THE NEW ARMENIAN MEDICAL JOURNAL
Vol.4 (2010), N 4 , p. 25-34

NEUTROPHIL F-ACTIN OSCILLATIONS AND IMPAIRMENT OF CHEMOATTRACTANT RECEPTOR DESENSITIZATION IN FAMILIAL MEDITERRANEAN FEVER

Avetisyan S.A.[1], Davtyan T.K.[2], Hakobyan G.S.[3], Ghazaryan D.M.[1], Petrosyan L.J.[1]

[1] *Department of Pathophysiology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia*
[2] *Laboratory of Immunology and Virology, "Armenicum" Research Centre CJSC, Yerevan, Armenia*
[3] *Department of Internal Medicine, Yerevan State Medical University after M. Heratsi, Yerevan , Armenia*

Abstract

Objective: *To examine neutrophil actin cytoskeleton oscillations and chemoattractant receptor desensitization in patients with Familial Mediterranean Fever (FMF), in an effort to understand the mechanisms that regulate switch of neutrophil activation program and therapeutic effect of colchicine (Col).*

Methods: *Whole blood neutrophils obtained from 28 FMF patients and 15 normal donors (ND) were activated with N-formyl-Met-Leu-Phe (fMLP), phorbolmyristate acetate (PMA) or lipopolysaccharide (LPS), and cellular F-actin content determined by flow cytometry before and after exposure to Col. F-actin oscillation amplitude and period were calculated from the curves generated by mathematical simulation giving the assumption that in neutrophils F-actin oscillates about a fixed point in a harmonic motion.*

Results: *Unstimulated neutrophil F-actin content was markedly increased in FMF patients which, unlike in ND, was significantly reduced by Col. fMLP- but not PMA- or LPS-stimulated and Col-pretreated neutrophils were characterized by different pattern of F-actin dynamics and delayed time period of F-actin oscillation during FMF. Neutrophils from FMF patients failed to induce chemoattractant receptor desensitization during repeated action of fMLP, while in ND it occurred with significant reduction of F-actin oscillation amplitude and period. Microtubule-dissolution by Col or cytoskeleton-disruption by cytochalasin B caused distinctive unequal pattern of fMLP-induced F-actin oscillations in FMF patients and ND.*

Conclusion: *Impaired neutrophil F-actin cytoskeleton oscillations amplitude and frequency that tightly regulate switch of neutrophil activation program during its encounter with increasing concentration of chemoattractants might be a potential pathogenic mechanism causing aberrant resolution of inflammation and could represent a potential target for colchicine therapy during FMF.*

Keywords: *Familial Mediterranean Fever (FMF), neutrophil, lipopolysaccharide (LPS), N-formyl-Met-Leu-Phe (fMLP), F-actin, phorbolmyristate acetate (PMA)*

INTRODUCTION

Despite the evident progress in investigating Familial Mediterranean Fever (FMF) pathogenesis, the causes of periodicity and self-limited nature of its attacks remain largely unknown. It can be assumed that there are counter balancing systems in inflammatory effectors activation programs, which play a role in the rhythmicity of the disease.

Although much has been learned about the periodicity of FMF clinical sings, the molecular mechanisms of oscillations in cellular pro-inflammatory activation and inflammation resolution during FMF are only beginning to be unraveled [*Davtyan T. et al., 2006 a;b*]. We hypothesized that periodicity and self-limited nature of FMF attacks could be a result of oscillations in activation-deactivation programs shift of neutrophils,

Address for Correspondence:
Yerevan State Medical University after M. Heratsi
2 Koryun Street, 0025 Yerevan, Armenia
Tel.: (+374 10) 587 -527
E-mail: samvelarcruni@yahoo.com

which are an important cellular participants in FMF attack [*Orbach H., Ben-Chetrit E., 2001; Korkmaz C. et al., 2002*]. One of their main properties is a rapid migration in response to gradients of chemoattractants, soluble molecules serving as "danger signal" [*Matsukawa A. et al., 2000*]. Neutrophils crawl through tissues in an amoeboid fashion and undergo regular cycles of actin polymerization and depolymerization [*Stossel T., 1993; Stark J. et al., 2007*]. Actin is an adenine nucleotide-binding protein, and the frequency of shape oscillations mirrors cellular activation, with a period of a few minutes for resting neutrophils to about 10 *s* for activated neutrophils [*Ehrengruber M. et al., 1996*]. The amplitude and frequency of these oscillations can be modulated by a presence of both exogenous ligands and endogenous cytokines in neutrophils leading to priming or desensitization [*Adachi Y. et al., 1999*]. According to the dynamic view of neutrophil activation, their capacity to switch from an activated state to state of anergy may cause resolution of inflammation without new activated neutrophils recruitment into the inflammatory burden [*Serhan C., Savil J., 2005*]. Therefore, the impaired actin cytoskeleton dynamics that tightly regulate switch of neutrophil activation program during encounter with increased concentrations of chemoattractant may be a potential pathogenic mechanism, causing aberrant resolution of inflammation and could represent a potential target for colchicine-based therapy during FMF.

To address this hypothesis we investigated neutrophil F-actin oscillations and actin cytoskeleton dynamics during activation-deactivation programs shift of neutrophils in FMF patients using whole blood cell culture technique that preserves native microenvironment, reduces the risk of pre-activation of neutrophils [*Davtyan T. et al., 2008 a; b*].

