

Complement activation in a model of chronic fatigue syndrome

Bristol Sorensen, MS,^a Joanne E. Streib, BA,^a Matthew Strand, PhD,^b
Barry Make, MD,^c Patricia C. Giclas, PhD,^d Monika Fleshner, PhD,^e and
James F. Jones, MD^a *Denver and Boulder, Colo*

Background: A need exists to identify biological markers in chronic fatigue syndrome (CFS).

Objective: To use an exercise and/or allergen challenge to induce the symptoms of CFS and to identify a biological marker that correlates with these symptoms.

Methods: Patients with CFS (n = 32) and age-matched, normal control patients (n = 29) exercised for 20 minutes on a stationary bike at 70% of their predicted max work load (Watts). Patients from each group with positive skin test results were also challenged with intranasally administered relevant allergens. Symptoms were recorded for 2 weeks before and 1 week after each challenge, using 3 different instruments. Blood samples were taken before, and 0, 1, 6, and 24 hours after challenges. Levels of complement split products, cell-associated cytokines, and eosinophilic cationic protein were measured. Mean preexercise and postexercise symptom scores were evaluated for each group.

Results: Exercise challenge induced significant increases of the complement split product C4a, but not C3a or C5a, at 6 hours after exercise only in the CFS group ($P < .01$), regardless of allergy status. Mean symptom scores were significantly increased after exercise through the use of a daily diary ($P < .03$) and a weekly diary ($P < .01$) for the CFS group only. Mean scores for the Multidimensional Fatigue Inventory categories "reduced activity" and "mental fatigue" were significantly increased in the CFS group only ($P < .04$ and $P < .02$, respectively).

Conclusions: Exercise challenge may be a valuable tool in the development of diagnostic criteria and tests for CFS. Establishment of a role for complement activation products as markers or participants in production of illness require further study. (*J Allergy Clin Immunol* 2003;112:397-403.)

Key words: Exercise, allergy, pathogenesis, symptom production, chronic fatigue syndrome, complement activation, eosinophilic cationic protein

Chronic fatigue syndrome (CFS) in the United States remains an illness identified by a clinical definition published in 1994.¹ Although this definition has been tested and does identify patients with chronic illness (M. Reyes, R. Nisenbaum, D.C. Hoaglin, E.R. Unger, C. Emmons, B. Randall, J.A. Stewart, S. Abbey, J.F. Jones, N. Gantz, S. Minden, W.C. Reeves. Prevalence and incidence of chronic fatigue syndrome in Wichita, Kansas. In press), identification of pathologic markers of the syndrome remains an unfulfilled goal. The symptoms are similar to those seen in a systemic infection or in an inflammatory disease, such as rheumatoid arthritis, systemic lupus erythematosus, and a variety of other disorders. A hallmark of these illnesses is host immune system production of symptoms. Therefore, considerable work has concentrated on identifying potential alterations in specific and non-specific immune function,²⁻⁶ levels of cytokines,⁷⁻¹¹ mediators of inflammation,¹² and other cell products, including autoantibodies in up to 56% of patients¹³ that could contribute to the etiological factors of CFS.

The frequency and type of the immune alterations vary from study to study, suggesting a need for a more direct or focused approach. In addition, most studies in which immune alterations were observed have not addressed possible relations between the immune perturbations and mechanisms of illness production. One possible reason for the lack of consensus in these reports is that measurements are obtained at random time points in a prolonged and episodic illness. This issue can possibly be remedied by exposing patients to a situation known to induce an increase in symptoms. One such known symptom trigger, also part of the CFS definition, is physical exertion; the other trigger known to reproduce symptoms of CFS is exposure to allergens.

