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Anticardiolipin Antibodies in the Sera of Patients with Diagnosed Chronic Fatigue Syndrome

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Examination of anticardiolipin antibodies (ACAs) in the sera of patients clinically diagnosed with chronic fatigue syndrome (CFS) using an enzyme-linked immunoassay procedure demonstrated the presence of immunoglobulin M isotypes in 95% of CFS serum samples tested. The presence of immunoglobulin G and immunoglobulin A isotypes were also detected in a subset of the samples. Future studies will focus on elucidating whether alterations to mitochondrial inner membranes and/or metabolic functions play a possible role in the expression of ACAs. J. Clin. Lab. Anal. 23:210–212, 2009.

Key words: chronic fatigue syndrome; anticardiolipin; cardiolipin; ELISA

INTRODUCTION

Recent competitive inhibition assays inferred the presence of the phospholipid cardiolipin (CL) in serological samples from patients clinically diagnosed with chronic fatigue syndrome (CFS), suggesting that "acute phase lipids" may be part of disease pathogenesis in patients with CFS (1). These lipids may be analogous to "acute phase proteins" triggered by cytokines involved in the inflammatory processes in the liver such as C-reactive protein and serum amyloid A, which have been reported in several disease states (1). This study examines the sera of CFS patients for anticardiolipin antibodies (ACAs) and demonstrates that 95% of CFS samples tested showed ACA of the immunoglobulin M isotype in patient sera.

Certain marine toxins such as ciguatoxin are taken up by the liver and produce symptoms similar to CFS. Testing for antibodies to CL is routinely performed as one of a panel of tests for autoimmune disorders (2). In our studies, the presence of ACA at relatively high titers in patients with CFS suggests the possibility of alterations to the inner membranes of liver mitochondria, thereby exposing CL in a manner so as to elicit an antibody response to CL.

MATERIALS AND METHODS

CFS Patient Serum Collection

A total of 40 serum samples from individuals (females, $n = 24$, age range: 25–71; males, $n = 16$, age range: 26–77) clinically diagnosed with CFS were obtained from patients' physicians from various regions of the United States. The criteria for clinical diagnosis of CFS were based on Fukuda et al. (3) as accepted by the Centers for Disease Control in Atlanta, Georgia.

Enzyme-Linked Immunoassay Method for ACA

The enzyme-linked immunoassay (ELISA) method was performed according to the instructions from the commercial ELISA kit to quantify IgA, IgG, and IgM ACA (QUANTA Lite ACA Anticardiolipin Kit, INOVA Diagnostics Inc., San Diego, CA).

Briefly, polystyrene microwell ELISA plates coated with a purified CL antigen were incubated with a dilution of the serum sample for 30 min at 18–22°C. The plates were washed three times with phosphate-buffered saline, and then incubated with 100 μL goat anti-human IgA, IgG, or IgM peroxidase conjugate under the same conditions. After additional washes, the plates were

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incubated with 100 μL hydrogen peroxide plus tetramethylbenzidine in the dark for 30 min at 18–22°C. The enzymatic reactions were stopped with 0.344 M sulfuric acid and absorbance at 450 nm was measured using a microplate reader. The ACA titer of each serum sample was calculated using a reference curve consisting of five standards of known concentrations of IgA, IgG, or IgM ACA. The ACA titer was reported as standard IgA anticardiolipin units (APL), standard IgG anticardiolipin units (GPL), or standard IgM anticardiolipin units (MPL), and was reported as positive for concentrations at or above 20 phospholipid (PL) units, and negative for concentrations fewer than 20 PL units.

RESULTS

The ELISA results for ACA isotypes of the 40 serum samples from patients previously diagnosed with CFS are shown in Table 1. In addition to CFS, several patients also had confirmed diagnoses of type 2 diabetes, chronic lymphocytic leukemia (CLL), and fungal allergies.

The IgM isotype of ACA was present in 95% of the samples tested (38/40). The IgG isotype was present in 10% of the samples tested (4/40), and the IgA isotype was found in 2.5% of the samples tested (1/40). All four serum samples that were positive for IgG were also positive for IgM. Only one patient sample was positive for all three isotypes.

DISCUSSION

A survey of the literature reports ACAs as common serological markers in many different types of diseases, including viral diseases such as illnesses resulting from chemical (1) and marine toxin exposure (4,5,6), HIV (7,8) and Epstein-Barr virus (9), hematological cancers including CLL and acute myelocytic leukemias, exposure to fungal organisms, malaria, and staphylococcus infections (10,11), and autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, autoimmune hepatitis, and more (2). This study demonstrates that a large percentage of patients clinically diagnosed with CFS have elevated levels of the IgM isotype to CL (95%), suggesting that CFS may be an autoimmune condition.

For comparison, in clinically "normal" individuals, which can be generally classified as those who meet the following criteria: (a) no severe diseases, (b) no drug or alcohol dependence, (c) no clinical or laboratory evidence of systemic lupus erythematosus or other autoimmune disorder, and (d) no antibodies that might cross react with ACAs, one study showed that 77.3% (180/233) of assayed individuals had negative ACA titers, 15.0% (35/233) were considered low positives, and 7.7% (18/233) had moderately high titers (12). The study identified 38 (16.3%) subjects with isolated IgG isotype and 11 (4.7%) with isolated IgM isotype elevations, with four participants (1.7%) found to have both ACA isotypes increased, although it should be noted that ACA-positive subjects were slightly older and had a somewhat higher rate of cardiac disease than ACA-negative subjects (12).

As a possible autoimmune disease, CFS patients may be treated by suppression of the ACA or by diminishing the antigen CL in serum. Previous studies have shown that treatment with monoclonal antibodies to B cells reduces ACA levels to normal in patients with autoimmune disease, leading to clinical improvements. Specifically, Rituximab, a chimeric monoclonal CD20 antibody, has been shown to normalize high ACA serum titers of patients with autoimmune systemic lupus erythematosus, rheumatoid arthritis, autoimmune thrombocytopenia, and autoimmune hemolytic anemia. Rituximab may serve as an effective therapeutic agent for ameliorating the symptoms of CFS (11,13). Therefore, classification of CFS as an autoimmune disorder may serve to increase the availability of treatment options for patients suffering from the disease.

Experiments are underway to further elucidate why ACAs are produced in individuals afflicted with CFS. Such studies include investigating the effects of specific chemical agents, marine toxins, and ACAs on mitochondrial metabolic pathways that are indicative of reduced or blocked energy production that may lead to the fatigued state in CFS. Such studies may lead to the development of potential therapeutic agents to block or reduce such interactions.

TABLE 1. Number of Patients With ACA ELISA Scores for Immunoglobulin Isotypes G, M, and A ($n = 40$)*, Where + Scores Indicate Results > or = to 20 Phospholipid (PL) Units

G+ M+	G+ M−	G− M+	G− M−
4	0	34	2

*One patient sample had IgM+ (34.97 PL units), IgG+ (98.28 PL units), and IgA+ (29.30 PL units). IgM+range: 23.83–98.28 PL units; IgG+range: 22.86–34.97 PL units.

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