PATIENTS AND METHODS

Patients and blood samples: Peripheral blood samples were obtained from 28 (17 male, 11 female, aged 18-41 years) attack-free patients with FMF, diagnosed according to the Tel-Hasomer criteria [*Livneh A. et al., 1997*]. *MEFV* mutations were identified in all patients (14 patients were homozygous for the M694V mutation, 14 remaining patients were compound heterozygous for the M694V and one of the V724A, M680I, E148Q, R761H and F749L mutations).

The following selection criteria were applied to the patients enrolled in the study:

1. age > 16 years,
2. onset of FMF attacks in early childhood,
3. absence of chronic diseases such as chronic renal failure, renal amyloidosis, diabetes mellitus, ischemic heart disease, malignancy, trauma, infections and rheumatic disease,
4. treatment naïve and no drug administration within 4 weeks before blood drawing.

The informed consent of all patients was obtained for their inclusion in this study. Heparinised peripheral blood from 15 sex- and age-matched normal donors (NDs) (9 male and 6 female) was provided by "Viola Company" (Yerevan, Armenia) blood bank. No significant differences existed between FMF and NDs with respect to mean levels of erythrocyte sedimentation rate (ESR) and white blood cell (WBC) counts.

Intracellular F-actin staining of neutrophils: The quantitative flow cytometry determination of intracellular F-actin content [*Advani A. et al., 2004*] was performed, using whole blood samples obtained from NDs and FMF patients. Whole blood samples (with total volume 100 μl) were incubated with 20 *ng/mL* phorbol 12-myristate 13-acetate (PMA) or 10^{-7} *M* N-formyl-Met-Leu-Phe (fMLP) or 1 $\mu g/mL$ lipopolysaccharide (LPS) from E. *coli 026:B6* or either 0.1-10 $\mu g/mL$ colchicine (Col) or cytochalasin B (CyB). All reagents were obtained from Sigma Chemical (St. Louis, Mo., USA). After adding reagents to 10 μl final volume the whole blood samples were incubated at 37°C in horizontal shaking water bath for 1-10 *min*. The reaction was stopped at the appropriate time by addition of BD FACS™ erythrocyte lysing solution and the remaining cells fixation with 1.5% paraformaldehyde for 15 *min* at room temperature. The cells were then washed 3 times in PBS, permeabilized with 0.2% Triton X-100 containing 2% paraformaldehyde for 30 *min* and stained with 3 μM fluorescein isothio-

cyanate (FITC)-conjugated phalloidin (Sigma Chemical., St. Louis, Mo., USA) for an additional 45 *min* at room temperature. The cells were washed in PBS, and the average F-actin content of the FSC-SSC and $CD11b^+$ (a protein present intracellularly, following permeabilization) gated neutrophil population was monitored by determining the phalloidin relative fluorescence intensities (expressed as a mean channel number - MCN) on FACSCalibur™ instrument using CellQuest™ software (Becton Dickinson). Upon studying the effect of Col on neutrophil F-actin dynamics 1.5 *mL* whole blood samples were incubated with 10 *μg/mL* Col for 2 hours at 37˚C and neutrophils counted using haematological analyser (Celly v 2.20, Hycel Diagnostics), in order to exclude Col cytotoxicity.

Neutrophils chemoattractants receptor desensitization: We monitored chemoattractants receptor homologous desensitization by measuring the intracellular F-actin dynamics in whole blood neutrophils after preincubation in the presence or absence of fMLP [*Orbach H., Ben-Chetrit E., 2001*]. Neutrophils in 100 *μl* of whole blood were incubated for 10 *min* at 37°C with or without 10^{-7} *M* fMLP and then equal concentrations of chemoattractant added to blood samples. Then blood samples were incubated for additional 1-10 *min*. The reaction was stopped at the appropriate time and the intracellular F-actin dynamics measured as described above.

Statistical analysis: Method of dispersion analysis with parametric and non-parametric procedures was used in this study. Results of independent experiments were used to calculate mean values ± SEM, and differences were defined as statistically significant by Student's t-test (P_t), paired t-test (P_p), Wilcoxon-Mann-Whitney, and Welch's test (P_W) at $P \leq 0.05$.

Oscillation curves describing the time-response/dependence of F-actin intracellular content were generated from calculated mean values ± SEM data for F-actin content using the equation (1):

$$Y = B + A \times \sin(F \times t + P) \quad (1)$$

where Y represents a relative F-actin content expressed as MCN; B is a base line; A is an amplitude; F is a frequency and P is a phase shift of oscillation and *t* is time in minutes. A and F values