The incidence of atopy, confirmed by either positive skin test results or positive RAST results, has been reported to be as high as 50% to 83% in patients with CFS¹⁴⁻¹⁷ compared with 20% to 35% in the general population.¹⁸ Furthermore, patients with CFS examined in the clinical setting often report exacerbation of fatigue during their allergy season and after immunotherapy treatment. It is interesting to note that immune activation

From the ^aDepartment of Pediatrics, the ^bDepartment of Biostatistics, the ^cDepartment of Medicine, and the ^dDiagnostic Complement Laboratory, National Jewish Medical and Research Center, Denver, Colo; and the ^eDepartment of Kinesiology and Applied Physiology and Center for Neuroscience, University of Colorado at Boulder.

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Reprint requests: James F. Jones, MD, 1400 Jackson St, K913 Denver, CO 80206.

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Abbreviations used

CFS:	Chronic fatigue syndrome
ECP:	Eosinophilic cationic protein
CRP:	C-reactive protein
NJMRC:	National Jewish Medical and Research Center
SCID:	Structural Clinical Interview for DSM IV
CBC:	Complete blood count
ESR:	Eosinophil sedimentation rate
ANA:	Antinuclear antibody
TSH:	Thyroid-stimulating hormone
GCRC:	General clinical research center
MOS SF-36:	Medical Outcomes Study Short Form
MFI:	Multidimensional Fatigue Inventory
FEIA:	Fluorescence enzyme immunoassay
PBMC:	Peripheral blood mononuclear cells
TNF- α :	Tumor necrosis factor- α
RID:	Radioimmunodiffusion
LPS:	Lipopolysaccharide
PHA:	Phytohemagglutinin

markers present in patients with CFS are often indistinguishable from allergy cohorts. In one report, altered cell-associated cytokine levels and symptoms were present in patients with CFS, but the altered cytokine level effect in CFS could not be separated from the same changes in allergic subjects who did not have CFS.¹⁷ In another report, a serum protein also elevated in allergic states, eosinophilic cationic protein (ECP), was increased in both allergic and nonallergic patients with CFS.¹⁵

Observations based on results of screening for infection/inflammation in our clinic at the National Jewish Medical and Research Center (NJMRC) directed us to measure innate immune system parameters. When a history of physical activity yielding malaise or "flu-like" symptoms is given by a patient, general screening for systemic inflammation is performed by measurement of C-reactive protein (CRP), erythrocyte sedimentation rates, serum protein electrophoresis, serum immunoglobulin isotypes, and complement (C) split products. If patients have altered values in several of these parameters, further evaluation of specific infection/inflammatory diseases is necessary. In a majority of subjects, however, only elevated complement split products, C3a, C4a, and C5a, were consistently found.

Since subjects under evaluation of their fatiguing illness have exertion and allergen exposure as exacerbating factors of their illnesses, we chose to challenge allergic and nonallergic patients with CFS and otherwise healthy control patients with a steady-state bicycle exercise period, and challenge the allergic subjects in each group with nasal inhalation of subject-specific allergens. We also chose to measure prechallenge and postchallenge levels of illness symptoms, fatigue, cell-associated cytokines, complement split products, and ECP. The approach was designed to ask, if symptoms can be induced in patients who have fatiguing illnesses, would concomitant changes in select biological parameters occur as a consequence of the challenges?

METHODS

Collection of clinical samples for complement split products

Blood samples were obtained from consecutive patients evaluated in the adult clinic at the NJMRC for ongoing inflammation beginning in 1990. Control samples were derived from the Diagnostic Complement Laboratory's (part of the Clinical Reference Laboratory at NJMRC) control population.

Patients with CFS

Thirty-two patients with CFS from our outpatient consultation clinic volunteered for this study. Each of these patients met the current working definition¹ that includes the following criteria: fatigue lasting 6 or more months, no other illness that could explain the fatigue, 4 of 8 concurrent symptoms, fatigue is not relieved by rest, and fatigue interferes with occupational, educational, social, or personal activities. Of the 32 subjects with CFS, 23 were allergic and 9 were nonallergic (refer to screening procedures for determination of allergic status in study subjects). The age range of individuals was limited to 18 to 45 years. The ratio of women to men in the CFS group was approximately 3.5:1, which is consistent with other studies. All persons in the CFS group were white.

