine* 1993; 5: 1-7.
28. PETERS M, ODENTHAL M, SCHIRMACHER P *et al*.: Soluble IL-6 receptor leads to a paracrine modulation of the IL-6 induced hepatic acute phase response in double transgenic mice. *J Immunol* 1997; 159: 1474-81.