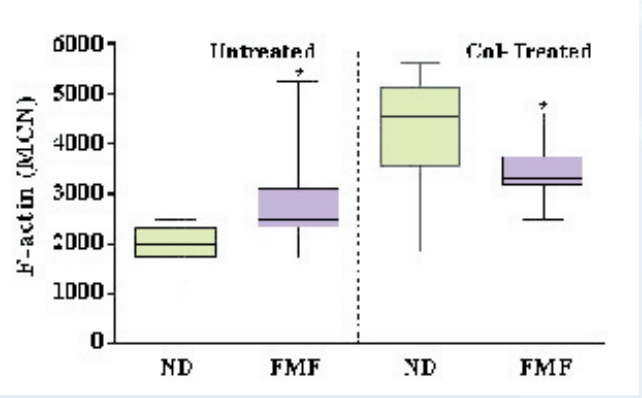

Figure 1. *Neutrophil F-actin content is increased in patients with FMF. Untreated and Col (10 μg/ml for 2 h) pretreated whole blood samples from ND (n=15) and FMF (n=28) patients were intracellulary stained for F-actin and FSC-SSC and $CD11b^+$ gated neutrophils were assayed by flow cytometry and expressed as a mean channel number (MCN). All data represent means ±SEM (error bars) and are significantly different at $P_w \leq 0.05$(*), comparing FMF with ND.*

were calculated using Graph Pad Prism v4.01 software and periods of oscillations (T) were calculated by equation (2) giving assumption that F-actin oscillates about a fixed point in a harmonic motion [*Stark J. et al., 2007*]:

$$T = 2\pi/F \quad (2).$$

RESULTS

Different pattern of activated neutrophil F-actin dynamics in FMF and ND: First, we studied the F-actin content in unstimulated neutrophils and activation-dependent F-actin dynamics in the presence or absence of Col. We observed that unstimulated neutrophils F-actin content in FMF patients was significantly higher than in ND (Figure 1). Pretreatment of whole blood with 10 *μg/mL* Col for 2 *h* increased neutrophil F-actin content both in FMF ($P_W<0.007$) and ND ($P_t<0.0001$). However, Col-pretreated neutrophils F-actin content in FMF patients was found to be significantly lower than in ND (Figure 1).

F-actin content in fMLP-stimulated ND neutrophils reached its maximal value during the first and third minutes and declined during the second and fifth minutes, whereas in FMF patient neutrophils it reached its maximal value during the first and second minutes and gradually declined during the third to fifth minutes (Figure 2). Col-pretreat-

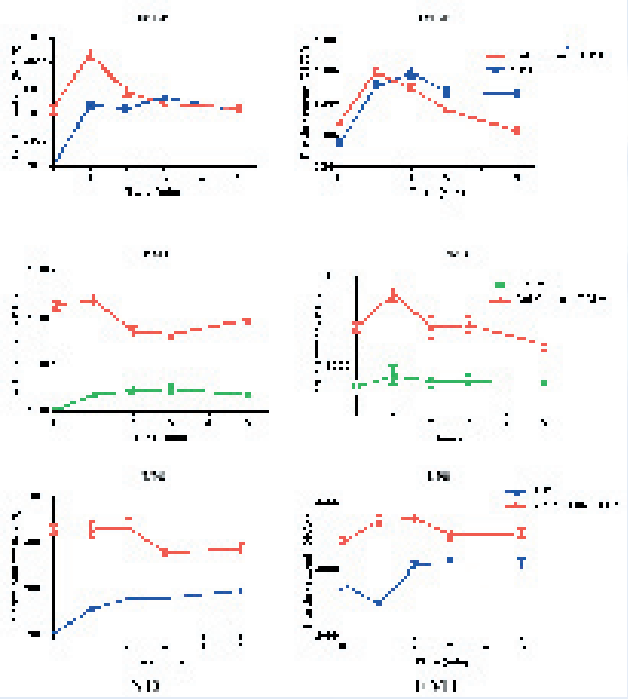

Figure 2. *Different pattern of activated neutrophil F-actin dynamics in FMF and ND. Untreated and Col (10 μg/ml for 2 h) pretreated whole blood samples from ND (n=15) and FMF (n=28) were incubated in the presence of fMLP, PMA or LPS for 1-10 min as indicated and F-actin content of FSC-SSC and CD11b⁺ gated neutrophils were assayed by flow cytometry and expressed as MCN.*

ment caused up-regulation of F-actin content in fMLP-stimulated neutrophils (P_t=0.01) during the first minute and gradual decline to the levels of Col-untreated cells during the second to fifth minute in ND. In FMF patients Col-treatment caused weak increase of F-actin content in fMLP-stimulated neutrophils (Pp=0.04) during the first minute and dramatic decline during the second to fifth minutes. In contrast to fMLP, LPS- and PMA-activation-dependent neutrophil F-actin dynamics either in the presence or absence of Col showed nearly similar pattern in both FMF patients and ND (Figure 2). One exception was observed in LPS-induced neutrophil F-actin dynamics that had undulating pattern in FMF patients, which contrasts with the linear pattern in ND. Col-pretreatment caused up-regulation of F-actin content in LPS- and PMA-stimulated neutrophils during the whole incubation period in both FMF patients and ND. Thus, fMLP-stimulated and Col-pretreated neutrophils were characterized by different patterns of F-actin dynamics and delayed shifts of maximums during FMF.

Activated neutrophil F-actin oscillations in FMF patients and ND: The existence of several shifted maximums in F-actin dynamics of ND and FMF patients activated neutrophils lead us to test, if this is due to different patterns of actin cytoskeleton polymerization and depolymerization regular cycles. To test it we assumed that in neutrophils F-actin oscillates about a fixed point in harmonic motion. Mathematical simulation for measuring its amplitude and periods was used. fMLP-induced neutrophil F-actin oscillation curves measured for FMF patients and ND using the equation (1) showed differences in baseline, but not in amplitude and frequency of F-actin oscillations (Figure 3).

