DETECTION OF CIRCULATING WUCHERERIA BANCROFTI ANTIGEN, FILARIA SPECIFIC IgG AND IgG4 IN CHYLURIA CASES IN JAPAN

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Abstract: Serum samples from Japanese chyluria patients were examined for filaria specific antibodies and a circulating filarial antigen in order to know if the symptom was filarial in origin. All the sera were negative for the circulating antigen. Anti-Brugia pahangi antibodies were detected in 6 out of 16 serum samples by ELISA after absorption of the sera with Anisakis and Dirofilaria immitis antigens. One of the positive sera showed a high titer for anti-B. pahangi IgG4, suggesting that Wuchereria bancrofti adults were surviving in the patient in recent years. Detection of antibodies would be helpful for immunodiagnosis of filarial chyluria in Japan, where filarial origin is often determined based simply on the history of residence in the past endemic areas.

Key words: Wuchereria bancrofti, chyluria, immunodiagnosis, IgG4, circulating antigen

INTRODUCTION

Filariasis caused by Wuchereria bancrofti was once a common parasitic disease in Japan, especially in its southern parts. In 1962, the national filariasis control program was launched and an extensive treatment campaign using diethylcarbamazine and mosquito control measures were carried out (Sasa, 1976). By the end of 1970's, microfilaria (mf) carriers were believed to have disappeared in Japan (Kobayashi, 1994). Twenty years after eradication of filariasis, chyluria cases are still encountered (Yagi et al., 1998; Sakakura et al., 1996; Ito et al., 1994; Kawahara et al., 1992; Koyama et al., 1990). In many cases of these reports, the illness was diagnosed or suspected as filarial chyluria simply based on patients' history of residence in the past endemic areas. In this study, a circulating W. bancrofti antigen, filaria specific IgG and IgG4 in the sera of chyluria patients were measured in order to obtain immunological evidence for the filarial origin, and to establish an immunological method useful to make a diagnosis of filarial chyluria.

MATERIALS AND METHODS

Serum samples:

Serum samples were obtained in 1994 from 16 chyluria patients and kept at −40°C until use. All the patients were born and brought up in Okinawa Islands, where W. bancrofti infection was highly endemic. Information on the patients is shown in Table 1.

Fourteen sera from healthy people living in central parts of Japan were used as healthy controls, and 8 sera from Sri Lankans who were known mf carriers of W. bancrofti were used as positive controls.

Detection of W. bancrofti circulating antigen:

An enzyme-linked immunosorbent assay (ELISA) for the detection of W. bancrofti circulating antigen was carried out using a commercially purchased kit (Trop-Ag W. bancrofti, JCU Tropical Biotechnology Pty Ltd., Australia), which is a sandwich ELISA using Og4C3 monoclonal antibody to capture the antigen. The kit has been tested worldwide and its high sensitivity and specificity have been established (More and Copeman, 1990).
Antigen preparation for ELISA: Female adult worms of Brugia pahangi collected from the abdominal cavity of Mongolian gerbils, Dirofilaria immitis adult worms from the heart of dogs, 3rd-stage larvae of Anisakis sp. recovered from mackerels and infective filariform larvae of Strongyloides ratti maintained in white rats were homogenized in 1/15 M PBS, pH 7.4, containing proteinase inhibitors: 1 mM of phenylmethane sulfonyl fluoride (PMSF), 10 μM of [I-3-trans-carboxyoxiran-2-carbonyl] -L-leucyl-agmatin (E-64), 0.5 mM of Pepstatin A and 5 mM of ethylenediaminetetraacetic acid (EDTA). Each of the homogenates was centrifuged at 15,000 rpm for 20 min at 4°C and supernatant was obtained. The supernatant was then measured for protein concentration using Bio-Rad Protein Assay Kit (Bio-Rad Lab., USA), adjusted to the required concentration and stored at -40°C until use.

