Clin. exp. Immunol. (1981) **43**, 357–361.

Suppressor cell function in a family with familial Mediterranean fever

D. N. ILFELD, S. WEIL & O. KUPERMAN *Department of Internal Medicine and the Clinical Immunology Laboratory, Shaare Zedek Medical Center, Jerusalem, Israel*

(Accepted for publication 29 August 1980)

SUMMARY

Defective suppressor cell function has been demonstrated in several diseases but has not been tested in familial Mediterranean fever (FMF). We tested the ability of concanavalin A-activated suppressor cells from one family with FMF to inhibit the proliferation of phytohaemagglutinin-stimulated responder cells from normal volunteers. Four FMF patients tested between acute attacks had a mean ($\pm$s.e.) per cent suppression (5 ± 2) which was significantly ($P < 0{\cdot}0005$) less than an FMF patient tested during a spontaneous remission (47 ± 3), 10 healthy family members (41 ± 6) and eight normal volunteers (45 ± 4). Since FMF is inherited as an autosomal recessive disorder, deficient suppressor cell function is expressed in homozygotes between acute attacks, but not in a homozygote in spontaneous remission, homozygotes who are phenotypically normal, nor heterozygotes. This suggests that the suppressor cell abnormality in this family is probably related to the pathogenesis of FMF rather than representing a genetic marker of FMF or non-specific depression by disease activity.

INTRODUCTION

Familial Mediterranean fever (FMF) is a disease of unknown aetiology characterized by recurrent attacks of fever and polyserositis (Sohar *et al.*, 1967). Untreated FMF patients' immunological parameters such as leucocyte pyrogen production, bacterial ingestion, migration in chemotactic chambers or skin windows, blastogenesis to mitogens and HLA typing are not significantly different than healthy controls (Dinarello *et al.*, 1976; Chaouat *et al.*, 1977). Defective concanavalin A (Con A) activated suppressor cell function has been found in other diseases such as systemic lupus erythematosus (Abdou *et al.*, 1976; Fauci *et al.*, 1978), Graves' disease (Aoki, Pinnameneni & DeGrott, 1979), psoriasis (Sauder, Bailin & Krakauer, 1980) and inflammatory bowel disease (Hodgson, Wands & Isselbacher, 1978) but has not been studied in FMF. Therefore, we measured the Con A-activated suppressor cell function in one family of five FMF patients and their 10 healthy family members. Evidence is presented that defective suppressor cell function occurs in these FMF patients and is probably related to the pathogenesis of FMF.

MATERIALS AND METHODS

Patients. We studied a Moslem Arab family. The proband is a 41-year-old man. Since the age of 20 he has had acute attacks of high fever (over 38·5°C) with joint pain for 2 or 3 days' duration with about eight to ten attacks per year. One year ago he developed the nephrotic syndrome and renal

Correspondence: Dr David Ilfeld, Department of Internal Medicine, Shaare Zedek Medical Center, Jerusalem, Israel.

0099-9104/81/0200-0357$02.00

biopsy showed amyloid. During our studies he was receiving conservative medical therapy for treatment of chronic renal failure.

The proband's 68-year-old father has had acute attacks of high fever and abdominal pain for more than 24 years, lasting 2 days each, with about 12 per year. The proband's 9-year-old son has had acute attacks of high fever and abdominal pain since age 2, each of 2 days' duration, with about 26 attacks per year. The proband's brother (age 29) for the past 4 years has had acute attacks of high fever, abdominal pain and joint pain for 2 days with about four attacks per year. Since age 1, the proband's 5-year-old cousin has suffered acute attacks of high fever and abdominal pain for 2 days' duration recurring about four times per year. Six months before testing suppressor cell function, he spontaneously stopped having attacks. All five FMF patients frequently have had erythematous papules on their face during acute attacks.

No patient was treated with colchicine. The proband received routine drugs for chronic renal failure. The other four FMF patients were not taking any drug.