Control patients

Twenty-nine healthy control patients who responded to posted advertisements volunteered for this study. Of the 29 control subjects, 17 were allergic and 12 were nonallergic. The control subjects were similar in age, sex, and activity level (sedentary to moderately active) to the patients with CFS. The ratio of women to men in the control group was approximately 2.5:1. Persons in the control group included both whites and Asians.

Screening procedures

Study patients signed an informed consent for this NJMRC Institutional Review Board–approved study. Screening procedures included (1) medical history and physical examination performed by a physician, (2) mental status determination through computerized administration of the Structural Clinical Interview for DSM IV (SCID),^{19,20} (3) laboratory screening including complete blood count (CBC) with differential, hemoglobin, and hematocrit, eosinophil sedimentation rate (ESR), urinalysis, blood chemistries, ANA, and TSH, and (4) allergy screening through a battery of 24 skin prick tests with antigens common to the geographic area performed by registered nurses employed in the general clinical research center (GCRC). An allergic subject was defined as having 1 positive skin prick test result (3 mm) and a history of allergic rhinitis symptoms consistent with natural exposure to antigens identified in the positive skin test result(s). Subjects with any abnormalities in screening tests were excluded. Because of the possible confounding influence of asthma on complement activation, patients with history of asthma were also excluded.

Exercise challenge

All subjects performed a submaximal, steady-state exercise consisting of 20 minutes on a stationary bicycle ergometer. The work load was 70% of the subject's predicted maximum work load in Watts. A physician was present to monitor heart rate and heart rhythm by electrocardiography. Blood pressure was recorded every minute during the challenge.

Allergen challenge

Only patients with CFS and control patients with positive skin prick test results performed the nasal allergen challenge. Nasal aller-

gen challenges were performed according to standard techniques in routine use at the NJMRC. Appropriate allergens were chosen on the basis of history and skin test reactivity. We used allergens that the subject had not had recent (<6 weeks) natural exposure (eg, out of season pollen extracts or animal danders). Preservative-free allergens were prepared from stock solutions (Greer Laboratories, Lenoir, NC; ALK ABELLO, Round Rock, Tex; ALS A/S, Hørsholm, Denmark) and were diluted with sterile buffered saline. Serial 10-fold dilutions (1:10 to 1:100,000) were prepared and sprayed intranasally after a control inhalation of saline. Increasing concentrations were sprayed at 10-minute time intervals until symptoms of allergic rhinitis developed that were maximal for the subject (a symptom score of 5) or the highest test dose was achieved. Pulmonary function testing was performed before and immediately after the challenge. Challenges were not performed if the FEV₁ was 80% of predicted. Subjects were observed for 6 hours after the challenge for late-phase responses.

Histamine challenge

The histamine challenge was performed in all subjects to serve as a negative control for systemic responses that should only occur in the allergen challenge and to test the effect of induction of symptoms in nonallergic persons. Histamine will reproduce rhinorrhea, nasal itching, and sneezing, although it will not produce systemic activation (eg, cytokine production) or prolonged or recurrent signs and symptoms. Serial 10-fold dilutions (1:10 to 1:100,000) were prepared and sprayed intranasally after a control inhalation of saline. The initial concentration was a 10e-1 Molar solution (30.7 mg/mL) made from histamine-diphosphate salt #H7357 (Sigma, St Louis, Mo) and sterile, filtered PBS (pH 7.56). Increasing concentrations were used with the same protocol outlined above for the allergen challenge.