The period of fMLP-induced neutrophil F-actin oscillation calculated using the equation (2) for FMF patients was 4.08 ± 0.46 *min* and 4.09 ± 0.18 *min* for ND. As expected, the period of F-actin oscillation in Col-pretreated neutrophils activated by fMLP increased by 1.31 *min* in FMF patients (5.39 ± 0.13) and by 1.1 *min* in ND (5.19 ± 0.21). In contrast to fMLP, LPS- or PMA-activated neutrophils F-actin dynamics were not characterized by undulating pattern and had linear time-dependence in ND (Figure 3). However, in FMF patients' neutro-

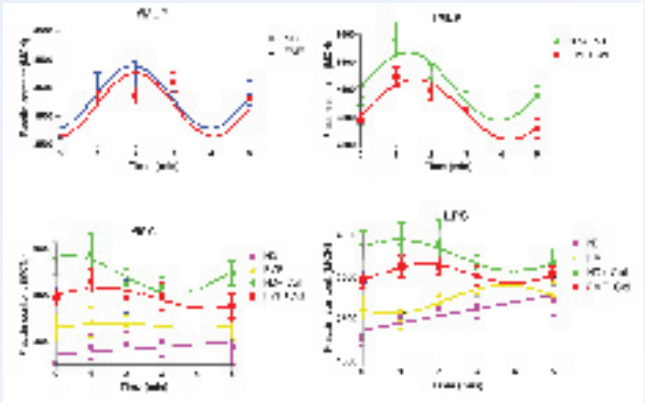

Figure 3. *Activated neutrophil F-actin oscillations in FMF and ND. Untreated and Col (10 μg/mL for 2 h) pretreated whole blood samples from ND (n=15) and FMF (n=28) were incubated in the presence of fMLP, PMA or LPS for 1-10 min as indicated and F-actin content of FSC-SSC and CD11b⁺ gated neutrophils was assayed by flow cytometry and expressed as MCN. Oscillation curves were generated from calculated mean values ±SEM data for F-actin content using the equation (1) and Graph Pad Prism v4.01 software and periods of oscillations were calculated by equation (2) giving assumption that F-actin oscillates about a fixed point in a harmonic motion.*

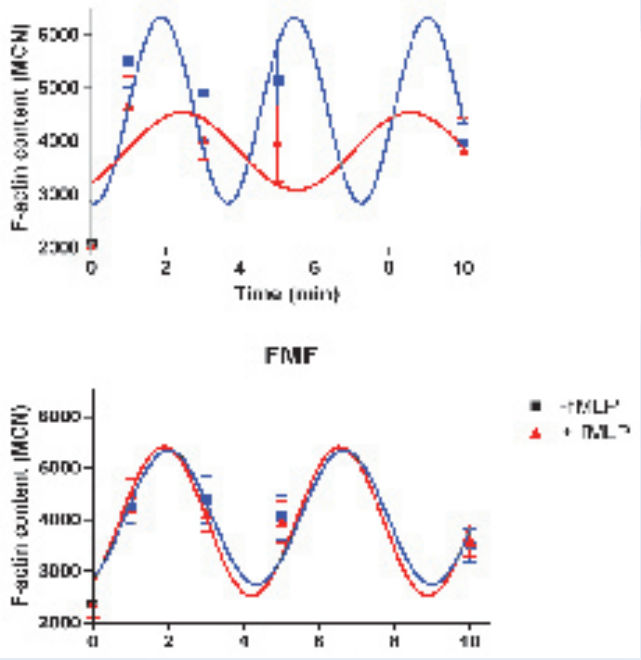

Figure 4. *Neutrophil F-actin oscillations during chemoattractant receptor desensitization in FMF and ND. Neutrophils from ND (n=15) and FMF (n=28) in 100 μL of whole blood incubated for 10 minutes with (+) or without (-) fMLP and then equal concentration of this chemoattractant added to blood samples and incubated for additional 1-10 min. The reaction stopped at the appropriate time and the intracellular F-actin dynamics was measured as described in Patients and Methods. Oscillation curves were generated from calculated mean values ±SEM data for F-actin content using the equations (1) and (2).*

phils, activated by PMA F-actin dynamics had undulating shape with low amplitude (54.9 ± 22.2 MCN) and 4.68 ± 0.3 *min* of period. Col-pretreatment caused increase in both, amplitude and period of PMA-activated neutrophil F-actin oscillation in FMF patients (6.15 ± 0.41) as well as in ND (6.02 ± 0.25). In contrast to ND, in FMF patients' LPS-activated neutrophil F-actin dynamics also had undulating shape with the period of 6.06 ± 0.28 *min*. However, Col-pretreatment caused decrease in the period of LPS-activated neutrophil F-actin oscillation in FMF patients for 1 *min* (4.59 ± 0.03), whereas in NDs it was the same as in PMA-activated neutrophils (6.04 ± 0.18). These results suggested that fMLP-, LPS- or PMA-stimulated neutrophil F-actin dynamics in FMF patients was characterized by oscillations with different amplitude and periods, whereas in NDs activated neutrophils F-actin dynamics has an undulating shape in presence of fMLP only. Further, we studied difference between ND and FMF patient's neutrophil F-actin oscillations in more details.