The proband's father, brother and son were repeatedly tested for suppressor cell function 4 days to 2 months after acute attacks of FMF. During this period between attacks they felt 100% well without any symptom or any abnormal finding on physical examination or any abnormality in routine clinical laboratory tests. They have continued to have acute attacks of FMF at the same frequency after measuring their suppressor cell function as they did prior to doing the assays. In contrast, the proband's cousin with FMF was tested for suppressor cell function 6, $6\frac{1}{4}$ and $6\frac{1}{2}$ months after his last attack of FMF. Subsequently, he has continued for another 9 months without any FMF attacks. Thus, only clinical criteria were applied to indicate that the proband's cousin with FMF was tested for suppressor cell function during a spontaneous remission while the other four FMF patients were tested between acute attacks of FMF.

We studied the proband's wife and the proband's first-degree relatives: six additional children (ages 3–17), another brother (age 26) and two sisters (ages 20 and 36). Regarding consanguinity, the proband's wife is the proband's first-degree cousin (father's brother's daughter). The proband's cousin with FMF is the son of the proband's sister. Also, his (proband's cousin's) father's grandfather was the brother of the proband's grandfather. Eight genetically unrelated normal volunteers (ages 23–69) served as a control group.

Suppressor cell assay. Fifteen millilitres of heparinized venous blood was collected from each test subject after obtaining informed consent. Five million mononuclear cells (isolated on Ficoll–Hypaque density gradients) were cultured for 40 hr (in culture dishes No. 3035, Costar, Cambridge, Massachusetts) with 1 ml of culture medium (RPMI 1640 containing penicillin, streptomycin, glutamine, 5×10^{-5} M 2-mercaptoethanol, and 10% heat-inactivated fetal calf serum (Biolab, Jerusalem, Israel)) plus 10 μg/ml of Con A (Miles Yeda, Rehovot, Israel). These Con A-activated cells were incubated with mitomycin C, washed three times, and then 100,000 cells were cultured for 72–80 hr at 37°C in 5% CO_2 in air, in 0·2 ml of culture medium in tissue culture microplates (3596; Costar, Cambridge, Massachusetts) with 100,000 fresh responder cells from normal volunteers plus 0·8 μg/ml of phytohaemagglutinin (PHA) (Wellcome, Kent, England). One microcurie of tritiated thymidine was added to each well for the last 16 hr of culture and the cells were harvested by a multiple automatic sample harvester. Incorporation of labelled thymidine into the cells was measured by liquid scintillation counting.

Triplicates or quadruplicates of cells from normal volunteers were tested in every assay along with cells from FMF patients and/or their healthy family members. Per cent suppression was calculated as the percentage difference between the thymidine uptake of PHA-stimulated responder cells from normal volunteers cultured with and without Con A-activated cells from the test subjects. There was no suppression (mean 2 ± 4% s.e.) by cells from the test subjects cultured in medium without Con A. Statistical analysis was performed by Student's *t*-test.

RESULTS

The mean ($\pm$s.e.) per cent suppression of PHA-stimulated cells from normal volunteers co-cultured with Con A-activated cells from the test subjects was 45 ± 4 for normal volunteers, 41 ± 6 for healthy

family members and 47±3 for the proband's cousin with FMF who was tested during a spontaneous remission (Fig. 1). There was no significant ($P > 0{\cdot}2$) difference among these three groups. Furthermore, if decreased levels of suppressor cell function are defined as a per cent suppression more than two standard deviations below the mean of the normal volunteers (18%), then all of the healthy family members and the FMF patient in remission had normal levels of suppressor cell function.

In contrast, the four FMF patients tested between attacks had a mean per cent suppression of 5±2 (s.e.) which was significantly ($P < 0{\cdot}0005$) less than the normal volunteers, the healthy family members, and the FMF patient in remission (Fig. 1). Only one FMF patient with active disease in one out of four assays had a per cent suppression within two standard deviations of the mean of the normal volunteers. The mean (±s.e.) per cent suppression by the proband (4±3), the proband's father with FMF (9±4), the proband's son with FMF (8±3), and the proband's brother with FMF (2±2) were all more than two standard deviations below the mean of the normal volunteers (Fig. 2).

