

Phagocytic Activity in Familial Mediterranean Fever

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Abstract

Familial Mediterranean fever (FMF) is an autosomal recessive disease. Although the possibility of multiple immunologic mechanisms have been studied, the actual mechanism is still unresolved. Forty-one patients with FMF (24 males and 17 females with a mean age and disease duration of 17.8 ± 4.1 and 4.7 ± 2.3 years, respectively) and 14 healthy controls (10 males and 4 females with a mean age 23.2 ± 5.1) were involved in the study. A phagotest was studied in both the patients and control groups with a FACScalibur Flow. All patients were in the acute stages of the disease and had not undergone colchicine treatment for 2 months. The percentage blood phagocytic activity of both granulocytes and monocytes were 84.23 ± 8.76 and 67.28 ± 10.15 in the patient group and 94.68 ± 3.24 and 76.23 ± 5.7 in the control group, respectively. There was no statistically significant difference in the percentage of phagocytic activity of the granulocytes and monocytes between the FMF patients and healthy controls ($p > 0.05$ and $p > 0.05$, respectively).

Key Words: FMF, phagotest, granulocyte, monocyte

INTRODUCTION

Familial Mediterranean fever (FMF) is a familial disease characterized by recurrent episodes of febrile serositis, peritonitis, arthritis and pleuritis. It appears to be transmitted by a recessive gene. The most striking feature of FMF is that it characteristically affects certain ethnic groups.¹⁻³ The differences in disease expression are probably due to both allelic heterogeneity and/or modifier genes as well as environmental factors.

Although the immunologic, serologic and metabolic factors have been studied,^{4,5} the pathogenesis of the disease is unknown. Many studies have attempted to understand the basis for the inflammatory attacks in FMF, but a single or multiple of factors capable of inducing these attacks has not been identified. Studies of the immunologic function have disclosed only nonspecific changes that are consistent with an

acute inflammatory reaction.⁶ Several studies have reported the role of the abnormal functions of polymorphonuclear (PMN) leukocytes in regards to the pathogenesis of various diseases.⁷

The main objective of this study is to investigate blood PMN leukocytes and monocytes and the % phagocytic activity in patients with FMF during attacks.

MATERIALS AND METHODS

Forty-one patients (24 male and 17 female with a mean age and disease duration of 17.8 ± 4.1 and 4.7 ± 2.3 years, respectively) at SSK Ankara İhtisas Hospital who were fulfilled the diagnostic criteria for FMF as described by Avi Livneh and colleagues were investigated.⁸ The patient demographics are shown in Table 1. The patients had not received colchicine treatments for at least for 2 months prior to this study. Amyloidosis was found in 10 patients and as these patients were in moderate renal failure, they were given 4 gr/day proteiuria. All patients were hospitalized during acute attacks at the time of study. Fourteen healthy volunteers (10 males, 4 females, mean age; 23.21 ± 5.13) served as controls. Five ml of venous blood was drawn from all of the patients and controls into heparinized tubes for the test. A

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Table 1. Demographics of the Patient Population

Male/Female	24/17
Mean age (yr)	17.8 ± 4.1
Mean disease duration (yr)	4.72 ± 2.31
Familial history	15/41
Peritonitis	41/41
Pleuritis (unilateral)	11/41
Arthritis	18/41
Fever	41/41
Splenomegaly	7/41
Vomiting	16/41
Erythema	8/41
Amyloidosis	10/41

phagotest was studied in both the patient and control groups using FACScalibur Flow Cytometry with Phagotest kit from the Oregan Pharma Company. This test kit allows the quantitative determination of leukocyte phagocytosis of both human whole blood and snovial fluid. The Phagotest kit contains fluoresceinisoithiocyanate (FITC) labeled opsonized bacteria (E. Coli-FITC) and other necessary reagents. It measures the overall blood percentage of monocytes and granulocytes showing phagocytosis in general (ingestion of one or more bacteria per cell) and individual cellular phagocytic activity (number of bacteria per cell). In our study, the overall blood percentage of monocytes and granulocytes were evaluated showing the ingestion of one or more bacteria per cell. Statistical evaluation was done with Student-t test.

RESULTS

All patients were studied during their acute attacks while they were not under treatment. The mean blood leukocyte number was 12543 ± 1662 and 6250 ± 545 in the patient and control group, respectively. In the patient group, the mean erythrocyte sedimentation rate was 57.17 ± 14.65 mm/h and the serum C reactive protein level was 19.37 ± 6.27 mg/L (N: 0–5 mg/L). The serum IgD levels were not high in any of the patients. The blood percentage phagocytic activity of granulocytes and monocytes in the patient group and healthy controls 84.23 ± 8.76 and 67.28 ± 10.15 , and 94.68 ± 3.24 and 76.23 ± 5.7 respectively (Table 2, 3). According to the results,

Table 2. The Blood Percentage Phagocytic Activity of Granulocytes and Monocytes of Patients with FMF and Healthy Controls

	% phagocytic activity of granulocytes	% phagocytic activity of monocytes
The patients (n=41)	84.23 ± 8.76	67.28 ± 10.15
Healthy controls (n=14)	94.68 ± 3.24	76.23 ± 5.70

*No difference between the groups.