Blood collection

Blood specimens for each challenge were obtained immediately before, immediately after, and 1 hour, 6 hours, and 24 hours after each challenge. Heparinized specimens were processed immediately to obtain mononuclear cells. EDTA tubes for complement were immediately centrifuged at 4°, and plasma was frozen at -70°C. Blood for ECP was allowed to clot at room temperature for 2 hours; serum was removed and frozen at -70°C. Each blood parameter (cell-associated cytokine, plasma complement, and serum ECP) was evaluated at each of the 5 time points.

Symptom diaries

Patient complaints were recorded by using 3 instruments. A 10-symptom diary with a 0 to 4 scale (daily diary) was filled out each day for 2 weeks before and 1 week after challenge. Additionally, the MOS SF-36, a 36-question health-related quality of life measure with a 0 to 9 scale (weekly diary),²¹ and the Multidimensional Fatigue Inventory (MFI), a 20-item questionnaire designed to measure general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue,²² were filled out on the last day of each of the 3 weeks.

Complement

The split products C3a des Arg, C4a des Arg, and C5a des Arg were measured with the use of kits by RIA from Amersham-Pharmacia Biotech (Birmingham, United Kingdom). The C proteins SC5b-9 and Bb were measured by ELISA, with the use of kits from Quidel (San Diego, Calif).

Eosinophilic cationic protein

ECP levels were determined through the use of the Pharmacia CAP System, a fluorescence enzyme immunoassay (FEIA) (Pharmacia Diagnostics AB, Uppsala, Sweden).

ELISAs for cytokines

Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Paque (Amersham-Pharmacia Biotech, Piscataway, NJ) separation of heparinized venous blood. PBMCs were washed, centrifuged, and the cell pellets collected. PBMCs were lysed through 3 cycles of freeze-thawing, and protein was extracted as previously described.¹⁷ Commercial ELISAs were obtained for IL-1β, IL-6, IL-10, TNF-α, and IFN-α (Intergen Co, Purchase, NY).

Determination of complement protein concentrations

The C proteins C1 esterase inhibitor (C1 Inh), C1q, C1r, C1s, C2, C4, C3, C5, C6, C7, C8, C9, FB, FH, and FI were measured by radioimmunodiffusion (RID).

Data analysis

We hypothesized that the CFS group would respond to the exercise and allergen challenges with an increase in cell-associated cytokines, complement split products, and/or ECP immediately after the challenge, with levels of these proteins continuing to rise for 24 hours. We evaluated these blood parameters separately for each of the 3 challenges with a repeated-measures analysis by using PROC MIXED in SAS (Cary, NC). The data analyses reflect all patients with CFS versus all control patients because no differences were observed between the allergic and nonallergic subjects in either group. The blood collections taken over time within subjects constituted the repeated measures, and the analysis accounted for the fact that the measurement time points were unequally spaced. The data were generally highly right-skewed, thus natural log-transformed variables were analyzed. Post hoc multiple comparisons were only performed on variables and challenges for which significant differences between CFS and control groups were found, including group-by-time interaction. The Tukey-Kramer multiple comparison method was used.

We also hypothesized that the CFS group would have an increase in symptoms after the exercise and allergen challenges. For each group, the average prechallenge symptom score was subtracted from the average postchallenge symptom score to obtain a symptom change score for the daily diary, weekly diary, and the 5 fatigue categories of the MFI questionnaire. For each challenge, average symptom change scores were compared between groups by using 2-sample *t* tests, for the daily and weekly diaries. The unequal variance method was used because the CFS group generally had a substantially greater variance than the control group. A multivariate ANOVA (or MANOVA) was used to analyze the MFI data, in which each symptom category was used as a separate outcome variable.

C-activation products from the clinical population versus control subjects were analyzed by using 2-sample *t* tests. Average C-protein concentrations between CFS and control groups for those who participated in the study were also compared by using 2-sample *t* tests.

All reported *P* values in this article are based on 2-tailed tests, unless otherwise mentioned. It should be noted that the use of a 2-tailed test when the hypothesis involves an expected increase (or decrease) yields a conservative *P* value.