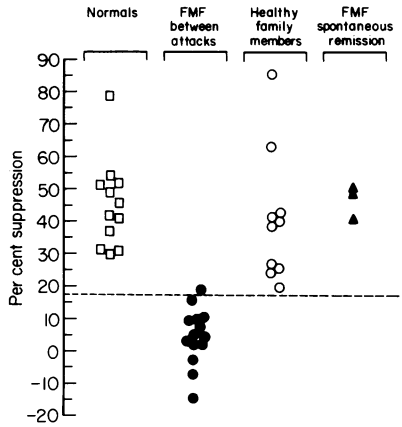

Fig. 1. Per cent suppression by Con A-activated cells from test subjects of PHA-induced proliferation of cells from normal volunteers. Each symbol represents one assay. Four FMF patients were tested between attacks three to four times each (●). One FMF patient was tested three times during a spontaneous remission (▲). Ten healthy family members were tested once each (○). Eight normal volunteers were each tested once or twice (□). The dashed horizontal line indicates 18% suppression (mean − 2 s.d. of normal volunteers).

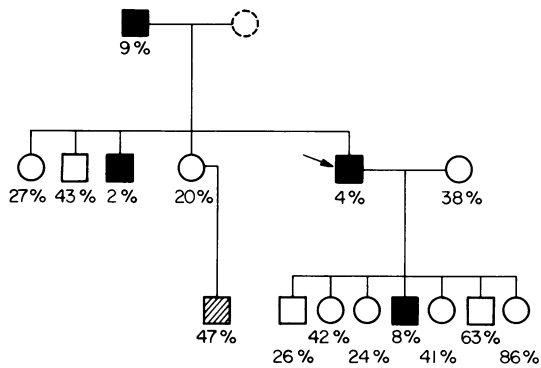

Fig. 2. Suppressor cell function in FMF patients and their healthy family members. The arrow points to the proband. Squares represent males and circles represent females. Closed symbols represent the four FMF patients tested between attacks. The striped symbol represents the FMF patient tested during a spontaneous remission. Open symbols represent healthy family members. The number under each symbol is the per cent suppression.

Since all four FMF patients with active disease were males, the question arises whether there was any difference between males and females in the control groups. The mean (±s.e.) per cent suppression was 48 ± 7 for normal male volunteers, 42 ± 3 for normal female volunteers, 44 ± 11 for healthy male family members, 40 ± 8 for healthy female family members and 47 ± 3 for the male FMF patient in remission. There was no significant ($P > 0{\cdot}2$) difference among any of these five groups. Routine tests of the FMF patients such as serum immunoglobulin levels and *in vitro* proliferation to Con A and PHA were normal.

DISCUSSION

Our results demonstrate that four FMF patients from one family tested between attacks have deficient suppressor cell function. Regarding the relationship between a suppressor cell deficiency and FMF, the major possibilities are that the suppressor cell deficiency is a genetic marker of FMF or is related to the pathogenesis or disease activity of FMF. We always tested FMF patients between acute attacks when they were clinically asymptomatic so that their suppressor cell deficiency would probably not be due to non-specific depression by disease activity. As for pathogenesis, the FMF patients' suppressor cell deficiency may lower their threshold in responding to the unknown brief recurrent stimuli that trigger the acute attacks of fever and polyserositis. In this case, the abnormal immunological response rather than the stimulus itself may be responsible for the systemic manifestations of the acute attack. Alternatively, suppressor cell deficiency in these FMF patients may be a genetic marker.

If suppressor cell deficiency in these FMF patients is one of several genetic markers, then one would expect to find suppressor cell deficiency in some of the healthy family members. For example, Miller & Schwartz (1979) demonstrated that 13 of 50 healthy members of patients with systemic lupus erythematosus had decreased suppressor cell function. They concluded that suppressor cell deficiency in SLE may be one of several genetic abnormalities needed to manifest SLE rather than an indicator of disease activity. In contrast, none of our 10 healthy family members had a suppressor cell deficiency.

