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Chronic Phase Lipids in Sera of Several Chronic Diseases Reacting with MAB–CTX (Antibody to Ciguatoxin)

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ABSTRACT

The membrane immunobead assay results on the acetone lipid fraction of serum from chronic fatigue syndrome (CFS) patients (60 samples) and normal individuals (with no clinical CFS or other disease symptoms) showed significant differences with 4 exceptions (4 normals showed 1:40 and 1:80 titres). This represented approximately 10.8% of the normal samples, with 3 samples at 1:20, the majority of the CFS titred 1:40 through 1:160. This represented 95.0% of the samples. The small numbers of hepatitis patients and chronic ciguatera fish poisoning patients also had titres of 1:40 to 1:80 in all of the serum samples examined. The weights of the lipids in mg/ml serum essentially are very similar, except 1 or 2 of CFS and hepatitis B showed values at the upper level. Comparison of sexes showed 65% females and 35% men with CFS, representing a ratio of approximately 2:1 (female/male). It is concluded that certain disease conditions and environmental exposures to deleterious factors (toxin, chemicals, microorganisms) trigger the release of lipids (probably by the liver) with similar epitopes to ciguatoxin, and that they react with MAb–CTX. We designate these lipids as ''chronic phase lipids'' comparable to ''acute phase protein'' in inflammatory and traumatic diseases.

Key Words: Chronic phase lipids; Chronic fatigue syndrome; Chronic ciguatera fish poisoning; Hepatitis B; Ciguatera; Membrane immunobead assay (MIA).

I. INTRODUCTION

The clinical literature on chronic fatigue syndrome (CFS) and chronic ciguatera fish poisoning (CCFP) show great similarities in symptomologies (Palafox and Buenconsejo-Lum, 2001; Pearn, 2000,2001). The clinical similarities reported in the literature suggested it might be useful to explore lipids in sera of patients with CFS, CCFP, as well as other diseases with the

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membrane immunobead assay (MIA) used for screening ciguatera fish (Yasumoto and Murata, 1993).

CFS is initiated by a variety of causative factors. The initial phase includes exposure to chemical and other organic pollutants (gulf war veterans), exposure to marine toxins (ciguatera fish poisonings), exposure to viruses (Epstein virus in infectious mononucleosis), exposure to allergenic antigens (sick building syndromes), and other hypersensitivity problems (Racciatti et al., 2001; Straus, 1988).

The MIA procedure for detecting ciguatoxin (Hokama et al., 1998) is used in this study with modification in the preparation of the lipids from the serum samples. One ml of serum was treated with 4 ml of absolute acetone, mixed thoroughly and the suspension centrifuged at 1000 rpm for 10 min at 20°C. The clear light-to-yellowish acetone solution was poured into a clean tared 15 × 100 mm test tube. The acetone phase was evaporated by an air jet in the hood for 16 hours. The dried sample was weighed with an analytical balance. Weight in mg/ml of serum was recorded for each specimen. The lipid residue was dissolved in 1 ml of methanol and tested in the MIA with MAb–CTX–latex suspension. The methanol-soluble lipid was tested undiluted and diluted 1 to 5 serially to 1 to 160 in methanol. The undiluted and diluted methanol solutions were tested in the MAb–CTX–latex suspension assay and the endpoint titer scored. The MIA procedure is described in the materials and method (Hokama et al., 1998).

In this study it was of interest to examine CFS and CCFP sera with the membrane immunobead assay (MIA) using MAb–CTX (monoclone antibody to ciguatoxin) for analysis of the lipids of these diseases (Hokama et al., 1998). Titers were obtained with extracted lipids from each sera examined. Using the MIA test, it was shown that serum samples from patients with CFS, CCFP and Hepatitis B appeared to have high titers in the acetone-soluble lipids. The normal individuals sera showed 11.0% with titers of 1:40 and 1:80, similar to CFS. Since the lipid(s) reacting with MAb–CTX is present in some normals and potentially other diseases, we are suggesting that this lipid be designated as ''chronic phase lipid'' comparable to ''acute phase proteins'' in inflammatory diseases and other traumatic injury (Hokama, 1982).