RESULTS

Clinical data

Levels of the C proteins C4a, C3a, C5a, SC5b-9, and Bb were all significantly elevated in patients seen in the adult clinic at the NJMRC compared with samples collected from healthy control patients (see Table I).

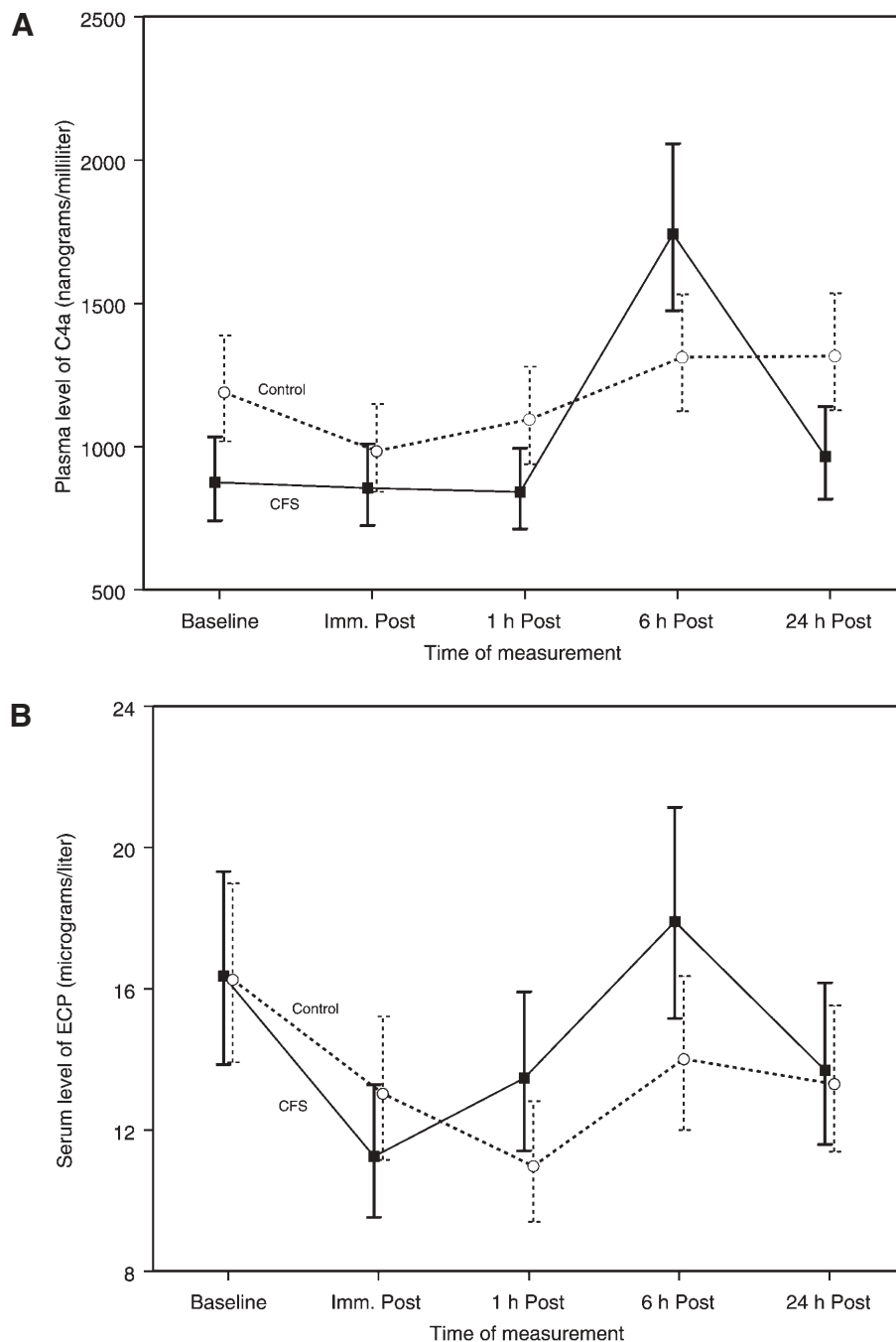


FIG 1. A and B, Estimates of mean C4a and ECP levels before and after exercise challenge for CFS and control populations, with standard error bars. Data were analyzed on the log scale and then inverted back for presentation, resulting in a longer +se bars than –se bars.