Neutrophil F-actin oscillations during chemoattractant receptor desensitization: F-actin oscillation periods in FMF patients and NDs that were measured for a relatively short time (during total 5 *min* of incubation) were found to be similar (approx. 4 *min*) in FMF patients and NDs, despite the facts that F-actin content in unstimulated cells was higher in FMF patients with delayed shift of maximums of fMLP-induced F-actin dynamics. At the next step we analyzed, if neutrophil F-actin oscillation amplitude and periods in FMF patients and NDs differed during 10 *min* of incubation, which is more than double the calculated period of oscillation. The period of fMLP-induced neutrophil F-actin oscillation in FMF patients was found to be 4.66 ± 0.11 *min* versus previously obtained 4.08 ± 0.46 *min* and 3.58 ± 0.17 *min* versus 4.09 ± 0.18 *min* for ND, respectively (Figure 4: black lines). These values do not significantly differ from each other, while increasing length of trial revealed an increase of fMLP-induced neutrophil F-actin oscillation period in FMF patients by 1 *min* (4.64 ± 0.11 *min* versus 3.58 ± 0.17 *min* in ND). Next, we analyzed how this delayed F-actin oscillation period in FMF could contribute to the impaired chemoattractant receptor desensitization in neutrophils during repeated fMLP action. Whole blood neutrophils of NDs and FMF patients were incubated with fMLP for 10 *min,* then equal amount of fMLP was added for 1-10 *min* and cellular F-actin content assayed by flow cytometry. We observed that repeated action of fMLP induced chemoattractant receptor desensitization in NDs (Figure 4) with significant reduction of cellular F-actin oscillation amplitude (1751 ± 166 in a single dose fMLP-treated cells versus 725 ± 120 MCN in double fMLP-treated cells, P_t=0.03). These changes paralleled with the increase of neutrophils F-actin oscillation period from 3.58 ± 0.17 *min* in the single dose fMLP-treated cells to 6.10 ± 0.26 *min* in double fMLP treated cells (P_t=0.02). In contrast to NDs, FMF patients' neutrophils failed to induce chemoattractant receptor desensitization during repeated action of fMLP. Indeed, neither F-actin oscillation amplitude (1298 ± 104 in a single dose fMLP-

treated cells versus 1442 ± 93 MCN in double fMLP treated cells), nor oscillation period (4.64 ± 0.11 versus 4.66 ± 0.09 *min*, respectively) were changed in FMF patients neutrophils in the presence of single or double doses of fMLP.

Unequal pattern of Col- and CyB-induced F-actin oscillations in FMF and ND: Finally, we tested how microtubule-dissolution by Col or cytoskeleton-disruption by CyB caused differential reorganization of fMLP-induced neutrophil F-actin oscillations in FMF patients and ND. The addition of 0.2-10 *μg/mL* Col or CyB to whole blood taken from NDs' or FMF patients (each sample size was 3), for 10 *min* caused dose-dependent increase of neutrophils F-actin content, which reached plateau at 1 *μg/mL* of CyB and 4 *μg/mL* of Col, respectively (data not shown). Thus, in future we used 400 *ng/mL* concentrations of either CyB and Col, which are placed on linear plots of corresponding dose-dependency curves. In NDs' neutrophils Col incubated for 1-10 *min* caused liner increase of cellular F-actin content (Figure 5). This linear time-dependent pattern of cellular F-actin dynamics in ND is still evident even in the presence of fMLP. In contrast to ND, FMF patients' neutrophils F-actin dynamics in the presence of Col had undulating shape (with 291.5 ± 28.4 MCN amplitude and 6.67 ± 0.02 *min* of period), in the same undulating shape there was registered the presence of fMLP with increase of the amplitude (1382 ± 129.3 MCN) and decrease of the period (4.73 ± 0.13 *min*) of F-actin oscillations. Thus, neutrophil microtubule-dissolution by Col in FMF patients does not change the period of fMLP-induced F-actin oscillation (4.73 ± 0.13 *min* versus 4.64 ± 0.11 *min* in the absence of Col, respectively). However, neutrophil F-actin dynamics in the presence of cytoskeleton-disrupting agent CyB revealed oscillation behavior both in FMF patients and NDs. The amplitude and frequency of cellular F-actin oscillation in the presence of CyB were found to be significantly higher in ND (P_t=0.02) than in FMF patients (526 ± 32 MCN and period of 3.81 ± 0.01 *min* versus 149 ± 30 MCN and 6.83 ± 0.04 *min*, respectively), whereas no difference was observed in fMLP-induced F-actin oscillations (Figure 5).

DISCUSSION

In the innate immune system the cellular adaptation regulates neutrophil chemotaxis and macrophage activation by endotoxins, which could play a pivotal role in FMF pathogenesis. The chemoattractants (formylated peptides, interleukin-8, C5a and platelet activating factor) that bind and activate specific receptors belong to a family of G-protein coupled receptors, leading up to directed migration, granule mobilization and activation of the neutrophil NADPH-oxidase [*Ye R., Boulay F., 1997*]. The chemotactic behavior of these cells is of great importance not only for outcomes of the continuously ongoing combat with invading pathogens, but also for the tissue damage, associated with activation of neutrophils during inflammatory disorders [*Werner E., 2004*]. The resolution of inflammation can now be regarded as an integral component of the neutrophil activation negative regulation programs [*Han J., Ulevitch R., 2005*]. The best-known example of this is the phenomenon known as neutrophil chemoattractant receptors desensitization [*Ali H. et al., 1999*]. When neutrophils encounter increasing concentration of chemoattractant, they gradually become nonresponsive to further stimulation by the same agent. Thus, the induction of homologous desen-