Since FMF has an autosomal recessive inheritance, all of the healthy family members tested must be at least heterozygous and approximately half homozygous for FMF. Therefore, the suppressor cell deficiency is expressed in homozygotes who phenotypically have FMF whereas no suppressor cell deficiency is expressed in heterozygotes who are phenotypically normal. Furthermore, it appears that homozygotes who are phenotypically normal have normal suppressor cell function. This is supported by the observation that the proband's cousin with FMF (who must be homozygous) had normal levels of suppressor cell function when tested during a spontaneous remission. This suggests that the decreased suppressor cell function in patients with active FMF in this family is probably related to the pathogenesis of their disease rather than to a genetic marker in linkage disequilibrium with the gene responsible for FMF.

Five patients with FMF, amyloidosis and chronic renal failure were reported to have a clinical remission of FMF during chronic treatment with haemodialysis (Rubinger, Friedlaender & Popovtzer, 1979). Our proband with FMF, amyloidosis and chronic renal failure has now been treated with haemodialysis for 10 months. Since starting haemodialysis he has had no attacks of FMF and therefore has a clinical remission of FMF. Since instituting haemodialysis, serial assays of his suppressor cell function have always been normal (Ilfeld, Weil & Kuperman, 1980). This observation supports the hypothesis that FMF patients in this family have a suppressor cell abnormality between acute attacks of FMF but not during remission.

In conclusion, FMF in this family is associated with defective suppressor cell function which is expressed in homozygotes between acute attacks, but not in a homozygote in spontaneous remission, homozygotes who are phenotypically normal, nor heterozygotes. This suggests that the suppressor cell abnormality in this family is probably related to the pathogenesis of FMF rather than representing a genetic marker of FMF or non-specific depression by disease activity. Further studies are needed to determine whether FMF patients from other families also have a suppressor cell abnormality.

REFERENCES

ABDOU, N.I., SAGAWA, A., HEBERT, J. & SADEGHEE, S. (1976) Suppressor T-cell abnormality in idiopathic systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **6,** 192.

AOKI, N., PINNAMENENI, K.M. & DEGROTT, L.J. (1979) Studies on suppressor cell function in thyroid diseases. *J. Clin. Endocrinol. Metab.* **48,** 803.

CHAOUAT, Y., TORMEN, J.P., GODEAU, P.G., CAMUS, J.P., KAHN, M.F., RYCHEWAERT, A., LAULA, J.E., MENKES, C.J. & SCHMID, J. (1977) HLA markers and periodic disease (familial Mediterranean fever (FMF)). *Nouv. Presse Med.* **6,** 2949.

DINARELLO, C.A., CHUSID, M.J., FAUCI, A.S., GALIN, J.I., DALE, D.C. & WOLFF, S.M. (1976) Effect of prophylactic colchicine therapy on leukocyte function in patients with familial Mediterranean fever. *Arthritis Rheum.* **19,** 618.

FAUCI, A.S., STEINBERG, A.D., HAYNES, B.F. & WHALEN, G. (1978) Immunoregulatory aberrations in systemic lupus erythematosus. *J. Immunol.* **121,** 1473.

HODGSON, H.J., WANDS, J.R. & ISSELBACHER, K.J. (1978) Decreased suppressor cell activity in inflammatory bowel disease. *Clin. exp. Immunol.* **32,** 451.

ILFELD, D.N., WEIL, S. & KUPERMAN, O. (1980) Suppressor cell dysfunction and the pathogenesis of familial Mediterranean fever. In *Immunoregulation and Autoimmunity* (ed. by R. S. Krakauer & M. K. Cathcart). Elsevier/North-Holland, Amsterdam.

MILLER, K.B. & SCHWARTZ, R.S. (1979) Familial abnormalities of suppressor cell function in systemic lupus erythematosus. *N. Engl. J. Med.* **301,** 803.

RUBINGER, D., FRIEDLAENDER, M.M. & POPOVTZER, M.M. (1979) Amelioration of familial Mediterranean fever during hemodialysis. *N. Engl. J. Med.* **301,** 142.

SAUDER, D.N., BAILIN, P.L. & KRAKAUER, R.S. (1980) Suppressor cell defect in psoriasis. *Arch. Dermatol.* **116,** 51.

SOHAR, E., GAFNI, J., PRAS, M. & HELLER, H. (1967) Familial Mediterranean fever: a survey of 470 cases and review of the literature. *Am. J. Med.* **43,** 227.