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Lymphocyte Markers and Natural Killer Cell Activity in Fibromyalgia Syndrome: Effects of Low-Dose, Sublingual Use of Human Interferon-α

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ABSTRACT

A clinical study was designed to utilize flow cytometric immunophenotyping and chromium release from cultured tumor target cells to characterize peripheral blood mononuclear leukocyte (PBML) subpopulations and natural killer activity in healthy normal controls (n = 18) and in patients with fibromyalgia syndrome (FMS) at baseline (n = 124) and again after 6 weeks of treatment with low-doses of orally administered human interferon-α (IFN-α). Volunteer subjects discontinued all analgesic and sedative hypnotic medications for 2 weeks prior to the baseline phlebotomy. Laboratory measures included a complete blood count; a phenotypic analysis of PBML by flow cytometry; and *in vitro* natural killer (NK) cell activity. After baseline blood sample collection, the FMS patients were randomized to one of four parallel treatment groups (n = 28/group) to receive sublingual IFN-α (15 IU, 50 IU, 150 IU), or placebo every morning for 6 weeks. The tests were repeated at week 6 to evaluate treatment effects. At baseline, FMS patients exhibited fewer lymphocytes and more CD25$^+$ T lymphocytes than did normal controls. By week 6, the main significant and consistent change was a decrease in the HLA-DR$^+$ CD4$^+$ subpopulation in the 15 IU and 150 IU treatment groups. These data do not support an immunologically dysfunctional PBML phenotype among patients with FMS as has been observed in the chronic fatigue syndrome.

INTRODUCTION

FIBROMYALGIA SYNDROME (FMS) is a chronic, painful disorder commonly seen in rheumatology practice and in the general population.[1–3] The etiology of FMS is not known but consideration has been given to genetic predisposition,[4,5] traumatic injury,[6,7] affective psychopathology,[8,9] viral infections,[10] and immunologic mechanisms[11] as possibilities.

Many studies, including two pilot studies from our laboratory, have suggested a potential involvement of the immune system in the pathogenesis of FMS.[12–17] Some of the reasoning behind studying immune function in FMS came from its proposed overlapping relationship to the chronic fatigue syndrome (CFS).[18–22]

Impaired lymphocyte responses to mitogen and reduced natural killer (NK) cell cytotoxicity are the most consistent findings in a substantial proportion of patients with CFS.[23–26] Gupta *et al.*[27] studied 20 CFS patients and 20 age-matched normal controls. They found that NK cells, as defined by CD16, CD56, and CD57 antigens, were significantly reduced. Their study of mononuclear cell phenotypes revealed a significant increase in the proportions of CD4$^+$ ICAM1$^+$ T cells, and increased density of intracellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1); decreased enhancing response to recombinant interferon-γ (rIFN-γ) *in vitro* were also found. They also found significantly enhanced production of (IL-6) by cultured monocytes from CFS patients compared with those from normal controls.[28]

IFN-α has been used for immunotherapy in patients with certain forms of hematological malignancies and in other clinical situations.[29,30] IFN-α can be cytotoxic/cytostatic for some malignant cells. It modulates immune response by exerting a di-

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rect effect on the immune system. It has been shown that IFN-α can inhibit antigen- or mitogen-induced cell proliferation and antibody production.[31,32] It can augment the cytotoxic activity of NK cells[33,34] and the production of certain cytokines.[35–39] However, in spite of extensive studies, the mechanisms by which the immunostimulatory actions of IFN-α occur are not fully understood.

See and Tilles[40] conducted a double-blind crossover therapeutic study of IFN-α involving 30 patients with CFS. After 12 weeks of IFN-α_{2a} therapy, the quality of life score was significantly improved in the 7 patients with isolated NK dysfunction compared to baseline. Because the pain component of CFS was believed by some authors to be similar to that in FMS, it was hypothesized that IFN-α might prove to be beneficial with the widespread discomfort associated with FMS and possibly in the proposed immunologic component of its pathogenesis as well.

The purpose of this prospective clinical trial is to evaluate rigorously the lymphocyte surface marker phenotypes and the NK cell activities in patients with FMS and to determine the effects of IFN-α treatment of FMS patients on those immunologic markers.

SUBJECTS AND METHODS

Informed consent

The study was approved by the Institutional Review Board for human subjects and participants signed informed consent before receiving any study-related intervention. There were no serious consequences to any study subject.

Patients

Sequential, primary FMS patients of either sex, between the ages of 18 and 69 years, were offered inclusion if they meet American College of Rheumatology (ACR) criteria[1] for that diagnosis. All patients were examined by one of us (IJR) to confirm the diagnosis of primary FMS and to exclude other diagnoses that would influence entry. Potential subjects were excluded if they were found to have a rheumatic disease such as rheumatoid arthritis[41] or systemic lupus erythematosus,[42] a criteria-based diagnosis of CFS,[43] an untreated endocrinopathy such as hypothyroidism, or prior treatment with IFN-α.

