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The immune system in familial Mediterranean fever

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SUMMARY

Familial Mediterranean fever (FMF) is a genetic disorder with an obscure aetiology. In attempts to investigate a possible immunoregulatory imbalance involved in this disease we tested 24 FMF patients for suppressor T cell activity and for chemotaxis of mononuclear cells. The suppressor T cell activity and chemotaxis were decreased in untreated FMF patients as compared to colchicine treated patients or normal controls. Amyloid FMF patients manifested significantly increased chemotactic activity, while the suppressor T cell activity was normal. This finding may extend our knowledge concerning the immune mechanism involved in FMF.

Keywords familial Mediterranean fever amyloidosis chemotaxis suppressor T cells

INTRODUCTION

Familial Mediterranean fever (FMF) is a genetic disorder with a predilection to certain ethnic groups and marked by recurrent episodes of fever and polyserositis, usually peritonitis. Other clinical characteristics include painful erythematous skin lesions and arthritis (Sohar *et al.*, 1967; Eliakim, 1970). An integral feature of FMF is the development of systemic amyloidosis. Prophylactic treatment with colchicine usually prevents the acute attacks of fever of polyserositis (Zemer *et al.*, 1974, 1976) and may also prevent the development of amyloidosis or reduce the severity of proteinuria.

In an attempt to delineate the aetiology of FMF some investigators tested the immunological parameters in such patients: blastogenic responses to mitogens, skin window and HLA typing. Leucocyte pyrogen production and leucocyte ingestion, migration in chemotactic chamber were not found to be significantly different from healthy controls (Bar-Eli *et al.*, 1977; Dinarello *et al.*, 1974; Territo, Peters & Cline, 1976). Ilfeld, Weil & Kuperman (1981a, 1981b) showed decreased suppressor T cell function in four FMF patients which was corrected by the addition of colchicine.

In the present study we examined the suppressor T cell activity and the chemotaxis of mononuclear cells in FMF patients.

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MATERIALS AND METHODS

Patients. The study included 24 patients (19 from the Tel-Hashomer Hospital series and five from the Hadassah pediatric outpatient clinic; 13 males and 11 females, all Jews of Sephardic origin, ranging in age from 12–41 years; mean 24). Six patients were examined prior to treatment and 18 during treatment with colchicine (1–2 mg/day). Evidence of nephropathy due to amyloidosis, which is very frequent among patients in the Tel-Hashomer series, was found in five patients. As controls served a group of 17 healthy persons from medical staff and medical students matched for age and sex and from the same ethnic origin.

Informed consent was obtained from all patients and controls who participated in the study.

Suppressor T cell activity. Heparinizided venous blood was drawn from each subject. The lymphocytes were isolated and purified as described previously (Böyum, 1968) and cultured at a concentration of 5×10^6/ml with and without 20 μg concanavalin A (Con A) (Miles Yeda Lab., Rehovot, Israel). Following 48 h incubation 50 μg of Con A/ml were added to the control cells cultured without Con A. Both pre-treated Con A and untreated peripheral lymphocytes were then washed four times in the presence of 5 mg/ml of methyl-D-mannoside. Subsequently, 0·05 ml untreated autologous lymphocytes at a concentration of 1×10^6/ml were cultured with an equal volume of Con A pre-treated or control lymphocytes in the presence of 0·1 ml Con A at 100 μg/ml or medium alone in microtitre wells. The cultures were pulsed with ^{3}H-thymidine (sp. act. 2 Ci/mmole) on day 3, and the degree of ^{3}H-thymidine incorporation into DNA was determined after 16 h with an automated harvester. Radioactivity was determined in a liquid scintillation counter and the stimulation index (SI) calculated according to the following formula

$$\text{SI} = \frac{\text{ct/min in stimulated cultures}}{\text{ct/min in control cultures}}.$$

The percentage of suppression of Con A proliferation was calculated according to the formula:

$$\%\ \text{suppression} = \frac{\text{SI control} - \text{SI Con A pre-incubated cultures}}{\text{SI control cultures}} \times 100.$$

In our laboratory in all normal subjects the % of suppressions is higher than 70, so we decided to define as 'low suppression' results below 70%.

Chemotaxis assay. Chemotaxis was measured in modified Boyden Chambers (Synderman & Pike, 1978). Briefly, isolated MNL (human mononuclear leucocytes) were suspended in RPMI 1640 (GIBCO, Grand Island, New York, USA) to a concentration of $1{\cdot}5 \times 10^6$ monocytes/ml. The cell suspension (0·2 ml) was placed in the upper well of the chamber which was separated from the chemotactic stimulant (Formyl-Met-Leu-Phe) in the compartment by a 5·0 μm polycarbonate filter. After incubation of 90 min in humidified air at 37°C, the chambers were emptied and the filters were removed, fixed and stained with haematoxylin. Chemotaxis was quantified by counting and averaging the number of cells which migrated completely through the filter in 10 microscopic fields ($\times$ 1,500) with the aid of eye piece grid. Chemotactic activity was determined as the average number of migrating cells per high power field in triplicate sample and is expressed as the percent of the control value. A dose–response for the chemoatractent (F-Met-Leu-Phe) was established between 10^{-7}–10^{-8}M and the concentration of 10^{-8}M was found as an optimal in our laboratory.

