Jpn. J. Trop. Med. Hyg., Vol. 27, No. 4, 1999, pp. 483-486

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

MAKOTO ITOH[1], XU-GUANG QIU[1], YUZO KOYAMA[2], YOSHIHIDE OGAWA[2], MIRANI V. WEERASOORIYA[3], HANESANA VISANOU[1], YASUNORI FUJIMAKI[4] AND EISAKU KIMURA[1]

Received May 25, 1999/Accepted August 3, 1999

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).

1 Department of Parasitology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan
2 Department of Urology, University of the Ryukyus School of Medicine, Nishihara, Okinawa 903-0215, Japan
3 Department of Papasitology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka
4 Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

Table 1 Information on the chyluria patients from Okinawa, Japan

Patient No.	Sex	Age	History of filarial infection	Duration of the latest chyluria
1	female	57	—	—
2	male	59	Yes	1 month
3	male	71	—	—
4	—*	—	—	—
5	female	78	—	1 month
6	—	—	—	—
7	—	—	—	—
8	female	60	Yes	2 weeks
9	male	83	Yes	—
10	female	58	—	1 week
11	—	—	—	—
12	female	62	Yes	3 months
13	—	—	—	—
14	male	71	Yes	1 year
15	—	—	—	—
16	male	61	—	1 year

*Information not available.

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 [L-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

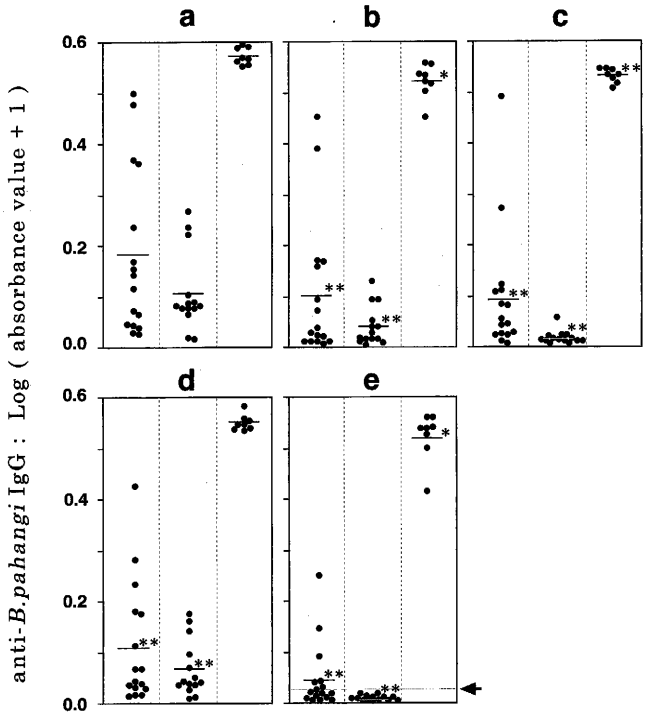

Figure 1 Anti-*B. pahangi* IgG antibodies in serum samples before and after absorption with antigens prepared from *D. immitis, Anisakis* sp. and *S. ratti.*

Note: Serum samples without absorption (a). Samples absorbed 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).

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, Anisakis* or *S. ratti* antigens significantly reduced absorbance values (Fig. 1b, c, d, e) with one exception: absorption with *S. ratti* antigens did not reduce the values in Sri Lankans (Fig. 1d). The results of statistical tests are shown in Fig. 1. Absorption with *Anisakis* antigens was most effective in reducing absorbance values. Although cross-reactivity of bancroftian filariasis sera with *D. immitis* antigens was well-documented (Grove *et al.,* 1977; Weller *et al.,* 1980), absorption with *D. immitis* antigens was not as effective as *Anisakis* antigens in this study. *S. ratti* was also not effective despite the fact that *Strongyloides stercoralis* is popular in Okinawa (Asato *et al.,* 1992). When cross-reactive antibodies were absorbed with a

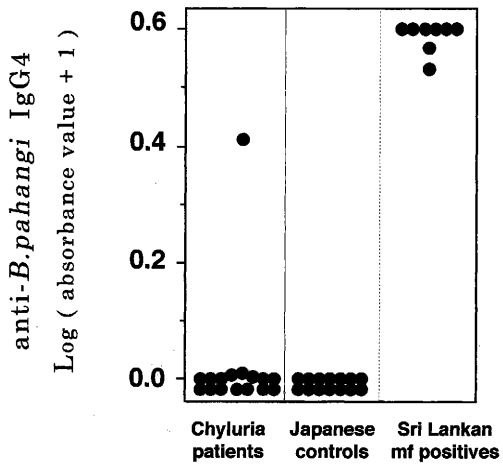

Figure 2 Anti-*B. pahangi* IgG4 antibodies in serum samples.

mixture of *Anisakis* and *D. immitis* antigens, mf positive Sri Lankans, chyluria patients and healthy controls were separated more clearly (Fig. 1e). The absorption by the mixture reduced absorbance values more than the absorption with a single antigen in chyluria patients and healthy controls (*t*-test, $p<0.01$), but not in Sri Lankans. Apparently higher IgG levels to *B. pahangi* in some chyluria patients than in healthy controls (Fig. 1e) would indicate that their clinical sign is filarial in origin.

If the average antibody level of the healthy control + 3SD is regarded as a cut-off point, 6 of 16 chyluria patients could be diagnosed as filarial.