Study data

Exercise challenge. Twenty patients with CFS (15 allergic, 5 nonallergic) and 23 control patients (14 allergic, 9 nonallergic) completed the exercise challenge. The average work load performed by the control group (2227.6 W) was significantly higher ($P < .01$) than the average work load performed by the CFS group (1650.7 W).

In the repeated-measures analyses, there was significant group-by-time interaction for the C4a (Fig 1, A) and ECP (Fig 1, B) variables ($P < .04$ and $P < .05$, respectively) but not for C3a and C5a. The main effect of group was not significant for any of the variables. Post hoc tests were performed between pairs of time points within each group and between groups within each time, only for C4a and ECP.

The average C4a level for the CFS group had

increased by 6 hours ($P < .01$ between 6 hours and each of the previous times), then decreased between 6 and 24 hours ($P < .05$). The 24-hour average was not significantly different from the baseline, postchallenge, and 1-hour time points, indicating that the levels were returning to baseline after the previous increase. For the control group, there were no significant differences found between any pair of time points.

ECP was also elevated at 6 hours for the CFS group relative to the other times. However, in this case, the average response dropped from baseline to the postchallenge measure ($P < .02$), followed by an increase from postchallenge to 6 hours ($P < .001$), and then a moderate but not significant decrease to 24 hours. The control group also had a drop and rise in ECP levels, but only the decrease between baseline and 1 hour was significant ($P < .01$) (see Fig 1, B).

No significant differences from baseline or between groups were observed for the cell-associated proinflammatory cytokines IL-1 β , IL-6, TNF- α , and IFN- α or the anti-inflammatory cytokine IL-10. However, an interesting trend was observed: The CFS group means were elevated at 6 hours after exercise for each of the proinflammatory cytokines, whereas the control group means were decreased at the same time point (data not shown).

Mean preexercise and postexercise symptom scores were evaluated for both symptom diaries and the MFI questionnaire. Mean symptom change scores from preexercise to postexercise were higher for the CFS group than the control group, for both the daily ($P < .03$) and weekly ($P < .01$) diaries (see Table II). Additionally, mean scores for the MFI categories "reduced activity" and "mental fatigue" were significantly higher for the CFS group than the control group ($P < .04$ and $P < .02$, respectively), although the joint category test (MANOVA) did not detect strong differences between patients with CFS and control patients ($P = .14$).

In a follow-up analysis, Pearson correlations between change in C4a (6 hours minus baseline) and change in daily diary symptom scores (day 1 after challenge minus baseline) were determined for the patients with CFS. The hypothesis was that for each symptom, an increase in C4a would be associated with increased symptom scores. A significant correlation was found between the increase in C4a and total symptom score ($P < .05$) and the following individual symptoms: headaches ($P < .02$), joint problems ($P < .05$), and thinking difficulty ($P < .03$), through the use of 1-sided tests.

Allergen challenge

Fifteen patients with CFS and 10 control patients completed the allergen challenge outside of their respective allergy season. Nonallergic subjects did not participate. Although the levels of C split products and ECP were generally higher across all time points in the CFS group, no significant differences between groups were observed (data not shown). Similarly, the average symptom change scores reported by patients with CFS in the daily and weekly diaries were higher than that of the control patients but not significant (see Table II). The joint cate-

TABLE I. Levels of complement (C) components ($\pm$ SE) from patients seen in NJMRC adult clinic for chronic fatigue and healthy controls