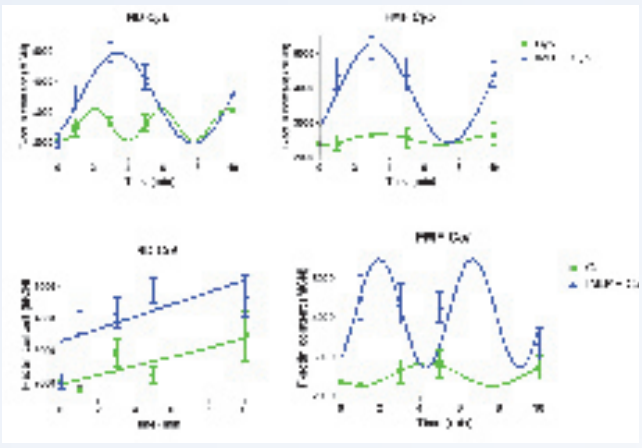

Figure 5. *Different pattern of Col- and CyB-induced F-actin oscillations in FMF and ND. Neutrophils from ND (n=15) and FMF (n=28) in 100 μL of whole blood incubated for 1-10 minutes either with 400 ng/mL Col or CyB in the presence or absence of fMLP. The reaction stopped at the appropriate time and intracellular F-actin dynamics was measured as described in Patients and Methods. Oscillation curves were generated from calculated mean values ±SEM data for F-actin content using the equations (1) and (2).*

sitization is important to limit or terminate the response to higher concentrations of an attractant, avoiding prolonged activation and thereby a continuation of an inflammatory event [*Fu H. et al., 2006*] and might be the potential pathogenic mechanism causing FMF febrile episodes and resolution of inflammation. The gene responsible for FMF, *MEFV* is expressed almost exclusively in neutrophils and cytokine-activated monocytes but not in serous tissues [*French FMF Consortium, 1997; Centola et al., 2000; International FMF Consortium, 1997*]. This finding was unexpected and opened new questions as to the involvement of the affected serous tissues during FMF. Namely, *what is the relationship between the mutant MEFV expression in neutrophils and the serous membrane inflammation or selective susceptibility of FMF patients to Col?* The finding that *MEFV*-encoded protein, pyrin, associates with microtubules and colocalizes with actin filaments [*Mansfield E. et al., 2001*] has important implications for understanding the role of pyrin in regulation of chemotactic behavior of neutrophils during FMF. Since actin has a central role in biological motility as an essential constituent of cytoskeleton and a partner of intracellular signaling pathways associated with chemoattractant-receptor activation [*Fu H. et al., 2006*], we investigated neutrophil F-actin oscillations and actin cytoskeleton dynamics during neutrophils activation-deactivation program shift in FMF patients. We found that F-actin content in unstimulated neutrophils was increased in FMF patients, thus suggesting the persistence of activation neutrophils associated with sub-clinical inflammation during the attack-free period of the disease. The importance of the actin cytoskeleton for regulation of neutrophil locomotion and phagocytosis [*Fu H. et al., 2006*] is supported by the facts that in FMF patients increased neutrophils phagocytosis [*Avetisyan S. et al., 2005*] and respiratory burst [*Davtyan T. et al., 2005*] are observed in parallel with enhanced neutrophil actin polymerization. However, unlike in NDs, Col significantly reduces neutrophils' F-actin content in FMF patients in conditions that previously have been shown to inhibit neutrophils phagocytosis [*Avetisyan S.. et al., 2005*], protein kinase-C-, and phagocytosis-dependent respiratory burst [*Hakobyan V. et al., 2005*] and surface expression of integrins [*Davtyan T. et al., 2006 a, b*]. During FMF, FMLP-stimulation of both untreated and Col-pretreated neutrophils was characterized by different patterns of F-actin dynamics and delayed time period of F-actin oscillation. Furthermore, we found that delayed F-actin oscillation period in FMF contributes to the impairment of chemoattractant receptor desensitization in neutrophils during repeated fMLP action, demonstrating that neutrophils from FMF patients fail to induce chemoattractant receptor desensitization, while in ND it occurred with significant reduction of F-actin oscillation amplitude and period. This finding together with our recent observation that monocytes from attack-free FMF patients failed to induce endotoxin homologous and heterologous tolerance [*Davtyan T. et al., 2006 a, b]* supports the idea that *MEFV* mutations may cause dissolution of cellular adaptation to environmental stimuli. Innate immune cells capable of adapting their responsiveness are concerned with rapid changes in concentration of extracellular ligands and use the steady state level to generate appropriate negative feedback that effectively shuts down cellular activation in the absence of change. Delay in generation of such a feedback, which is evident during FMF [*Davtyan T. et al., 2006 a, b*]., *Davtyan T. et al., 2008 a, b*], extends the duration of response, thereby allowing the cellular response states over a broad range of ligand concentration. For instance, in every individual the serosal tissues are repeatedly exposed to various non-significant physical stimuli, which initiate a cascade of cytokine excretion and recruits neutrophils for the potential inflammatory process in serosal tissues. In NDs the signals received by neutrophils do not enhance migration of these cells due to their depression by generation of the appropriate negative feedback. In FMF patients, when pyrin or its functions are lacking such signals cause an effective migration of neutrophils to the affected serous tissues leading to a full-blown attack [*Ben-Chetrit E., Levy M., 2001*].