RESULTS

Comparison of the immunological parameters of FMF patients with or without amyloidosis is summarized in Table 1. In Table 2 we summarized the immunological parameters of the colchicine treated group as compared with the untreated group.

Suppressor T cell activity was significantly decreased in two groups (Fig. 1): (a) patients with amyloidosis and (b) patients who were not treated with colchicine.

The suppressor T cell activity in the colchicine treated group and in the group without

Table 1. Detailed results on percentage of suppression and chemotaxis assay in FMF patients with or without amyloidosis

	With amyloidosis ($n=5$)			Without amyloidosis ($n=19$)		
	Mean ± s.d.	Control	*P*	Mean ± s.d.	Control	*P*
Chemotaxis						
No. of migrating monocytes /HPF × 1,500	44 ± 6·8	23 ± 5·7	0·005	15 ± 6·2	29 ± 4·8	0·05
Mean percentage of suppression FSD	69 ± 7·1	82 ± 6·4	0·05	76 ± 9·1	82 ± 9·1	NS

Table 2. Detailed results on percentage of suppression and chemotaxis assay in FMF patients treated or untreated with colchicine

	Treated with Colchicine ($n=18$)			Not treated ($n=6$)		
	Mean ± s.d.	Control	*P*	Mean ± s.d.	Control	*P*
Chemotaxis						
No. of migrating monocytes /HPF × 1,500	25 ± 4·7	29·5 ± 6·1	0·05	11·5 ± 5·1	28 ± 4·5	0·05
Mean percentage of suppression FSD	79 ± 4·5	84 ± 6·5	NS	68 ± 9·5	84 ± 6·5	0·05

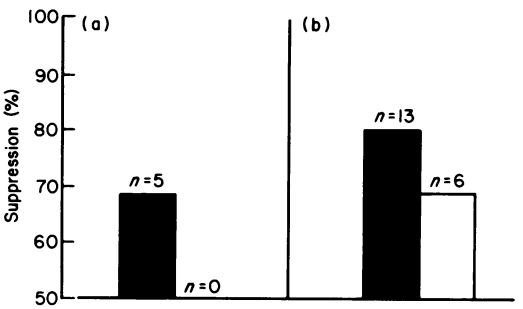

Fig. 1. Percentage of suppression in four groups of FMF patients (a) with amyloidosis and (b) without amyloidosis. ■ = treated with colchicine; □ = not treated with colchicine.

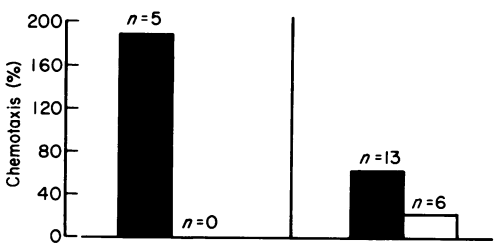

Fig. 2. Percentage of chemotaxis (of the control value) in four groups of FMF patients (a) with amyloidosis and (b) without amyloidosis. ■ = treated with colchicine; □ = not treated with colchicine.

amyloidosis was not different from the control group. In the untreated group without amyloidosis the suppressor T cell activity was significantly lower as compared to the treated group.

The chemotactic activity was significantly increased in the amyloidotic group as compared to the group without amyloidosis (Fig. 2). The chemotactic activity was unimpaired in the colchicine treated group while it was significantly lower in patients not treated with colchicine.

DISCUSSION

The diagnosis of FMF is based on definite clinical criteria. Although it was suggested that FMF is an inborn error of metabolism, the inducing agent of the mechanism of the acute attacks remain unknown, yet an immunoregulatory imbalance has been recently reported (Ilfeld *et al.*, 1981a, 1981b).

The chemotaxis of leucocytes from untreated FMF patients without amyloidosis was lower than that of normal or colchicine treated patients. Dinarello *et al.* (1976) found that both random and direct migration of neutrophils from FMF patients treated with colchicine was normal. The finding that colchicine disrupts microtuble assembly but does not prevent pseudopode formation which is essential for chemotasis (Synderman & Goetzl, 1981) may serve as a possible explanation for the unimpaired chemotaxis of the colchicine treated patients. Yet, the increased chemotactic activity in the amyloid FMF patients is a unresolved interesting point. A possible explanation is that the amyloid protein may serve as a chemotactic stimulus. This assumption is under investigation.

Kuperman, using a different method and in a small number of patients, demonstrated a decrease in suppressor T cell activity in FMF patients. He was able to reverse this abnormality by adding colchicine to the lymphocyte cultures. Our study confirmed the decreased suppressor cell activity in patients with FMF. In addition FMF patients who were treated with colchicine had normal suppressor cell function. FMF patients with amyloidosis had significantly lower suppressor cell activity. The possible explanation for this finding is under investigation.

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