II. MATERIALS

A. Chronic Fatigue Syndrome (CFS) Sera

Patients who were selected satisfied the criteria of diagnosis for chronic fatigue syndrome. CDC guidelines were adhered to and the CFS definitions of

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Fukuda et al. (1994) and the text Harrison's 15th edition, Principles of Internal Medicine (Braunwaed et al., 2001) were used as references for defining CFS. Sixty CFS samples were obtained from various parts of the country, although a large majority was from the state of New York. A female-to-male ratio of approximately 2:1 sera samples were obtained in the 60 samples.

Blood was withdrawn by veni-puncture. The serum was separated and shipped frozen with dry ice to the Department of Pathology in Honolulu, Hawaii within 48 hours by Federal Express. The serum was immediately thawed upon arrival and was treated with acetone (see methods).

B. Acute Hepatitis B Sera

Eight sera samples examined from local hospitalized patients undergoing interferon therapy for acute hepatitis B. The sera were stored at $-76^{\circ}C$ until ready for use.

C. Chronic Ciguatera Fish Poisoning (CCFP) Sera

Four samples were from patients with initial acute phase ciguatera which then converted to a chronic status. These patients were exposed to a ciguatera-positive fish initially.

D. Normal Sera

Thirty-three sera samples, frozen at $-76^{\circ}C$ previously used for other studies, were thawed and used as negative controls. Four recently obtained samples not in the CFS clinical category were also examined in this study and placed in this category.

III. METHODS

The MIA was primarily established for the testing of ciguateric fish. It was carried out as follows: a piece (50 ± 0.5 mg) of fish soaked in absolute methanol (0.5 ml) for 20 min in the presence of a hydrophobic membrane attached to a plastic template. The membrane was air dried and then soaked in the MAb–CTX coated latex polystyrene bead (blue colored) for 10 min. This is followed by washing the membrane in tap water, drying and examining for blue color (Hokama et al., 1998). The procedure for testing lipids from serum was identical except for the preparation of serum lipids. One ml of serum was treated with 4 ml of acetone and the precipitate,

mainly proteins, was centrifuged at 1000 rpm for 10 min at 20°C. The yellowish acetone supernatant was decanted into a tared 15 × 100 mm test tube. The acetone was then evaporated with a jet stream of air in the hood for 16 hours. The sample in the test tube was weighed and the sample weight in mg obtained minus the tared weight of the test tube.

A. Assessment of the Acetone Soluble Serum Fraction in the MIA Procedure

The air dried lipids were dissolved in 1 ml methanol. The membrane plastic end of the test stick was immersed in the methanol solution for 10 min. The membrane was removed and air dried, then immersed into the blue latex MAb–CTX suspension for 10 min. The membrane was then removed and washed with tap water, dried and the color intensity was recorded. Titration was carried out by diluting 0.2 ml of the undiluted lipid solution with 0.8 ml methanol to give a 1:5 dilution. This 1:5 dilution was diluted further serially up to 1:160 in methanol. Each of the dilutions was tested in the MIA with the MAb–CTX–latex solution. The blue color intensity recorded for each dilution and the undiluted scored as 2+, +, ±, or – and the results recorded for each serum sample. The endpoint represented the last dilution with a ± value.

IV. RESULTS

The MIA assay results for sera from normal individuals, CFS, CCFP and acute hepatitis are summarized in Figure 1. The 60 CFS sera examined showed 57 (95%) with titers of 1:40 or higher, with 3 (5%) with 1:20 titer, in contrast 33 (89%) normal sera showed titers of less than 1:10, while 4 (11%) had titers of 1:40 (Pearn, 2000) and 1:80 (Pearn, 2000). The small

Table 1. Distribution of titres with Mab–CTX of male and female in the CFS samples.

Titre	Male	Female	Total
160	4	12	16
80	13	16	29
40	2	10	12
20	2	1	3
Total (%)	21 (35%)	39 (65%)	60 (100%)

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Table 2. Comparison of total lipids in mg/ml serum sample (chronic phase lipids).