In an attempt to understand the mechanisms resulting in delay of F-actin oscillation time period and impaired chemoattractant receptor desensitization during FMF we used pharmacological

approach to study F-actin dynamics in neutrophils stimulated by different agents. We found that neutrophil F-actin dynamics in FMF patients is characterized by oscillations with different amplitude and periods in the presence of all stimulators used, whereas in NDs' activated neutrophils F-actin dynamics has an undulating shape in the presence of fMLP only and has linear time-dependence in the presence of LPS and PMA. The oscillation shape of F-actin dynamics in FMF patients neutrophils stimulated by LPS could be associated with the enhanced endotoxin susceptibility of neutrophils during FMF due to increased surface retention of CD11b receptors [*Davtyan T. et al., 2008 a,b*]. Rapid activation of CD11b/CD18 function and its subsequent down-regulation in neutrophils regulated by means of bi-directional signaling between integrins and actin cytoskeleton [*Schoenwaelder S., Burridge K., 1999; Anderson S. et al., 2000*]. Both neutrophil actin polymerization and CD11b down-regulation during stimulation with LPS are impaired in FMF futher suggesting that *MEFV* mutations may cause dissolution of cellular adaptation to endotoxins. Similarly, different pattern responses of cellular F-actin in the presence of PMA (a protein kinase C activator, which bypasses the receptor activation level) during FMF may be associated with the increased oxidative response [*Davtyan T. et al., 2005*], which have been shown unaffected by the presence of cytochalasins [*Fu H. et al., 2006*].

It has been shown that neutrophils desensitization is induced by actin-dependent/-driven segregation of active ligand-receptor complex from amplifying G proteins. Desensitized/deactivated neutrophils can be reactivated to produce pro-inflammatory factors by the secondary addition of cytoskeleton-disrupting agent such as CyB [*Bylund J. et al., 2003; 2004*]. Short term treatment of neutrophils by CyB caused distinctive pattern of cellular F-actin oscillations in FMF patients and NDs. The amplitude and frequency of F-actin oscillation in the presence of CyB were found to be significantly higher in NDs, than in FMF patients further supporting the notion that desensitization relies on an intact actin cytoskeleton. Although, no difference was observed in fMLP-induced F-actin oscillations in the presence of CyB, microtubule-dissolution by Col caused unequal pattern of neutrophils F-actin oscillations in FMF patients and NDs. In NDs F-actin dynamics has an undulating shape in activated neutrophils pretreated with Col for a relatively long (Figure 3), but not for short time (Figure 5). Col not only disrupted microtubular structure, but also caused increased F-actin content and the characteristic cellular viscosity suggesting that actin filaments but not microtubules are the primary structural determinants of neutrophil mechanical properties [*Tsai M. et al., 1998*]. Therefore, we proposed that association of *MEFV*-encoded protein, pyrin, with microtubules and actin filaments may have contributed to different mechanical properties, actin cytoskeleton dynamics and activation-dependent chemotactic behavior of neutrophils during FMF.

About 65% of FMF patients respond to Col with complete remission and 20-30% experience significant improvement with reduction in the number and severity of the attacks [*Ben-Chetrit E., Levy M., 2001*], however, causes of Col non-responsiveness in remaining 5-10% FMF patients are still unknown. In conclusion, we suggest that in future determination of neutrophil F-actin oscillation by a simple whole blood based method described here could serve as an excellent tool for prediction of Col responsiveness during FMF treatment.

ACKNOWLEDGEMENTS

This study was supported by the Yerevan State Medical University and "Armenicum+" CJSC, Yerevan, Armenia. We thank Professor Tamara Sarkisian, PhD, Sc.D., Center of Medical Genetics and Primary Health, for her critical reading of the manuscript.