Detection of antibodies to B. pahangi with ELISA:

A 96-well microtiter plate was coated with B. pahangi antigens (5 μg/ml) at 4°C overnight. The surface of the plate was blocked by casein buffer (1% casein in 0.05 M Tris–HCl buffer with 0.15 M NaCl, pH 7.6) for two hours at room temperature. Serum samples were prepared in five different ways, that is, the sera diluted 400 times with the casein buffer were mixed with the same volume of (1) the buffer itself, (2) the buffer containing D. immitis antigens (1 mg/ml), (3) the buffer containing Anisakis antigens (1 mg/ml), (4) the buffer containing S. ratti antigens (1 mg/ml), or (5) the buffer containing both D. immitis and Anisakis antigens (1 mg/ml for each antigen). The procedures from (2) to (5) were to absorb antibodies to the corresponding nematodes which may cross-react with the filarial antigens. These mixtures were incubated for 2 hrs at 37°C, then applied to the plate (100 μl/well) and incubated for one hour at 37°C. The plate was washed three times with Tween-PBS (0.05% Tween 20 in 1/15 M phosphate buffered saline, pH 7.4) and affinity purified goat anti-human IgG conjugated with peroxidase (Bio Source International, Inc., Tago Products, USA), which was diluted 4,000 times, was added. After incubation at 37°C for one hour, the plate was washed three times and then ABTS Peroxidase Substrate (KPL, Inc., USA) was added as a substrate for coloration, and absorbance at 415 nm was measured. All antibody levels were expressed as log [absorbance value +1].

For the detection of anti-B. pahangi IgG4 antibodies, serum samples were not absorbed as above but simply diluted 500 times. The mouse monoclonal antibody to human IgG4 conjugated with peroxidase (Caltag Lab., Inc., USA) was used as a second antibody.

RESULTS AND DISCUSSION

All chyluria cases in this study were regarded clinically as filariasis patients based on the history that they were from the endemic areas. However, there was no concrete evidence to confirm the diagnosis. Many years after eradication of filariasis, it is difficult to differentiate filarial chyluria from that of other etiologies. As adult parasites were reported to survive for up to 17 years, or be reproductive for 5-40 years (Carme et al., 1979), it is still interesting to test if there is any active filarial infection in Japan. For this test, the Og4C3 ELISA which mainly detects W. bancrofti adult circulating antigen is applicable. The ELISA has high sensitivity and specificity, and sera collected in the daytime can be used (Lammie et al., 1994). The antigen assay revealed that all the sera from chyluria patients were negative, indicating that there were no surviving parasites in the chyluria patients.

B. pahangi has been a source of antigen for use in serological tests for human lymphatic filariasis (Au et al., 1982; Nuti et al., 1982; Estambale et al., 1994). Immunoglobulin G levels to B. pahangi antigens are shown in Figure 1. Without absorption with the nematode antigens (Fig. 1a), IgG levels of mf positive Sri Lankans were clearly higher than those of chyluria patients and healthy controls. Some healthy people
showed relatively high antibody levels, suggesting that they had been exposed to antigens which produced cross-reactive antibodies to *B. pahangi*. Absorption of these antibodies with *D. immitis* antigens (b), *Anisakis* antigens (c), *S. ratti* antigens (d), and both *D. immitis* and *Anisakis* antigens (e). The left column of each panel is for chyluria patients, the middle column for Japanese healthy controls and the right column for Sri Lankan mf positives. An arrow in panel (e) indicates a cut-off point. A horizontal bar in each column indicates the mean of antibody titers. The means were compared before and after absorption with *t*-test. Asterisks in panels (b), (c), (d) and (e) indicate levels of significance: *for p<0.05, **for p<0.01 compared with panel (a).

For immunodiagnosis of filarial chyluria in Japan, there would be two steps to undertake; (1) measurement of *B. pahangi*–reactive IgG 4 and (2) measurement of *B. pahangi*–reactive IgG after absorption of cross-reactive antibodies with *Anisakis* and *D. immitis* antigens. Without having a gold standard of confirmed
filarial chyluria cases, the sensitivity of these immunodiagnoses could not be evaluated. However, in Japan where diagnosis of filarial chyluria is often made without evidence, the present diagnostic method can provide an immunological evidence to support it.

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