Normal controls

For each fourth randomized patient, an age-, sex-, race-matched, physically inactive, but healthy normal control (HNC, $n = 18$) was identified from the community to complete all of the screening visit assessments. Each HNC subject was asked to donate a single blood sample for research laboratory testing. The HNC did not receive IFN-α treatment. The fluorescence-activated cell sorting (FACS) laboratory values obtained from these normal subjects were compared with quality control normal values from the FACS Laboratory.

Medication

With only minor exceptions, all analgesic and sedative hypnotic medications typically used for the treatment of FMS[44–46] were discontinued at the screening visit and proscribed for the duration of the study. Patients were permitted to take acetaminophen occasionally as a rescue for severe headache, and low-dose (85 mg/day) aspirin was allowed if previously prescribed for its antiplatelet effect.

Those who successfully discontinued prestudy medications were randomized to take one of the coded study medications. The study lozenges, prepared by Hayashibara Biochemical Laboratories Inc. (Okayama, Japan), contained IFN-α at 15, 50, or 150 IU. The manufacturer also provided an identically appearing and tasting placebo lozenge.

Interventions

After clinical evaluation and baseline immunological studies, each FMS patient participant was to dissolve one lozenge in his/her mouth every morning for 6 weeks. The treatment group designations were defined by the dosage of the administered lozenges contained 15, 50, or 150 IU of IFN-α, whereas the placebo group subjects received placebo lozenges.

Immunological measures

Heparinized whole blood for analysis of peripheral blood mononuclear leukocytes (PBML) was obtained at weeks 0 (baseline) and 6. All of the laboratory measurements were conducted without knowledge of the diagnosis or of the randomization code, which was not revealed to the investigators until after the last patient completed the study and the database was complete.

Flow cytometric analyses

On week 0 and 6, FACS analysis was performed on EDTA anticoagulated blood. Whole blood was stained using fluorochrome-labeled antibodies tagged with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Antibodies to CD2, CD3, CD4, CD8, CD16, CD25, CD54, CD56, CD57, and HLA-DR were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA). Antibodies to CD5 and CD19 were obtained from Gen Track, Inc (Plymouth Meeting, PA).

The following antibody combinations were used in sequential tubes for each analysis: CD3-FITC/HLA-DR-PE, CD2-FITC/CD16-PE, CD4-FITC/CD8-PE, CD5-FITC/CD19-PE, CD4-FITC/HLA-DR-PE, CD8-FITC/HLA-DR-PE, CD54-FITC/CD56-PE, CD3-FITC/CD25-PE, and CD3-FITC/CD54-PE. After staining with antibody, the red blood cells were lysed using FACSlyse (Becton Dickinson Immunocytometry Systems) and the white blood cells were washed free of reagents with phosphate-buffered saline (PBS). The white blood cell pellet was then resuspended in 1% formalin and stored at 4°C until analyzed. Flow cytometric analysis was performed on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems) using forward and right-angle side scatter to gate on the lymphocytic region. The results were routinely reported as the percentage of the total number of gated lymphocytes identified by a given antibody in any flow cytometry experiment. The absolute number of cells staining with each antibody was also calculated using complete blood count (CBC) data obtained with a Coulter STK S Counter (Coulter Scientific Instruments, Hialeah, FL).

Double-staining analysis allowed characterization of dual markers on selected lymphocyte subpopulations. For example, the subpopulation identified by dual staining with antibodies to CD8 and to HLA-DR was composed of activated suppressor/cytotoxic T lymphocytes.

NK cell activity assay

On weeks 0 and 6, 20 cc of blood was anticoagulated with 0.2 cc of liquid, sodium heparin (1,000 IU/ml). The heparinized whole blood was mixed with equal volumes of RPMI 1640 (Gibco BRL, Grand Island, NY, USA) layered onto a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) cushion and centrifuged at 500 × *g* for 25 min. The PBML layer was removed, placed into a clean 15-ml conical tube, and diluted with RPMI-1640. The PBMLs were then washed three times by centrifugation at 500 × *g* for 10 min at 4°C. After the last wash, the PBMLs were resuspended in a known volume of complete culture media (RPMI 1640 containing 10% fetal calf serum, supplemental L-glutamine, and penicillin/streptomycin), counted on a hemocytometer, and adjusted to 2×10^6 PBMLs/ml.

K-562 cells (target cells) were labeled with ^{51}Cr by the addition of 25 μl of ^{51}Cr (New England Nuclear Life Science Products, Boston, MA) to K-562 cells that have been spun and resuspended in 0.5 ml of complete culture media. The target cells were then incubated at 37°C, 5% CO_2 for 60 min. The target cells were then washed three times in RPMI 1640, counted, and adjusted to a concentration of 2×10^5/ml in complete culture media.

