

Short communication

Eosinophil cationic protein serum levels and allergy in chronic fatigue syndrome

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Chronic fatigue syndrome (CFS) is a syndrome of uncertain etiopathogenesis characterized by disabling fatigue associated with a variable number of somatic and/or neuropsychologic symptoms. In patients with CFS, several immunologic abnormalities can be detected, including a higher prevalence of allergy. The aim of this study was to determine whether CFS patients, well studied for their allergy profile, show signs of eosinophil activation, as detectable by the measurement in serum of eosinophil cationic protein (ECP) levels. In 35 consecutive CFS outpatients (diagnosis based on the Centers for Disease Control case definition), ECP was measured in serum by a competitive enzyme immunoassay (ECP-IEIA kit, Kabi Pharmacia Diagnostics, Uppsala, Sweden). Fourteen disease-free subjects with no history of CFS or allergy were selected as controls. ECP serum levels were significantly higher in CFS patients than in controls ($18.0 \pm 11.3 \mu\text{g/l}$ vs $7.3 \pm 2.1 \mu\text{g/l}$; $P < 0.01$). In the CFS population, the prevalence of RAST positivity to one or more allergens was 77%, while no control showed positive RAST. Twelve of the 14 CFS patients with increased ECP serum levels were RAST-positive. However, CFS RAST-positive patients had no significantly higher ECP serum levels than CFS RAST-negative patients ($19.3 \pm 12.4 \mu\text{g/l}$ vs $13.6 \pm 3.7 \mu\text{g/l}$; $P = 0.4$). This is the first report of increased serum levels of ECP in CFS. On the basis of the available data, it is discussed whether eosinophil activation has a pathogenetic role in CFS or is linked to the frequently associated allergic condition, or, finally, whether a common immunologic background may exist for both atopy and CFS.

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Chronic fatigue syndrome (CFS) is a pathologic condition characterized by disabling fatigue associated with various somatic and/or neuropsychologic symptoms such as myalgia, arthralgia, sore throat, low-grade fever, sleep disturbance, confusion, and depression (Table 1) (1–3). Specific diagnostic tests for CFS are not available; therefore, diagnosis is usually made by ruling out other diseases after a series of diagnostic investigations (1, 4).

The etiopathogenesis of CFS is unknown. However, several immunologic abnormalities have been detected in patients with CFS, suggesting the possible participation of the immune system in the pathogenesis of this disease (5, 6). It has also been suggested that in CFS immune activation can be linked to various agents, both infectious (e.g., Epstein-Barr virus, cytomegalovirus, herpes simplex

virus) (7, 8) and chemical (e.g., tryptophan, silicone) (9, 10).

CFS has been reported to be associated with allergy: almost 65% of patients report a history of inhalant, food, or drug allergies and show IgE antibodies to common allergens, as revealed by skin tests and/or RAST (radioallergosorbent test) (5, 11, 12). However, in allergic diseases, it is known that IgE antibody plays a role in the activation and degranulation of mast cells mainly responsible for the early immediate phase of the allergic reaction. On the other hand, eosinophil inflammation is more important for the late phase of allergic reaction and persistence of symptoms (13). Therefore, eosinophils – more than IgE antibodies – might be relevant in diseases with a chronic evolution.

Eosinophils release several granule cationic pro-

Table 1. Diagnosis of CFS must fulfill both major criteria and four or more minor criteria

Major criteria

1. New onset of persistent or relapsing fatigue lasting 6 months and not substantially alleviated by rest
2. Other conditions producing fatigue must be ruled out

Minor criteria

Symptoms criteria – beginning at or after onset of fatigue and persisting or recurring for at least 6 months

1. Impaired memory or concentration
2. Sore throat
3. Tender cervical or axillary lymph nodes
4. Muscle pain
5. Multijoint pain without joint swelling or redness
6. Headache of new type
7. Unrefreshing sleep
8. Postexertional malaise lasting more than 24 h

Adapted from Fukuda et al. (12).

teins, which are harmful to tissues. Among these mediators, eosinophil cationic protein (ECP) has been well characterized and studied (14). ECP can be easily measured in serum (15), and increased levels of ECP have been reported in several diseases with eosinophil participation such as allergic conditions and parasitosis (16–22).

