Th1 Polarization in Familial Mediterranean Fever

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ABSTRACT. ***Objective.*** Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by fever and serosal inflammation accompanied with an outburst of acute phase inflammatory products and cytokines. We studied the role of T helper (Th) 1 and 2 cells in FMF to elucidate the character of the inflammation. The cytokine products of Th1 and Th2, interferon-γ (IFN-γ) and interleukin 4 (IL-4), respectively, were analyzed by intracellular cytokine staining and FACS analysis.

Methods. We studied 34 Turkish patients with FMF (18 asymptomatic, 8 during an attack, and 8 with amyloidosis) and 14 age matched controls, as well as 11 parents of the patients who were accepted as heterozygotes for MEFV (Familial Mediterranean gene) mutations. Peripheral blood mononuclear cells were isolated and stained with monoclonal antibodies for IFN-γ and IL-4. The percentage of IL-4 positive T cells was not significantly different between the groups. However, the percentage of IFN-γ positive T cells in FMF patients experiencing an attack (median 25.8%, range 8.9–50.5%) was significantly higher than asymptomatic FMF patients (median 12%, range 0.1–70.7) ($p = 0.04$) and age matched controls ($n = 7$, median 0.4%, range 0–3.9%) ($p = 0.0001$). The percentage of IFN-γ positive T cells in asymptomatic FMF patients was also significantly higher than age matched controls ($p = 0.008$). Heterozygotes for FMF had significantly higher IFN-γ production (median 2.6%, range 0–42.4%) compared to age matched controls ($n = 7$, median 0.2%, range 0–1.4) ($p = 0.001$). IFN-γ production in FMF patients with secondary amyloidosis was also markedly increased but had a large range of variation.

Conclusion. Inflammation in FMF shows a Th1 polarization. We suggest that in patients with FMF the IFN-γ concentrations may remain higher because the defective pyrin is not able to inhibit this Th1 mediated inflammation. (J Rheumatol 2003;30:2011–3)

Key Indexing Terms:
FAMILIAL MEDITERRANEAN FEVER Th CELLS Th1 Th2 INTERFERON-γ

Familial Mediterranean fever (FMF) is an autoinflammatory disease with an autosomal recessive inheritance, characterized by periodic self-limited attacks of fever and serositis[1,2]. The gene causing FMF, designated MEFV, encodes a protein called pyrin or marenostrin that is expressed mainly in myeloid bone marrow precursors, neutrophils, and monocytes[1,3]. Mutations in MEFV may result in defective inflammation suppression[4]. The exact pathogenesis of FMF has not been clearly elucidated. An outburst of acute phase inflammatory products and cytokines accompanies the clinical inflammation. Untreated individuals with ongoing inflammation may develop secondary amyloidosis.

T helper (Th) cells are classified into 2 subsets according to their cytokine production pattern. Th1 clones produce proinflammatory cytokines interleukin 2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-ß (TNF-ß), favor T cell mediated cellular immunity, and activate monocytes. Th2 clones produce IL-4, IL-5, IL-6, IL-10 and IL-13, favor B cell mediated humoral immunity, activate eosinophils, and deactivate monocytes, leading to production of antiinflammatory cytokines[5]. A Th1 profile has been found to be predominant in adult and juvenile rheumatoid arthritis and in infections due to some intracellular bacteria, whereas a Th2 profile is predominant in allergic disorders and partially in asthma.

We investigated Th subsets in FMF to elucidate the character of the inflammation. The cytokine products of Th1 and Th2, IFN-γ, and IL-4 were identified by intracellular cytokine staining and FACS (fluorescence activated cell sorter) analysis. This method determines the actual frequency of individual cytokine-producing cells, in contrast to traditional bioassays that determine the extracellular presence (in supernatant) of secreted cytokines only[6]. We also studied the aforementioned cytokines in the parents who were accepted as carriers for the disease.

MATERIALS AND METHODS

The study included 6 groups: Group 1: 8 symptomatic FMF patients during an attack period; Group 2: 18 asymptomatic FMF patients; Group 3: 7 child controls; Group 4: 7 adult controls for the heterozygotes; Group 5: 11 heterozygotes for MEFV mutations who were parents of patients; and Group 6: 8 FMF patients who developed secondary amyloidosis.

There were 34 FMF patients who were diagnosed according to defined criteria[7] and followed in our department. Mutation analysis for the MEFV gene was carried out in 25 patients: 20 had mutations for the FMF gene in both alleles, 5 had a mutation in one allele only. The patients who were not confirmed genetically had typical phenotype for FMF, and all fulfilled the

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Tel-Hashomer diagnostic criteria[7] and responded to colchicine therapy. Patients with infectious diseases were excluded. None was taking any medication other than colchicine.

Eleven carriers for MEFV mutation had no symptoms of FMF. They were the mothers or fathers of the patients who had mutations in both alleles for the MEFV gene.