Antibodies of IgG4 subclass to filarial antigens were specifically detected in filariasis patients (Lal and Ottesen, 1988). In addition, the IgG4 antibodies were reported to be associated with active infection of filarial parasites, and disappeared in a relatively short period after treatment (Estambale *et al.,* 1994; Kwan-Lim *et al.,* 1990). In this study, the detection of this subclass resulted in a clear difference between actively infected Sri Lankans and Japanese control subjects (Fig. 2). One chyluria patient, whose sample always showed the highest antibody level before and after absorption, maintained a very high IgG4 level. It is possible some parasites survived until recent years and were stimulating the antibody production. In other words, the detection of IgG4 might be less useful in Japan 20 years after eradication of filariasis.

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.

ACKNOWLEDGEMENTS

The study was supported in part by Grant-in-Aid for International Scientific Research (Field Research) no. 09041193 from the Ministry of Education, Science, Sports and Culture in Japan.

REFERENCES

1) Asato, R., Nakasone, T., Yoshida, C., Arakaki, T., Ikeshiro, T., Murakami, H. and Sakiyama, H. (1992): Current status of *Strongyloides* infection in Okinawa, Japan. Jpn. J. Trop. Med. Hyg., 20, 169-173
2) Au, A.C., Draper, C.C., Denham, D.A., Rao, C.K., Ismail, M.M. and Mak, J.W. (1982): Detection of filarial antibodies in Malayan and Bancroftian filariasis by the indirect fluorescent antibody test, using different filarial antigens. Southeast Asian J. Trop. Med. Public Health, 13, 142-147
3) Carme, B. and Laigret, J. (1979): Longevity of *Wuchereria bancrofti* var. *pacifica* and mosquito infection acquired from a patient with low level parasitemia. Am. J. Trop. Med. Hyg., 28, 53-55
4) Estambale, B.B., Simonsen, P.E., Vennervald, B.J., Knight, R. and Bwayo, J.J. (1994): Bancroftian filariasis in Kwale District of Kenya. II. Humoral immune responses to filarial antigens in selected individuals from an endemic community. Ann. Trop. Med. Parasitol., 88, 153-161
5) Grove, D.L., Cabrera, B.D., Valeza, F.S., Guinto, R.S., Ash, L.R. and Warren, K.S. (1977): Sensitivity and specificity of skin reactivity to *Brugia malayi* and *Dirofilaria immitis* antigens in Bancroftian and Malayan filariasis in the Philippines. Am. J. Trop. Med. Hyg., 26, 220-229
6) Ito, T., Katoh, A., Hanai, H., Kaneko, E., Kino, H. and Terada, M. (1994): A case of chyluria probably caused by chronic filariasis. Clin. Parasitol., 5, 157-160 (in Japanese)
7) Kawahara, K., Kubo, H., Kakinoki, T., Chan, Y.F., Saita, H., Yamashita, J., Shirahama, T., Kayajima, T., Kawahara, M., Ikeda, K., Makinose, S., Matsusako, T., Yagi, S., Nishida, M., Yamauchi, D. and Eta, S. (1992): Statistical observations on inpatients and operations at the urological clinic of Kagoshima Prefectural Oshima Hospital from 1981 to 1990. Nishinihon J. Urol., 54, 2283-2288 (in Japanese)
8) Kobayashi, T. (1994): Filariasis, TBS Britanica, Tokyo (in Japanese)
9) Koyama, Y., Chinen, Y., Ogura, H., Shishido, S., Higa, I., Hatano, T., Hayakawa, M., Osawa, A. and Igarashi, M. (1990): Immunological study on chyluria. Jpn. J. Urol., 81, 1212-1216 (in Japanese)
10) Kwan-Lim, G.E., Forsyth, K.P. and Maizels, R.M. (1990): Filarial-specific IgG4 response correlates with active *Wuchereria bancrofti* infection. J. Immunol., 145, 4298-4305
11) Lal, R.B. and Ottesen, E.A. (1988): Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment. J. Infect. Dis., 158, 1034-1037
12) Lammie, P.J., Hightower, A.W. and Eberhard, M.L. (1994): Age-specific prevalence of antigenemia in a *Wuchereria bancrofti*-exposed population. Am. J. Trop. Med. Hyg., 51, 348-355
13) More, S.J. and Copeman, D.B. (1990): A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. Trop. Med. Parasitol., 41, 403-406
14) Nuti, M., Ferrari, J.D. and Au, A.C. (1982): Sero-epidemiology of bancroftian filariasis in the Seychelles Islands. Tropenmed. Parasitol., 33, 25-27
15) Sakakura, T., Ikeuchi, T. and Watanabe, H. (1996): Chyluria treated with stripping of the renal pedicle: a case report. Acta Urol. Jpn., 42, 221-223 (in Japanese)
16) Sasa, M. (1976): Human filariasis. A global survey of epidemiology and control. University of Tokyo Press, Tokyo.
17) Yagi, S., Goto, T., Kawamoto, K., Miyawaki, I., Tanaka, I., Mori, K., Hayami, H. and Ohi, Y. (1998): Endoscopic treatment of refractory filarial chyluria: a preliminary report. J. Urol., 159, 1615-1618
18) Weller, P.F., Ottesen, E.A. and Heck, L. (1980): Immediate and delayed hypersensitivity skin test responses to the *Dirofilaria immitis* filarial skin test (Sawada) antigen in *Wuchereria bancrofti* filariasis. Am. J. Trop. Med. Hyg., 29, 809-814