In a 96-well, round-bottomed microliter plate, 100 μl of target cells were plated in triplicate with either 100, 50, 25, or 0 μl of effector cells to give a effector-target ratio of 20:1, 10:1, 5:1, or 0:1 (spontaneous release), respectively. Culture medium RPMI 1640 was added as necessary to increase the volume of each well to 200 μl. The positive control (100% lysis) was 100 μl of target cells and 80 μl of RPMI 1640. Complete lysis was accomplished by the addition of 20 μl of a 12% Triton-X100 (Sigma Chemical Company, St. Louis, MO) in normal saline solution during the last 45 min of incubation. The plate was centrifuged at 250 × *g* (1,000 rpm) for 4 min, and then incubated for 4 h at 37°C, in an atmosphere of moist 5% CO_2. At the end of the culture period, the plate was centrifuged at 250 × *g* (1,000 rpm) for 4 min and 100 μl of supernatant was removed from each well without disrupting the cell pellet. The supernatant was placed into 12 × 75-mm glass culture tubes and counted for 2 min in a gamma counter (Packard Instruments, Downers, Grove, IL). Specific ^{51}Cr release was calculated by the following formula:

$$\left[\frac{\text{CPM Sample}^{51}\text{Cr release} - \text{CPM Spontaneous }^{51}\text{Cr release}}{\text{CPM 100\% Lysis} - \text{CPM Spontaneous }^{51}\text{Cr release}}\right] \times 100$$

The data resulting from the specific release formula was reported as NK activity at the various killer to target ratios (see Table 2, below). In addition, the results from a standard CBC were compared at each of the phlebotomy visits (see Table 2, below).

Sample size

Sample size needs were determined analytically, based on the requirement of the study to achieve a clinical endpoint. Assuming a 17% mean for nonspecific improvement in the tender point index (TPI) among the placebo-treatment FMS subjects in a prior study conducted by the same investigative team,[46] it was determined that 23 evaluable patients in each group would support a 94% power for detecting a 52% average improvement (*i.e.*, about three-fold greater improvement than expected with placebo) in response to at least one of the IFN-α dosage regimens. Because our previous studies with our own patient population have exhibited a 20% loss to follow-up, it was considered prudent to enroll 28 patients per group to complete the study with at least 23 patients per treatment group.

Statistical analysis

Assessment of treatment clinical efficacy was based on within-patient differences or changes in outcome from baseline (week 0) to the time of the evaluation (week 6). All analyses were adjusted for age. Separate linear models were applied at the week 6 follow-up to assess the significance of differences in outcome variable changes among the treatment groups (15, 50, and 150 IU IFN-α) compared with placebo (P). All pairwise contrasts with placebo (P vs. 50 IU, P vs. 100 IU, P vs. 150 IU) were examined. Multiple comparison procedures were not applied.

The primary null hypothesis predicted that there would be no difference in the amount of TPI improvement with treatment between active and placebo interventions. The implied null hypothesis relevant to laboratory analyses was that there would be no significant differences in any of the following: the distribution of cell-surface markers in PBML of FMS or HNC by FACS analysis; the functional NK activity of PBML from FMS relative to HNC; and changes in phenotype or NK function with IFN-α treatment relative to placebo treatment.

RESULTS

Human subjects

One hundred twenty-four FMS patients were screened for eligibility. Of those, 12 were not randomized because they proved not to have primary FMS ($n = 2$), could not tolerate the washout ($n = 2$), or elected not to participate ($n = 8$). One hundred twelve patients did qualify for study participation and were enrolled. These FMS participants exhibited a female:male ratio of 103:9, and mean age of 46.9 years. The ethnic distribution (Hispanic 39.3%) of the participants was slightly lower than that of the overall San Antonio community (about 60% Hispanic). After baseline evaluations at week 0, 28 subjects were randomly assigned to each of four treatment groups.

Table 1 summarizes the baseline values of the demographic variables and the results of the clinical measurements at the time of study entry. Analysis of treatment groups disclosed no significant differences with regard to sex, age, and race at either of the visits (baseline week 0 or week 6) at which laboratory samples were obtained. It should be noted, however, that the HNC group was marginally ($p = 0.08$) younger than the average for all of the randomized FMS patients.

Table 1. Demographic Characteristics and Clinical Variables for Patients with FMS and HNC

Variable	*HNC*	*FMS (All)*	*FMS (PBO)*	*FMS (15 IU)*	*FMS (50 IU)*	*FMS (150 IU)*
Age	42.3	46.9	49.5	47.0	43.7	47.3
Gender (F:M)	17:1	103:9	27:1	25:3	25:3	26:2
Race						
Caucasian	13	65	17	14	19	5
Hispanic	5	44	10	13	8	13
Other	0	3	1	1	1	0
TPI	0.3	32.9	31.5	34.7	32.3	33.1
APT	7.0	2.1	2.2	1.9	2.2	2.1
Pain	0.0	6.7	7.1	6.8	6.5	6.6
Function	9.6	7.6	7.7	7.9	7.9	7.0
Stiffness	0.2	6.8	6.1	7.4	6.5	7.1

Abbreviations: FMS, fibromyalgia syndrome; HNC, healthy normal control; PBO, placebo; IU, international units; TPI, tender point index (sum of tenderness severity, 1–4 scale, at 18 tender points; APT, average pain threshold (kilograms) by dolorimetry at 18 tender points; Pain, pain severity (0 = no pain to 10 cm = severe pain) by visual analog scale (VAS); Function, physical function by the Stanford Health Assessment Questionnaire; Stiffness, morning stiffness severity (0 = no stiffness to 10 cm = severe stiffness) by VAS; F:M, female:male ratio; Caucasian, persons of Northern European Caucasian descent; Hispanic, Persons of New World Spanish descent or American Indian descent; Other, African American or Oriental descent.