The aim of this study was to determine whether CFS patients well studied for their allergy profile show signs of eosinophil activation as detectable by the measurement of ECP serum levels.

Material and methods

Thirty-five consecutive CFS outpatients (25 women and nine men; mean age 37.7 years; age range 24–54 years) diagnosed on the basis of the CDC (Centers for Disease Control) case definition (Table 1) were enrolled in this study, after informed consent. For exclusion of other possible causes of persistent fatigue, each subject underwent clinical evaluation and several tests, i.e., complete blood count, serum electrolytes, blood urea nitrogen, glucose, creatinine, thyroid function tests, ESR, ALT, AST, bilirubin, alkaline phosphatase, CPK, LDH, aldolase, iron, ferritin, transferrin, serum total protein levels and electrophoresis, HIV, HBV, and HCV antibodies, antinuclear antibodies, C3 and C4 complement levels, serum IgG, IgA, and IgM concentration, and lymphocyte subsets immunophenotyping.

Fourteen disease-free, age- and sex-matched subjects with no history of CFS or allergy were selected as controls.

Venous peripheral blood (10 ml) was drawn into preservative-free tubes. Blood was centrifuged at 1500 g for 10 min at room temperature, and serum was stored at -20°C until assayed.

ECP was measured in serum samples by means

of a competitive enzyme immunoassay (ECP-ELISA kit, Kabi Pharmacia Diagnostics, Uppsala, Sweden), according to the manufacturer's instructions. The standard curve range was 2–200 $\mu\text{g/l}$. With this procedure, normal ECP serum ranges are 2–16 $\mu\text{g/l}$, as stated by the manufacturer.

Serum measurement of specific IgE (RAST) to a panel of common food and inhalant allergens – including ryegrass, *Dermatophagoides pteronyssinus*, *Parietaria officinalis*, *Olea europaea*, *Alternaria*, milk, egg, and fish – was performed by the RAST-RIA test (Pharmacia, Uppsala, Sweden). We considered RAST-positive those patients showing even class I positivity for at least one of the allergens tested.

For statistical analysis, a nonparametric test (U Mann-Whitney) was performed. A *P* value of 0.01 or less was considered statistically significant.

Results

CFS patients had significantly higher serum ECP levels than controls: $18.0 \pm 11.3 \mu\text{g/l}$ ($\pm\text{SD}$) vs $7.3 \pm 2.1 \mu\text{g/l}$ ($\pm\text{SD}$); $P < 0.01$ (Fig. 1). Fourteen CFS patients (40%), but no control, showed increased ECP serum levels when 16 $\mu\text{g/l}$ was considered the upper limit of normality.

Twenty-seven of 35 CFS patients (77%) presented specific IgE directed against one or more allergens, while no control showed positive RAST. Of 27 RAST-positive patients, only nine had a history of allergies to foods or inhalants after a detailed interview; however, establishing a relationship between symptoms of allergy and CFS was impossible.

Twelve of the 14 CFS patients with increased ECP serum levels were RAST-positive to one or more allergens. ECP serum levels were $19.3 \pm 12.4 \mu\text{g/l}$ in RAST-positive vs $13.6 \pm 3.7 \mu\text{g/l}$ in RAST-negative patients ($P = 0.4$).

Discussion

ECP serum levels have been reported to be elevated in several diseases and were considered a marker of eosinophil activation and disease severity (16–22). This is the first report of increased ECP serum levels in CFS, a disease still awaiting clarification of its pathophysiologic mechanisms and suitable diagnostic tests.