The first control group included 7 children and the second group included adults serving as controls for the heterozygotes. They were of comparable age and sex. Neither the heterozygotes nor the controls had infections or were taking any drugs for at least 2 weeks prior to the study.

In the patient group, blood samples for erythrocyte sedimentation rates (ESR) and serum C-reactive protein (CRP) were withdrawn simultaneously while sampling for peripheral blood mononuclear cells (PBMC). Ethical consent was given by all subjects.

Intracellular cytokine staining using flow cytometry. Heparinized venous blood samples were collected. PBMC were isolated and were stimulated by phorbol-myristate-acetate (PMA; Sigma) and ionomycin (Sigma). Cells were washed, centrifuged, and resuspended with appropriate solutions as described[6]. The fixed/permeabilized cells were added in 100 µl of Perm/Wash solution containing fluorochrome conjugated anticytokine antibodies [phycoerythrin (PE) conjugated anti-human-IL-4; Becton Dickinson, Pharmingen, San Diego, CA, USA] and FITC conjugated anti-human-IFN-γ (Becton Dickinson) or appropriate negative control and incubated at 4°C for 30 min in the dark. Cells were washed and resuspended in staining buffer prior to flow cytometric analysis.

Samples were analyzed on a 3-color FACS Calibur (Becton Dickinson) using Cell Quest software (Becton Dickinson); 10,000 cells were collected per sample. The percentages of IL-4 and IFN-γ positive T cells were analyzed by typical forward and side angle scattered light gating. Gates were set to exclude any dead cells or contaminating non-CD4+ lymphocytes. Quadrant statistics were determined on the basis of the staining of negative controls.

For analysis, the frequencies of cytokine-producing cells were given as median and range. The Mann-Whitney U test was used to evaluate differences between the groups; Spearman's correlation coefficient test was used to evaluate correlations.

RESULTS

The demographic features and results of the groups are summarized in Table 1. The percentage of IL-4 positive T cells was not significantly different between the groups. However, the percentage of IFN-γ positive T cells in FMF patients experiencing an attack (median 25.8%, range 8.9–50.5%) (Figure 2) was significantly higher than asymptomatic FMF patients (median 12%, range 0.1–70.7%; p = 0.04) and age matched controls (n = 7; median 0.4%, range 0–3.9%; p = 0.0001) (Figure 1).

Heterozygotes for FMF had significantly higher numbers of IFN-γ positive T cells compared to their age matched controls (p = 0.001) (Table 1).

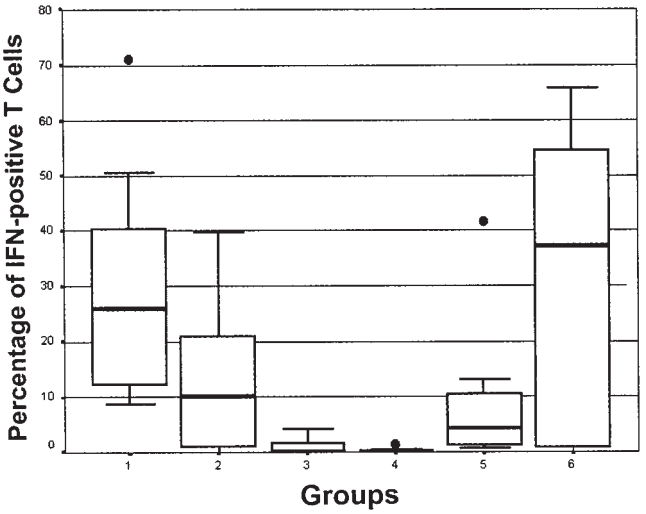

Figure 1. Comparison of IFN-γ expression in the 6 different clinical groups. Bars represent the interquartile range, whiskers represent the range, and thick horizontal bars show median value for each group. Extreme cases of individual variables are also plotted.

The percentage of IFN-γ positive T cells in FMF patients who developed amyloidosis was numerically higher than those without amyloidosis (median 37.2%, range 0.1–65.8%). Statistical analysis was not performed in this group, because of the large range of variation; this was mainly due to 2 of the patients with endstage renal failure.

No significant correlation was found between simultaneously measured ESR and CRP levels and IFN-γ levels.

DISCUSSION

This is the first report suggesting that the inflammation in FMF shows a Th1 polarization. This was suggested by the increased IFN-γ production in FMF patients compared to healthy controls. The Th1 activation is accompanied by a proinflammatory state, and Th1 cells specifically activate phagocytes. The increased IFN-γ production in asymptomatic FMF patients during attack-free periods may reflect the ongoing inflammation in FMF patients. This was suggested by studies where increased serum CRP concen-

Table 1. The clinical features of the groups and percentages of IL-4 and IFN-γ expression.