Blood cells and NK cell activity

In the left-hand column of Table 2 is a listing of blood cell populations measured in the CBC and the functional NK activities at each of the three killer:target ratios. The two columns to the right provide the mean values and standard deviations obtained with the HNC (n = 18) and all FMS (n = 112) at the baseline, week 0 visit.

The mean total number of lymphocytes in the blood from all FMS was significantly (p = 0.01) lower than the mean among the HNC group. All of the treatment groups exhibited this difference from the HNC, ranging from p = 0.003 for HNC:150 IU FMS Group, to p = 0.08 for the HNC:15 IU FMS Group comparison.

NK activity was consistently lower in the FMS treatment groups than in the HNC (significantly for HNC:150 IU FMS at 5:1 killer to target cell ratios and numerically at the 10:1 and the 20:1 ratios).

Lymphocyte phenotypic markers

In the left column of Table 3, is a listing of the reagents used in FACS analysis to tag cell-surface markers of PBML. The center column describes the cell population identified by each assay. The two columns to the right provide the mean values and standard deviations obtained with the HNC (n = 18) and all FMS (n = 112) at the baseline, week 0 visit. For example, the active T-suppressor/cytotoxic subpopulations (CD8 and HLADR) comprised 6.67 ± 1.11% of the HNC and 8.17 ± 0.53% of all randomized FMS.

As noted in Table 2, where the mean total of lymphocytes in the blood from all FMS was low, that decrease was paralleled by numerically decreased levels of T lymphocyte surface markers in all groups for CD2 (significant in HNC:15 IU FMS, p = 0.007), CD3 (nearly significant in HNC:15 IU FMS, p = 0.07), CD4 (significant in HNC:50 IU FMS, 0.02), and CD5 (significant in all three treatment groups p = 0.02–0.06). The CD8 subpopulation of T lymphocytes was not lower than normal.

By contrast, the FMS patients appeared to have increased levels of T lymphocyte/NK cells exhibiting CD57 and especially in the T lymphocyte subset expressing the IL-2 receptor ($CD3^+$:$CD25^+$), where all of the FMS treatment groups exhibited higher values than the HNC group (p = 0.02–0.08).

Thus, at baseline week 0, patients with FMS exhibited fewer total lymphocytes, fewer helper/inducer T lymphocytes, lower NK activity, a measurably larger number of B lymphocytes and a greater proportion of activated T helper/inducer subpopulations. Finally, there is evidence to suggest an increase in the expression of IL-2 receptors on $CD3^+$ T lymphocytes in FMS. The ratios of NK activity to NK phenotype for all three NK conditions were consistently lower for FMS than for HNC (data not shown).

Table 2. Comparisons of Blood Cell Populations and NK Cell Activities in the Peripheral Blood of Healthy Normal Controls and All Randomized FMS Patients at Baseline Week 0

Assay performed	*HNC*	*FMS*
Whole blood		
RBC (10^6/mm^3)	4.54 ± 0.09	4.51 ± 0.03
WBC (10^3/mm^3)	6.85 ± 0.40	7.03 ± 0.18
Lymphocytes (%)	36.72 ± 1.51	31.26 ± 0.85
Monocytes (%)	3.56 ± 0.66	4.55 ± 0.25
NK cell activity		
5:1 killer:target ratio	12.26 ± 2.21	10.61 ± 0.72
10:1 killer:target ratio	21.54 ± 3.56	18.51 ± 1.16
20:1 killer:target ratio	35.49 ± 5.06	33.40 ± 1.71

Abbreviations: HNC, healthy normal control; FMS, fibromyalgia syndrome; CD, cluster designation; NK, natural killer cell.

Statistics: Values shown are mean ± SD of gated cells stained by a listed antibody system or ± SD of NK activity.

Effects of low-dose sublingual use of IFN-α

Ninety (80.4%, placebo $n = 20$, 15 IU $n = 25$, 50 IU $n = 23$, 150 IU $n = 22$) of the 112 randomized FMS patients completed week 6 evaluation. Only 19.6% of the randomized patients were lost to follow-up by the end of the study, confirming the accuracy of the prestudy loss-to-follow-up projections. Chi-square testing found no association between treatment group and the occurrence of protocol violations or loss to follow-up.