C component	n =	Chronic fatigue	n =	Control subjects
C4a (ng/mL)	83	2202.1 $\pm$ 278.4*	31	1121.1 $\pm$ 98.6
C3a (ng/mL)	77	887.7 $\pm$ 102.7†	31	488.3 $\pm$ 32.8
C5a (ng/mL)	66	12.9 $\pm$ 0.5*	32	6.3 $\pm$ 0.4
SC5b-9 (μ g/mL)	74	154.2 $\pm$ 13.9*	26	91.8 $\pm$ 42.4
Bb (μ g/mL)	83	0.91 $\pm$ 0.03*	26	0.46 $\pm$ 0.03

*Significant relative to control patients, at $P < .001$.

†Significant relative to control patients, at $P < .005$.

TABLE II. Mean prechallenge to postchallenge change scores ($\pm$ SE) for daily and weekly symptom diaries for all challenges

	CFS	Control
Daily diary		
Exercise	2.10 $\pm$ 0.61*	0.10 $\pm$ 0.56
Allergen	1.53 $\pm$ 1.03	0.17 $\pm$ 1.26
Histamine	1.00 $\pm$ 0.76	0.61 $\pm$ 0.80
Weekly diary		
Exercise	27.7 $\pm$ 5.57†	0.20 $\pm$ 5.17
Allergen	10.5 $\pm$ 7.30	-0.20 $\pm$ 8.94
Histamine	11.9 $\pm$ 3.82	3.63 $\pm$ 4.02

*Significant relative to control patients at $P < .05$.

†Significant relative to control patients at $P < .01$.

gory test for the MFI did not indicate differences between patients with CFS and control patients, but the individual categories "physical fatigue" and "reduced motivation" were significantly higher for patients with CFS than for control patients ($P < .05$ and $P < .04$, respectively).

Histamine challenge

Twenty-two patients with CFS (15 allergic, 7 nonallergic) and 20 control patients (10 allergic, 10 nonallergic) completed the histamine challenge. As expected, no significant differences between the CFS group and control group were observed for C split products, ECP, or symptom scores for either the daily or weekly diaries or the MFI questionnaire.

Complement concentrations

After the study was completed, concentrations of C proteins were measured from baseline serum samples for 16 of the patients with CFS and 14 of the control patients to determine if there were mean differences between groups that might explain why C4a levels were elevated in the CFS group after exercise. There were no significant differences found between the CFS and control groups for any protein.

DISCUSSION

In this study, we hypothesized that challenges with either exercise or nasally administered allergens would exacerbate the defining symptoms of CFS and induce the

production of a biological marker or markers that correlate with an increase in these symptoms. The exercise challenge significantly exacerbated symptoms associated with CFS and generated reliable increases in the complement protein C4a. Furthermore, the increases in C4a were significantly correlated with postexercise symptom scores. The detection of this single protein (C4a) after exercise in conjunction with increased symptoms makes the development of a diagnostic test with an exercise challenge a real possibility.

The exacerbation of symptoms after exercise is seen only in the CFS population. Exercise does not appear to exacerbate symptoms in other disease states associated with fatigue such as depression or autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis. In fact, depressed persons usually report a relief of symptoms after exercise.²³⁻²⁶ Patients with rheumatoid arthritis tolerate moderate exercise as part of treatment without an exacerbation of clinical markers, immune markers, pain, or disease activity.^{27,28} Additionally, exercise intervention in patients with lupus and multiple sclerosis does not significantly increase fatigue²⁹ and in some cases reduces fatigue.³⁰⁻³²

The allergen challenge, as in the exercise challenge, was designed to exacerbate symptoms in patients with CFS. Even though increased physical fatigue and reduced motivation accompanied the allergen challenge, the lack of measurable changes in the biological parameters raises questions regarding the uniformity of mechanisms of fatigue production. It is possible that symptoms in the CFS group after the allergen challenge were related to untested allergy-related processes, including T_H2-mediated and related mechanisms. Future studies could evaluate patients with CFS and control patients undergoing allergen immunotherapy, a stimulus that might provoke a more robust response to allergens, allowing measurement of cytokines and other products related to atopy.