Our data also indicate that in CFS the prevalence of sensitization to common allergens (as shown by a positive RAST) is particularly high (77% of cases), and significantly higher than that of our control healthy population. This prevalence is still definitely higher than expected even if compared to that of a random population, where the prevalence of a positive RAST has been reported to be 12.8%

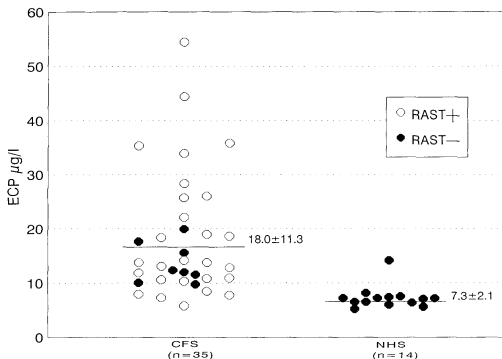


Fig. 1. ECP serum levels in 35 CFS patients and in 14 NHS (normal human subjects). Straight lines indicate mean values $\pm$ SD. White symbols represent RAST-positive sera and black ones RAST-negative sera.

in subjects without a family history of atopy and 15.0% in subjects with a family history of atopy (23, 24). Therefore, the association between CFS and allergy does not seem to be casual, confirming previous findings of a high prevalence of allergy in CFS patients (5, 11, 12).

Interestingly enough, however, in our study, only nine of the 27 CFS patients with a positive RAST also had a history of clinical allergy. Therefore, a high prevalence of positive RAST, even in the absence of clinical symptoms, should prompt the routine performance of allergy tests in CFS patients, in order to clarify the clinical or prognostic relevance of subclinical sensitization in these patients.

The relationships among eosinophils, CFS, and allergy might have important pathophysiologic and clinical implications. On the basis of our data, three different, although not mutually exclusive, interpretations can be discussed.

Firstly, eosinophil activation plays a pathogenic role in CFS, and some clinical manifestations of this syndrome might be ascribed to eosinophil neurotoxic and immunomodulatory products. In fact, ECP and other inflammatory mediators of eosinophils are released after antigen exposure in both allergic and nonallergic conditions and cause tissue inflammation and damage (25). Moreover, by increasing their *in vitro* activity, ECP shows immunomodulatory effects on T-suppressor cells (26). Finally, as observed in a murine model after

intrathecal injection, ECP possesses neurotoxic properties and causes a paralytic syndrome (27).

Secondly, the increased ECP serum levels in CFS are not a distinct feature of this disease but, on the contrary, represent an epiphenomenon related to the coexisting allergic condition, which itself causes an increase in ECP serum levels. In fact, high ECP serum levels were more frequently found in RAST-positive subjects, and ECP serum levels in CFS RAST-positive patients seem to be higher than in CFS RAST-negative patients. However, not all CFS patients with high ECP serum levels were RAST-positive, and the difference in ECP serum levels between RAST-positive and RAST-negative patients did not attain statistical significance. Moreover, the increase of ECP was not related to the severity of the concurrent allergic disease.

Thirdly, a common pathophysiologic background including eosinophil participation might be shared by both CFS and allergy. It has been shown that allergic diseases are characterized by a distinct cytokine profile, called Th2, and including interleukin (IL)-5, IL-4, IL-3, and GM-CSF (28, 29). In particular, IL-5 plays a key role in regulating eosinophil differentiation, activation, degranulation, and survival (30). It might be speculated that a common Th2 profile is present in both allergy and CFS. In fact, no final evidence of the cytokine profile of CFS patients is available (31, 32).

In conclusion, we have shown increased serum

levels of ECP and increased allergy prevalence in CFS patients. CFS is probably a multifactorial syndrome in which various stimuli, as well as different predisposing factors, interact in inducing the wide spectrum of its clinical manifestations. We suggest that a better understanding of the role of eosinophil activation and allergy and/or of their pathophysiological basis in CFS may be of help for a more suitable approach to the individual CFS patient.

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