All immunological variables were assessed for treatment effects in the form of changes from week 0 to week 6 among the randomized patients. The NK assay failed for 12 patients, so the n for that set of assays was slightly lower than for the FACS assays (placebo $n = 10$, 15 IU $n = 22$, 50 IU $n = 17$, 150 IU $n = 14$, total $n = 63$).

The changes observed with treatment (week 6 minus week 0) among the IFN-α FMS patient subgroups are shown in Table 4 (see also Fig. 1). The statistical analysis of these changes in each of the IFN-α-treated groups, contrasted with placebo treatment (value from IFN-α-treated group minus value from placebo-treated group), are also shown. The total numbers of red blood cells in the IFN-α-treated FMS patients consistently increased, but that may have been spurious because the placebo group value decreased and other groups remained stable.

The proportion of activated, $CD4^+$ T lymphocytes ($CD4^+$:$HLADR^+$) decreased among IFN-α-treated FMS patients relative to placebo (significant for 150 IU, $p = 0.05$ and marginally with 15 IU, $p = 0.06$). Because the subpopulation was elevated at baseline, it changed toward normal with IFN-α treatment. The $CD3^+$:$HLADR^+$ subset appeared to change in the other direction, increasing further with 15 IU IFN-α, $p = 0.06$. There was no treatment-specific change in the numbers of total NK cells (CD16, CD56, CD57) or B cells (CD19).

DISCUSSION

All FMS patient participants in this study met the published ACR criteria for primary FMS[1] and exhibited the typical clinical features of that disorder, with a female:male ratio of 11:1, a mean age of 46.9 years, moderately severe widespread body pain, prolonged morning stiffness, and insomnia. Therefore, the phenotypic and functional findings with the PBMC subpopulations are likely to be representative of persons with FMS. The main limitation of the data and the conclusions derived from this study would be sample size of the HNC group. Is the control group sufficiently large and well enough matched to the FMS group to conclude that it is representative of the general HNC population?

The comparisons of PBMC subpopulation-specific surface markers of medication-free FMS patients with those of HNC at the beginning of the study disclosed interesting differences of

TABLE 3. COMPARISON OF LYMPHOCYTE SUBPOPULATION DISTRIBUTIONS IN THE PERIPHERAL BLOOD OF HNC AND ALL RANDOMIZED FMS PATIENTS AT WEEK 0 BASELINE

Antisera Used in Assay	*Population Identified by Antisera*	*HNC* (n = *18)*	*FMS* (n = *112)*
T lymphocyte markers			
CD 2 (Leu 5) (%)	Pan-T, subset of NK, 80% of lymphs	80.89 ± 1.01	78.59 ± 0.64
CD 3 (Leu 4) (%)	Pan-T, 61–85% of all lymphs	73.83 ± 1.56	71.04 ± 0.76
CD 4 (Leu 3) (%)	Helper/inducer T, 28–58% of all lymphs	52.17 ± 2.61	49.01 ± 0.83
CD 5 (Leu 1) (%)	Pan-T, 70% of all lymphs	72.61 ± 1.39	66.23 ± 1.29
CD 8 (Leu 2) (%)	Cytotoxic/suppressor T, 19–48% of lymphs	28.22 ± 1.54	27.58 ± 0.67
CD4:CD8 (Th:TS ratio)		1.85	1.78
CD3 and HLA-DR (%)	Activated Pan-T	8.61 ± 0.96	10.90 ± 0.63
CD4 and HLA-DR (%)	Activated Helper/inducer T	7.56 ± 0.53	9.34 ± 0.39
CD8 and HLA-DR (%)	Activated Cytotoxic/suppressor T	6.67 ± 1.11	8.17 ± 0.53
B lymphocyte markers			
CD19 (Leu 12) (%)	Pan-B cells	10.11 ± 0.67	12.03 ± 0.44
CD19 and CD5 (%)	B cells with T cell markers, or vice versa	2.50 ± 0.41	6.46 ± 1.24
NK cell			
CD 16 (Leu 11a) (%)	IgG FcR3, on all NK, 15% of all lymphs	11.44 ± 1.18	12.50 ± 0.54
CD 16 and CD 2 (%)	Subset of NK with Fc Receptors	7.78 ± 0.98	8.54 ± 0.42
CD 56 (Leu 19) (%)	NCAM, 10–25% of lymphs	14.61 ± 1.35	15.46 ± 0.60
CD 57 (Leu 7) (%)	Subset of NK, subset of T lymphs	11.33 ± 2.11	17.10 ± 1.20
CD 56 and CD 57 (%)	Subset of NK or T lymphs, surface NCAM	6.56 ± 0.81	7.92 ± 0.47
Other markers			
CD 3 and CD 25 (%)	Subset of T, surface IL-2 receptors	9.33 ± 1.76	13.71 ± 0.69
CD 3 and CD 54 (%)	Subset of T, surface ICAM-1	24.78 ± 2.94	27.01 ± 1.25
CD 54 (Leu 54) (%)	ICAM-1 surface marker	20.39 ± 1.43	22.46 ± 0.70

Abbreviations: HNC, healthy normal control; FMS, fibromyalgia syndrome; CD, cluster designation; NK, natural killer cell; ICAM, intercellular adhesion molecule; NCAM, neural cell adhesion molecule, B, B lymphocyte; T, T lymphocyte; M, monocyte; HLA-DR, histocompatibility leucocyte antigen D region; IL-2R, interleukin 2 receptor; IgG FcR3, Fc receptor for immunoglobulin class G-3; lymphs, lymphocytes; surface, lymphocyte cell surface.