Exercise challenges triggered the activation of the classic pathway of the complement system generating C4a only in patients with CFS. In healthy, trained subjects, intense exercise such as a 30-minute run,³³ a 2.5-hour run,³⁴ and a graded maximal exercise test on a bicycle ergometer³⁵ led to increased C4a levels, with those levels returning to baseline values between 30 minutes and 3 hours after exercise. In our study, all subjects were untrained, and the intensity of the exercise bout was much less than what has been previously studied. These factors probably explain why our control patients did not show an elevation of C4a after the exercise challenge; these same factors make the elevation of C4a in patients with CFS even more unique.

Notably, mean levels of C4a before the exercise challenge for the CFS group were less than the mean levels before their allergen and histamine challenges, and less than the mean levels seen in patients with CFS from our clinical sample. Two possible explanations for this phenomenon include (1) patients with CFS only participated in the exercise challenge when they were feeling up to

the task or had rested in anticipation of increased exertion, or (2) this observation could represent a random phenomenon, since patients with CFS tend to fluctuate very dramatically in their illness states. These parameters are amenable to future testing.

Exercise increases circulating immune complexes containing IgG and IgM antibodies³³ and generates a variety of substances associated with muscle damage and the acute phase response that can activate the classic pathway of complement. These substances include CRP, DNA, cell membranes of altered cells, and plasmin.³⁴ CRP levels are rare in patients seen in our clinic. Their presence actually constitutes an exclusion to consideration of a diagnosis of CFS, and no subjects in this study had elevated levels of CRP. Autoantibodies are also known to activate the C system, but our subject population did not test positive for ANAs or rheumatoid factors, making at least these autoantibodies unlikely triggers of the C cascade.

Cell-associated proinflammatory cytokine levels in the CFS group were elevated from baseline values 6 hours after exercise, although those values were not statistically different from baseline values. We chose to assay levels of cytokines in unstimulated PBMCs to address possible *in vivo* changes. Past studies have demonstrated that *in vitro* stimulation with lipopolysaccharide (LPS) or phytohemagglutinin (PHA) significantly increase the levels of IL-1 β , IL-6, TNF- α , and IL-10 produced by PBMC cultures from patients with CFS versus control patients^{7,9,11}; however, these studies did not specifically address T_H2-mediated cytokines or allergic status of patients. Thus, the role of cytokines in the production of fatigue should be further investigated.

Even though patients with CFS were symptomatic during the 2 weeks before each challenge, their symptoms were significantly increased only after the exercise challenge. Symptoms scores at 24 hours after exercise were significantly correlated with the increase of C4a levels at 6 hours after exercise. Our study design did not have subjects report their symptoms in conjunction with blood draws during the 24-hour challenge period. Clusters of symptoms may change over the course of the first 24 hours after exercise; thus, documenting which symptoms are the most correlated with increases in C4a may give us clues as to which processes may be contributing to fatigue after exercise.

Challenging patients with CFS with exercise allows the study of this population during an exacerbated state of their illness. Correlation of an increase in symptoms after exercise with biological markers may be a key factor in devising diagnostic tests for CFS and other fatiguing illnesses. If standardized exercise reliably induces symptoms in patients, measurement of processes associated with the production of symptoms would naturally follow. Tools that are now available to address the latter issue include evaluation of gene expression at the messenger RNA and protein levels and protein function. At this point, we are regarding exercise-induced C4a as a potential marker of CFS. Until further research is done on the biology of C4a, assumptions about its possible role in the pathophysiology

of CFS are premature. Evaluation of a role for atopy per se or the cellular and innate humoral components seen in atopy in conjunction with factors such as exercise remains a more complex task, but one that seems important in view of the frequency of atopy in CFS.

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