Category	Number	Mean weight ± S.D.	Range
CFS	56	10.22 ± 2.46	6.3–18.2
Hepatitis B	8	11.9 ± 3.4	7.4–18.0
Normal	37	6.44 ± 2.19	1.5–14.0

number of acute hepatitis B (Hokama, 1982) and CCFP (Yasumoto and Murata, 1993) all showed titers of 1:40 (Straus, 1988) and 1:80 (Straus, 1988), none less than 1:40. The CFS, CCFP and acute hepatitis B sera samples all appeared to be similar with higher levels of lipid.

Further, the sex distribution showed 39 (65%) specimens of female sera in contrast to 21 (35%) males with a ratio of approximately 2:1, F:M. This is shown in Table 1 for the CFS samples analyzed in the MIA test.

Table 2 summarizes the weights of lipid in mg/ml of serum from each specimen studied. The weights represent the mean ± S.D. of each category including the normal sera. There appears to be slight but significant differences among the diseases as compared to normal values with a higher concentration in CFS and hepatitis B than in the normal specimens.

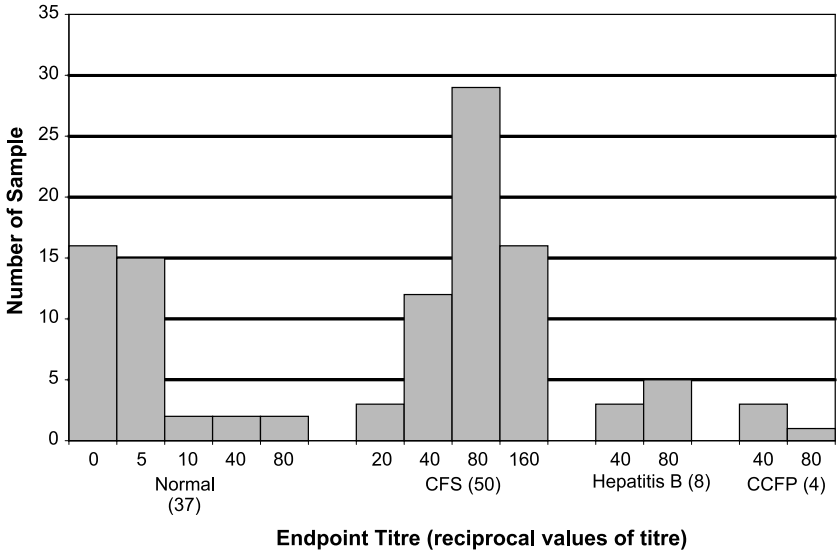

Figure 1. Summary of each category of diseases showing numbers (y-axis) and endpoint titres (x-axis).

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V. DISCUSSION

The data obtained with the MIA test for ciguatoxin with MAb–CTX definitely showed higher levels of acetone-soluble lipid resembling ciguatoxin in CFS, CCFP, and acute hepatitis B sera. The nature of this observation is an intriguing one and will require much more study. The immunological interaction between MAb–CTX and CFS lipids may merely be a cross-reaction, but this nevertheless, suggests common structural epitopes in CFS and ciguatoxin from moray eels and other ciguateric reef fishes.

This increase in ''chronic phase lipids'' in CFS, CCFP, and acute hepatitis B poses an interesting phenomenon. At this time, it is not known whether the lipids are abnormal in structural conformation or merely an increase in normal lipids of significant physiological function causing the symptomology showing in these diseases. Further studies in the chemistry of the acetone soluble ''chronic phase lipids'' will be needed to resolve the questions left unanswered. It is postulated that the ''chronic phase lipid'' involved may merely be comparable to the ''acute phase proteins'' such as c-reactive protein of inflammatory and traumatic diseases (Hokama, 1982). In addition, further MIA tests will be carried out with sera of as many diseases as possible to determine the extent or presences of ''chronic phase lipids'' (CPL). Patients will be examined for CPL over a long period to determine the concentrations in blood samples of CFS or CCFP patients.

Observation of high levels in acute hepatitis B and CCFP suggest the liver as a possible source of CPL, and as it is the major organ in the toxin detoxification process. It is suggested that routine laboratory liver function test be thoroughly examined in patients with CFS, CCFP, and hepatitis in addition to the MIA for CPL.

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