Statistics: Values, mean ± SD of gated lymphocytes staining with the listed antibody system.

TABLE 4. STATISTICAL CONTRASTS BETWEEN FMS GROUPS TREATED WITH DIFFERENT DOSAGES OF IFN-α OR PLACEBO: DIFFERENCES AT BASELINE AND CHANGE TO WEEK 6

Treatment group	*Placebo*		*15 IU*		*50 IU*		*150 IU*	
Assay	*Wk 0*	*Wk 0–Dif*	*Wk 0*	*Wk 0–Dif*	*Wk 0*	*Wk 0–Dif*	*Wk 0*	*Wk 0–Dif*
Whole Blood								
$^{RBC}/10^6$ mm^3	4.46	−0.32	4.42	−0.01*	4.56	0.05*	4.61	−0.05*,*
WBC/10^3 mm^3	6.59	0.03	7.15	0.34	7.33	−0.36	7.07	−0.06
Lymphocytes (%)	31.78*	−1.63	32.36*,*	−0.44	31.86*,*	3.43	29.04*	−3.95
Monocytes (%)	4.89	−0.05	4.25	−0.32	4.36	−0.43	4.71	−0.05
T lymphocyte markers								
CD 2 (Leu 5) (%)	80.79	−1.53	75.39*	0.08	78.50	−1.74	79.70	0.20
CD 3 (Leu 4) (%)	72.29	−1.53	68.82*,*	−0.24	70.50	−0.52	72.54	−0.71
CD 4 (Leu 3) (%)	52.14	−0.32	49.00	−0.76	45.54*	−1.09	49.36	−0.10
CD 5 (Leu 1) (%)	69.54	−1.58	67.11	−1.88	63.36*	−0.09	64.93*,*	1.67
CD 8 (Leu 2) (%)	27.14	−0.32	25.64	0.40	28.68	2.57	28.86	0.67
CD4:CD8 (Th:TS ratio)								
CD3 and HLA-DR (%)	11.04	−0.84	10.5	1.16*,*	10.71	−0.87	11.36	−0.24
CD4 and HLA-DR (%)	9.07	0.47	9.68	−1.00*,*	8.21	−0.43	10.39*	−1.14*
CD8 and HLA-DR (%)	7.89	0.05	7.82	0.64	7.79	0.43	9.18	−1.29
B lymphocyte markers								
CD19 (Leu 12) (%)	10.25	0.11	13.82*	0.20	11.93	−0.83	12.11	−0.38
CD19 and CD5 (%)	2.93	−0.53	4.61	1.28	10.21*	−1.43	8.07	−2.48
NK cell								
CD 16 (Leu 11a) (%)	13.39	0.53	11.75	−0.04	12.86	−0.22	11.96	0.90
CD 16 and CD 2 (%)	9.59	0.11	7.41	0.04	9.00	−0.30	8.14	0.71
CD 56 (Leu 19) (%)	15.07	−0.32	13.82	0.76	16.46	−0.30	16.46	0.33
CD 57 (Leu 7) (%)	15.79	3.21	19.36*	−1.36	17.14	3.39	16.11	−0.29
CD 56 and CD 57 (%)	7.39	−0.05	7.93	0.48	7.75	0.00	8.61	−0.52
Other markers								
CD 3 and CD 25 (%)	13.68*,*	−0.68	13.32*,*	−0.36	14.07*	−2.09	13.79*	0.24
CD 3 and CD 54 (%)	26.04	1.79	25.93	−1.88	29.57	−2.83	26.50	1.00
CD 54 (Leu 54) (%)	21.07	1.16	23.04	−0.08	23.43	−1.26	22.32	−0.67
NK cell								
NK 51	12.32	1.13	11.11	1.05	10.87	0.87	7.95*	1.51
NK 101	21.54	1.25	18.42	1.46	18.93	0.48	15.13*,*	0.94
NK201	38.55	0.56	32.53	0.32	33.46	0.29	29.31	2.50

Abbreviations: Wk0, baseline; Wk0–6Dif, changes from baseline to week 6 (week 6 minus baseline); IU, International Units of IFN-α activity; Others as in Table 3.

Statistics: Values at wk0, Mean. The values represent the percent of gated lymphocytes identified by a given antibody system; $^*p < 0.05$; $^{*,*}0.05 < p < 0.1$; week 0 contrasts were versus HNC; changes with IFN-α treatment from week 0 to week 6 were compared to changes with placebo treatment.

small magnitude. The FMS patients exhibited phenotypically fewer total lymphocytes and fewer resting helper/inducer T lymphocytes, whereas activated T lymphocyte subpopulations appear to be increased relative to the controls.