Table 3. The Blood Percentage Phagocytic Activity of Granulocytes and Monocytes FMF Patients with Amyloidosis and the Other Patients

	% phagocytic activity of granulocytes	% phagocytic activity of monocytes
The patients with amyloidosis (n=10)	78.42 ± 5.25	48.58 ± 3.65
The other patients (n=31)	89.24 ± 5.02	72.39 ± 3.22

*Significant difference between the groups ($p < 0.05$ and $p < 0.01$, respectively).

there was no statistical differences between the patient and control groups (in all $p > 0.05$ respectively). Ten patients were diagnosed with amiloidosis using a renal biopsy. The blood percentage phagocytic activity of the granulocytes and monocytes (78.42 ± 5.25 and 48.58 ± 3.65) in these patients were significantly low compared to both the control and other patients ($p < 0.05$ and $p < 0.01$ respectively).

DISCUSSION

The aetiology and pathogenesis of FMF are as yet unknown. The success of colchicine in preventing or ameliorating the course of the disease has provided new research directions into the disease pathogenesis. One of these directions is to investigate any pathological events in the defence cells in FMF patients. Colchicine treatment prevents attacks in most of patients but its mechanism is not clear. Most researchers have suggested that colchicine inhibits the lysosomal degranulation of neutrophils⁹ and impairs

chemotaxis but this is not a random movement.¹⁰ Bar-Eli et al.¹¹ suggested that the increased lysozyme release from the neutrophils may be of importance in FMF pathogenesis. However, many studies have shown that colchicine generally does not reduce the rate of phagocytosis by neutrophils¹²⁻¹⁵ and that the neutrophil function has generally been found to be normal in patients with FMF.¹⁶

Pathogen microorganisms activate PMN leukocytes chemotaxis directly or by humoral factors. Granulocyte and monocytes are important elements in febrile inflammatory diseases. Therefore the cells are influential in inflammation and their contents are capable of provoking acute inflammatory reactions and tissue injury.^{17,18} C5a, produced with the effects of bacterial endotoxines, increase the chemotaxis of PMN leukocytes with endothelial originated leucotriene and IL-1. The deficiency of C5a inhibitors surely has an effect on this event. The C5a fragment is recognized as a major inflammatory mediator of which its presence in tissues provokes a full-scale inflammatory response. Recent reports showed an abnormal response of polymorphs and monocytes in patients with FMF.^{5,11,16,19,20} Menashe et al.⁵ reported that during the attacks in untreated FMF patients, PMN leukocytes chemotaxis were increased and this affected the FMF inflammatory processes. In patients with FMF, in two studies have shown that during attacks, the chemotaxis of PMN leukocytes was elevated in 5 patients and normal in 20. In one study, it has been reported that the spontaneous O₂ production by neutrophils from active FMF patients was increased and that this increase was positively correlated with the clastogenic effects exerted by the patients' plasma (15 patients). Bar-Eli et al.²¹ reported that monocytes derived from the peripheral blood of patients with FMF demonstrated a lower phagocytic capacity for *Shigella flexneri* and *S. Albus* and this function was unaltered by colchicine.

In our study, there was no difference in phagocytic function of monocytes and granulocytes between the patients during attacks and the controls. These patients had not undergone colchicine treatment for 2 months. Quie et al reported that the neutrophile functions were decreased in patients with chronic renal failure.⁷ Moreover, in our patients with amyloidosis and who were suffering from moderate renal failure, the percentage of phagocytic activity, and the value of granulocytes and monocytes were signifi-

cantly low compared to controls and the other patient group. This may be related to impaired leukocyte chemotaxis or phagocytosis function. Many studies have found that in renal failure, leukocyte chemotaxis is impaired.^{7,22,23} Impairment of this function may be associated with a decrease in the intracellular cyclic GMP/cyclic AMP ratio or plasma factor blocking granulocyte membrane receptors.^{22,24} Davidson et al reported a depressed phagocytic activity and respiratory burst in patients with renal failure.²⁵ Furthermore, it has been known for many years that patients with renal failure have an acquired immunodeficiency characterized by abnormal T cell proliferation in response to antigenic challenges. This defect may be related to a monocytes dysfunction as T cell activation is monocyte dependent.

The gene responsible FMF, called MEFV, was found in 1992.²⁶ It encodes a protein that may be a neutrophil-specific transcription factor. Although there has been much research, the role of an abnormal neutrophil in the etiology of FMF remains speculative. In this study, we found a normal blood percentage of the phagocytic activity of the granulocytes and monocytes in patients with FMF compared with healthy controls. In the future, the pathogenesis of the disease may be explained from studies involving leukocytes.

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