REFERENCES

1. *Adachi Y., Kindzelskii A.L., Ohno N., Yadomae T., Petty H.R.* Amplitude and frequency modulation of metabolic signals in leukocytes: synergistic role of IFN-gamma in IL-6- and IL-2-mediated cell activation. J. Immunol. 1999; 163: 4367-4374.
2. *Advani A., Marshall S.M., Thomas T.H.* Increasing neutrophil F-actin corrects CD11b exposure in Type 2 diabetes. Eur. J. Clin. Invest. 2004; 34: 358–64
3. *Ali H., Richardson R.M., Haribabu B., Snyderman R.* Chemoattractant receptor. Cross-desensitization. J. Biol, Chem. 1999; 274: 6027-6030.
4. *Anderson S.I., Hotchin N.A., Nash G.B.* Role of the cytoskeleton in rapid activation of CD11b/CD18 function and its subsequent downregulation in neutrophils. J. Cell Sci. 2000; 113: 2737-2745.
5. *Avetisyan S.A., Hakobyan V.P., Davtyan T.K.* [The effect of colchicine and iodine-lithium-a-dextrin on the phagocytosis of granulocytes and monocytes in patients with familial Mediterranean fever] [published in Russian]. Exp. Klin. Pharm. 2005; 68: 36-39.
6. *Ben-Chetrit E., Levy M.* Enigmas in familial Mediterranean fever (FMF). Clin. Exp. Rheumatol. 2001; 19(24): S1-5.
7. *Bylund J., Bjorstad A., Granfeldt D., Karlsson A., Woschnagg C., Dahlgren C.* Reactivation of formyl peptide receptors triggers the neutrophil NADPH-oxidase but not a transient rise in intracellular calcium. J. Biol. Chem 2003; 278: 30578-30586.
8. *Bylund J., Pellme S., Fu H., Mellqvist U.H., Hellstrand K., Karlsson A., Dahlgren C.* Cytochalasin B triggers a novel pertussis toxin sensitive pathway in TNF-alpha primed neutrophils. BMC Cell Biol. 2004; 5: 21.
9. *Centola M., Wood G., Frucht D.M., Galon J., Aringer M., Farrell C., et al.* The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. Blood 2000; 95: 3223-3231.
10. *Davtyan T.K., Avetisyan S.A., Hakobyan G.S., Haruthunyan V.M.* [A respiratory splash of monocytes and neutrophils in Familial Mediterranean fever] [published in Russian]. Ter. Arkh. 2005; 77(12): 39-43.
11. *Davtyan T.K., Hakobyan G.S., Avetisyan S.A., and Harutyunyan V.A.* Engaging anti-inflammatory mechanisms and triggering inflammatory effector apoptosis during Familial Mediterranean fever attack. Inflamm. Res. 2008a; 57(2): 65-74.
12. *Davtyan T.K., Hakobyan G.S., Avetisyan S.A., Mkrtchyan N.R.* Impaired endotoxine tolerance induction in patients with familial Mediterranean fever. Pathobiology 2006a; 73: 26-39
13. *Davtyan T.K., Harutyunyan V.A., Hakobyan G.S., Avetisyan S.A* Heightened endotoxin susceptibility of monocytes and neutrophils during Familial Mediterranean fever. FEMS Immunol. Med. Microbiol. 2008b; 52: 370-378.
14. *Davtyan T.K., Mkrtchyan N.R., Manukyan H.M., Avetisyan S.A* Dexamethasone, colchicine and iodine-lithium-a-dextrin act differentially on the oxidative burst and endotoxin tolerance induction in vitro in patients with Behcet's disease. Int. Immunopharmacol. 2006b; 6(3): 396-407.
15. *Ehrengruber M.U., Deranleau D.A., Coates T.D.* Shape oscillations of human neutrophil leukocytes: characterization and relationship to cell motility. J. Exp. Biol. 1996; 199:741-747.
16. *French FMF Consortium*: A candidate gene for familial Mediterranean fever. Nat. Genet. 1997; 17: 25-31.
17. *Fu H., Karlsson J., Bylund J., Movitz C., Karlsson A., Dahlgreb C.* Ligand recognition and activation of formyl peptide receptors in neutrophils. J. Leuk. Biol. 2006; 79: 247-256.
18. *Hakobyan V.P., Avetisyan S.A., Davtyan T.K.* [The effect of colchicine on the spontaneous and induced respiratory burst of granulocytes and monocytes in patients with familial Mediterranean fever] [published in Russian. Exp. Klin. Pharm. 2005; 68: 43-46.
19. *Han J., Ulevitch R.J.* Limiting inflammatory responses during activation of innate immunity. Nature Immunol. 2005; 6: 1198-1205.
20. *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.

21. *Korkmaz C., Ozdogan H., Kasapcopur O., Yazici H.* Acute phase response in familial Mediterranean fever. Ann. Rheum. Dis. 2002; 61: 79-81

22. *Livneh A., Langevitz P., Zemer D., Zaks N., Kees S., Lidar T.* Criteria for a diagnosis of familial Mediterranean fever. Arthritis Rheum. 1997; 40: 1879-1885.

23. *Mansfield E., Chae J.J., Komarow H.D., Brotz T.M., Frucht D.M., Aksentijevich I., Kastner D.L.* The familial Mediterranean fever protein, pyrin, associates with microtubules and colocalizes with actin filaments. Blood 2001; 98: 851-859.

24. *Matsukawa A., Hogaboam C.M., Lukacs N.W., Kunkel S.l.* Chemokines and innate immunity. Rev. Immunogenet. 2000; 2: 339-358.

25. *Orbach H., Ben-Chetrit E.* Familial Mediterranean fever. Minerva Med. 2001; 92: 421-430

26. *Schoenwaelder S.M., Burridge K.* Bidirectional signaling between the cytoskeleton and integrins. Curr. Opin. Cell Biol. 1999; 11: 274-286.

27. *Serhan C.N., Savil J.* Resolution of inflammation: the beginning programs the end. Nature Immunol. 2005; 6: 1191-7

28. *Stark J.,Chan C., George A.J.T.* Oscillations in the immune system. Immunol. Rev. 2007; 216: 213–23

29. *Stossel T.P.* On the crawling of animal cells. Science 1993; 260: 1086–1094.

30. *Tsai M.A., Waugh R.E., Keng P.C.* Passive mechanical behavior of human neutrophils: effects of colchicines.and paclitaxel. Biophys. J. 1998; 74: 3282–3291.

31. *Werner E.* GTPases and reactive oxygen species: switches for killing and signaling. J. Cell Sci. 2004; 117:143-153.

32. *Ye R.D., Boulay F.* Structure and function of leukocyte chemoattractant receptors. Adv. Pharmacol. 1997; 39: 221-289.