These findings are different from those reported in an abstract published by our group in 1988.[12] In that study, PBML from 21 FMS patients and matched controls were evaluated by FACS. The findings indicated increased numbers of resting CD4 and CD8 T lymphocytes and decreased numbers of T lymphocytes co-expressing HLA-DR. The current study was performed nearly 6 years after the first study, using slightly different technology, but the patients should have been comparable. No other explanation is readily apparent. On the other hand, our earlier study of NK activity in FMS[13] showed decreased NK activity as was observed in the present study.

A third abstract pertained to a portion of the data provided in the present study.[47] That abstract reported that $CD5^+$ T lymphocytes from FMS patients were co-labeled by antibody to the B cell antigen CD19. This binding of an anti-B lymphocyte antibody to a T lymphocyte was variable. It depended on the commercial source of the monoclonal antibody and upon a serum soluble factor of molecular weight >10,000 daltons.

The clinical relevance of these laboratory findings to the symptoms or overall health of people with FMS is unknown. FMS patients do not appear to be more or less susceptible to infection or to malignancy than the general population. The same is true for people with CFS in whom have been observed even more dramatic immune function abnormalities.[48,49]

The present study documented an increase in the proportion of $CD25^+$, $CD3^+$ T lymphocytes as might occur in response to a relative deficiency of IL-2. It was observed earlier that incu-