Group	Clinical Feature	No. of Patients	Age, yrs, mean ± SD	Percentage IL-4 Positive T Cells, median (range)	Percentage IFN-γ Positive T Cells, median (range)
1	FMF patients during an attack period	8	10 ± 4.41	0.1 (0–1.1)	25.8 (8.9–50.5)
2	Asymptomatic FMF patients	18	9.53 ± 5.54	0.4 (0–4.4)	12 (0.1–70.7)
3	Child controls	7	9.2 ± 2.39	0.3 (0–0.7)	0.4 (0–3.9)
4	Adult controls	7	31 ± 6	0 (0–0.8)	0.2 (0–1.4)
5	Heterozygotes for FMF	11	42.5 ± 5.4	0.1 (0–1.5)	2.6 (0–42.4)
6	FMF patients with amyloidosis	8	24.88 ± 10.19	0.1 (0–1.7)	37.2 (0.1–65.8)

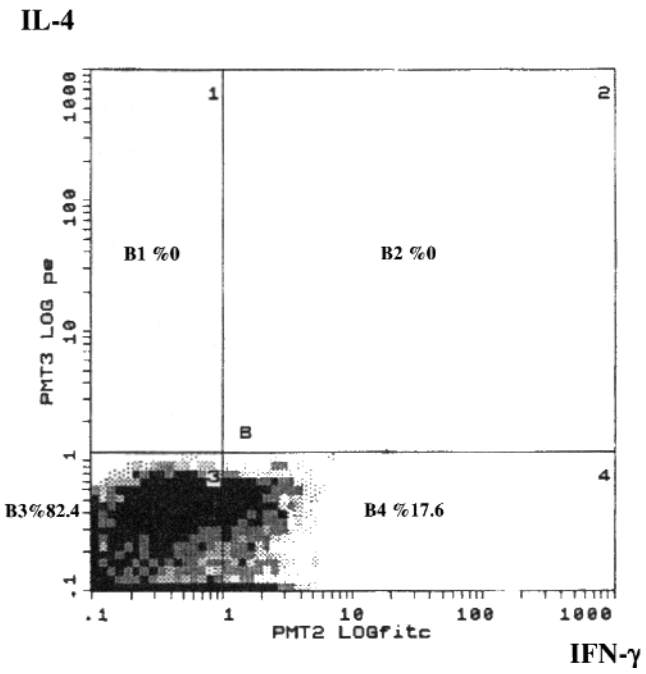

Figure 2. Flowcytometric profile of a patient with FMF during an attack period. The polarization toward IFN-γ (B4 quadrant) production suggests a Th1 cell predominance at the single cell level. The x-axis shows the logarithmic scale of expression of IFN-γ (FITC, B4 quadrant) and y-axis shows the logarithmic scale of expression of IL-4 (PE, B1 quadrant).

trations were observed for asymptomatic patients and indeed for the heterozygotes as well[8].

We analyzed intracellular staining of cytokines by FACS, which offers many advantages over traditional measures of cytokine populations such as ELISA and other bioassays that fail to distinguish between a high number of low level cytokine-producing cells and a few cells that produce a very high level of cytokine. Further, T cell purification is not necessary with this technique, making it appropriate to use with samples of limited size, as with pediatric samples.

The IFN-γ production varied within a rather large range, which may be attributed to the difference of the host response and the difference in timing. It is known that the acute phase reactants also vary within a wide range in FMF patients, and this is explained partly by the mutations and partly by the host response.

Centola, *et al*[3] have recently shown that MEFV plays a role in IFN-γ mediated inflammation, being a downstream element in this cascade, and is effective in phagocyte response in general. The authors concluded that MEFV functions in a negative feedback loop for Th1. Considering this along with our results, it may be deduced that when pyrin is defective the negative feedback may not be established, resulting in more pronounced Th1 activity and IFN-γ.

Another important result of our study was the increased IFN-γ production in heterozygotes compared to age matched controls. The carrier rate for FMF is as high as 1/5 in certain populations[9,10]. This high carrier rate for a mutation led us to believe that the heterozygous carriers for the mutations had an advantage to be selected over the course of time. We had originally postulated a heterozygote advantage for FMF carriers against tuberculosis, since tuberculosis was an ancient disease and an intracellular organism. However, our study disproved this theory[11]. On the other hand, Bar-Eli, *et al* have shown that patients with FMF had decreased phagocytic activity against *Shigella flexneri*[12]. It has been suggested by some authors that patients with FMF and heterozygotes had a possible protection against asthma[13]. However, our results suggest that the decreased allergic responses in patients with FMF are a result and not the cause of the underlying pathophysiology. The Th1 polarization in patients with FMF and carriers may be protecting them from diseases of pronounced Th2 response. The advantage of the carriers selected in biblical times may have been simply that these patients were able to mount a better Th1 inflammatory response that might have helped them react better to the various pathogens they encountered.

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