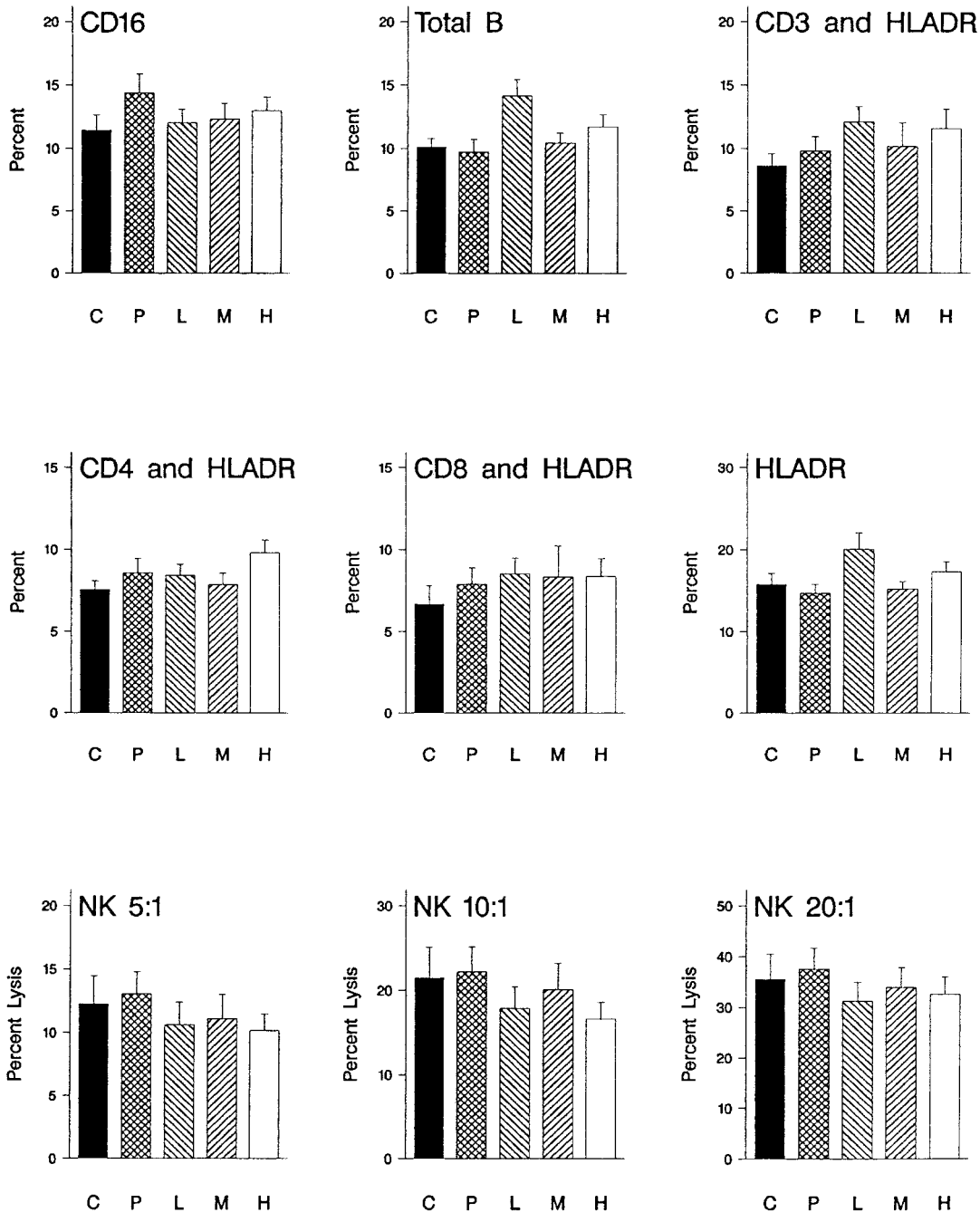

FIG. 1. Representative graphs from lymphocyte subpopulation distributions and from NK cell assays performed on blood collected from HNC (C) at week 0, and on blood from FMS patients at week 6 after daily treatment with either placebo (P) or IFN-α administered orally at 15 IU (L), 50 IU (M), or 150 IU (H). See Table 3 for identity of cell subpopulations detected by the named antibody systems. The values, plus 1 SEM, represent the percent of gated lymphocytes identified by a given antibody system. CD16, an FcR3$^+$, NK subpopulation, shows that the numerically higher values seen at week 0 normalized with all dosages of IFN-α but not with placebo. Total B cells that were high at week 0 seemed to decrease in all groups except the L group. CD3$^+$ and HLA-DR$^+$ (activated pan-T cells) were numerically elevated among FMS patients on week 0 and did not substantially change with treatment. The same can be said for CD4$^+$ and HLA-DR$^+$ (activated helper/inducer T cells) and CD8$^+$ and HLA-DR$^+$ (activated cytotoxic/suppress or T cells). The pattern with all HLA-DR$^+$ cells is similar to that of total B cells. NK activity tended to be numerically lower at week 6 for all IFN-α-treated patients compared with normal controls at week 0 or with FMS patients treated with placebo.

bation of NK cells from FMS patients with IL-2 increases NK activity.[13]

Indeed, other investigators[50] have found evidence for an IL-2-related defect in FMS. In their study, human PBML were stimulated by mitogens (concanavalin and/or phorbol myristate acetate) and the kinetics of IL-2 production by lymphocytes and $CD4^+$ T lymphocytes were characterized. Cell cultures from the FMS patients required higher concentrations of mitogen to achieve optimal IL-2 secretion and they exhibited a delay in the peak time of optimal IL-2 secretion. Addition of phorbol myristate acetate, which augments cytokine production independently of accessory cells, restored normal IL-2 secretion. These findings suggested that T lymphocytes from people with FMS may have a defect in the IL-2 pathway related to protein kinase C activation.

Our findings contrast with those of Hernanz *et al.*[51] who studied 65 FMS and 56 healthy controls with flow cytometry to document lymphocyte subpopulations. They reported that the number of T lymphocytes expressing activation markers CD69 (activation inducer molecular marker) and CD25 (IL-2 receptor) were significantly decreased in patients with FMS, and they proposed a defect in the T cell activation.

A clinical, therapeutic study of IFN-α treatment of CFS patients[52] indicated that a proportion of CFS patients may benefit from 3 months of thrice-weekly therapy with IFN-α. Although the number of patients involved in that study were too small to draw a firm conclusion, the temporal relationship of recovery with therapy and a significant association with coxsackie virus B immunoglobulin M (IgM) antibody in the same subjects raised the possibility of a treatment-mediated response. The authors proposed two explanations. If persistent coxsackie virus B IgM antibodies reflect chronic enterovirus infection, IFN-α may have acted by suppressing enterovirus replication. An alternative explanation was that the positive IgM antibody was due to an aberrant immune response and that the observed improvement related to the immunoregulatory properties of IFN-α.[40]

Because the pain component of CFS was believed to be that of FMS,[53] we hypothesized that IFN-α might prove to be beneficial with the widespread discomfort associated with FMS. In this case, FMS was studied instead of CFS because FMS is substantially more prevalent than CFS.[54–56] In conducting a phase III clinical study to follow-up on two apparently successful phase II studies conducted using inadequate sample sizes, it is recognized that the odds are only about 25% in favor of a statistically similar outcome for the phase III study.[57] Perhaps the same may apply to assessment of lymphocyte subpopulations.

In summary, a systematic assay of lymphocyte subpopulation phenotypes and NK activity in FMS patients and normal controls was conducted. The purposes were to determine whether there were differences between groups and to assess whether any potentially important changes would result from low doses of orally administered IFN-α in FMS patients. At baseline, before treatment, we found FMS patients have fewer than normal total lymphocytes, fewer resting helper/inducer T lymphocytes, and lower NK activity. By contrast, they exhibited a measurably larger number of B lymphocytes, a greater proportion of activated helper/inducer T lymphocytes, and increased numbers of IL-2 receptors on $CD3^+$ T lymphocytes. Treatment with low doses of orally administered IFN-α may have partially normalized the abnormal elevation of activated CD4 T lymphocytes. None of these changes in mononuclear leukocyte subpopulations can be directly associated with the clinical symptoms of FMS, but they do add additional fuel to the concept that subtle immunologic abnormalities may be present in FMS.

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