Parasitol Res (1996) 82:382–384 © Springer-Verlag 1996

SHORT COMMUNICATION

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The sheath of the microfilaria of *Brugia malayi* from human infections has IgG on its surface

Received: 20 October 1995 / Accepted: 20 October 1995

Abstract Microfilariae (mf) of *Brugia malayi* from microfilaraemic people had human IgG on their sheath. Fluorescent antibody studies showed that the predominant IgG isotype was IgG3, with IgG4 and IgG1 being present in lower quantities. Human albumin could not be detected. The sera of patients with chronic disease contained high levels of an IgG2 antibody that reacted with the sheath of mf taken from other people.

Microfilariae (mf) of *Wuchereria bancrofti*, the major lymphatic filarial nematode affecting humans, and *Brugia pahangi*, a natural filarial parasite of the cat in South East Asia, have host proteins bound to their sheath (Maizels et al. 1984; Premaratne et al. 1989; Kar et al. 1993). *W. bancrofti* mf are coated mainly with human albumin together with low levels of IgG1 and IgG4 (Kar et al. 1993). *B. pahangi* mf from cats are not coated with albumin but have variable amounts of feline IgG (mainly IgG2) on their surface (Premaratne et al. 1989; Malecela et al., unpublished observation). Piessens et al. (1980) have reported that a variable proportion of mf from 25% of patients infected with sub-periodic *B. malayi* have human Ig on their sheath.

On the other hand, there is a correlation between the presence of antibodies in the serum that react with the sheath of the mf antibody and the absence of mf from the blood in *W. bancrofti, B. malayi* and *B. pahangi* infections (Ponnudurai et al. 1974; McGreevy et al. 1980; Das et al. 1987). In *W. bancrofti* and *B. malayi* infections the highest levels of anti-sheath antibodies are found in patients with chronic pathology. In *W. bancrofti* patients the anti-sheath antibody is IgG2 (Ravindran et al. 1990, 1994). In *B. malayi* infections the anti-sheath antibody is either IgM or IgG (Piessens et al. 1980). In *B. pahangi*-infected cats, anti-sheath surface antibodies may be either feline IgG1 or feline IgG3, depending on how long after clearance of mf the observations are made (Baldwin et al. 1994).

The following studies were carried out to determine which proteins are present on the mf of periodic *B. malayi*, the causative organism of lymphatic filariasis in Kerala State, South India, and to determine which isotypes of IgG from mf-negative (mf-ve) patients with chronic pathology react with the sheath of *B. malayi* mf.

Mf were collected from residents of Alleppey district of Kerala, which includes Shertallai taluk. The studies were approved by the Ethical and Scientific Review Committee of the institute, and informed consent was obtained from all donors prior to their being bled for either mf or serum. In all, 5–10 ml of blood was collected in excess heparin from 11 mf-positive (mf+ve) patients at or after 2130 h. After they had donatet blood the patients were treated with diethylcarbamazine. The blood was kept at 4°C for between 12 h and 2 days. Mf were isolated from the blood on a 5-µm-pore Nuclepore membrane (Kar et al. 1993).

Sepharose 4B beads (Pharmacia 17-0430-01) coated with either human IgG (Sigma I4506) or human albumin (Sigma A 3782; Johnstone and Thorpe 1988) were used as both positive and negative controls with all of the conjugates.

Mf were tested by a direct fluorescent antibody test (FAT) to detect host proteins on the sheath. A total of 100 active, sheathed mf in 100 µl RPMI were incubated with 100 µl of the various anti-human fluorescein isothiocyanate (FITC)-conjugated reagents diluted in RPMI as described by Kar et al. (1993) with the addition of monoclonal anti-human IgG3 (Sigma F4641). After 1 h of incubation in a dark environment at room temperature the mf and the Sepharose beads were washed twice in RPMI, centrifuged into a pellet and viewed with an inci-

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dent light fluorescent McArthur microscope (Kirk Technology). The degree of fluorescence was subjectively scored as 0, 1, 2 or 3, with 0 being negative and 3 being the most fluorescent.

An indirect fluorescent antibody test (IFAT) was used to detect anti-sheath antibodies in the serum of mf-ve individuals. Serum was collected from eight patients with classic pathological sequelae of Brugian filariasis. Lymphoedema was classified as recommended by the WHO (1985), whereby Grade I lymphoedema is pitting with no fibrosis, Grade II lymphoedema is pitting with fibrosis and Grade III lymphoedema (elephantiasis) is non-pitting with a great increase in volume. Six patients had Grade III lymphoedema, one had Grade I disease and one had Grade II lymphoedema. The patients had been mf-ve for at least 6 months.

In the IFAT, 50–100 mf were spotted onto slides, allowed to dry and fixed in acetone. The slides were washed with RPMI, probed with the 10% test human serum and incubated for 1 h at room temperature, after which they were washed three times in RPMI and probed with one of the following conjugates diluted 1/5 in RPMI: polyclonal anti-human IgG, monoclonal anti-human IgG1, monoclonal anti-human IgG2, monoclonal anti-human IgG3 and monoclonal anti-human IgG4. The slides were washed three times in RPMI and viewed as described for the FAT. As a negative control, slides were probed with each of the conjugates alone.

Host proteins were detected on the sheath of microfilariae of *B. malayi* (FAT) as follows. The control IgG-coated beads fluoresced brightly with all the anti-IgG reagents and the albumin-coated beads reacted only with the anti-albumin reagent. The anti-IgA, anti-IgM and anti-C3c reagents did not react with either of the control beads. The mf observed were fully active and only those that were sheathed were read for fluorescence. All mf were negative for IgA, IgM, albumin and C3c. A variable amount of IgG and its isotypes was seen on the mf: 69% were positive for IgG; 23%, for IgG1; 6%, for IgG2; 91%, for IgG3; and 60%, for IgG4.

Anti-sheath antibodies were detected in the serum of mf-ve individuals as follows. Acetone-fixed mf probed directly did not fluoresce with any conjugate, demonstrating that fixation removed the host proteins that had previously been detected on the sheath by direct FAT. All mf fluoresced in IFAT with all the sera from mf-ve patients when developed with the polyclonal IgG reagent, although the degree of fluorescence was variable. Altogether, 98% of the mf fluoresced with the anti-IgG2 reagent, with 45% fluorescing at levels 2 or 3; 38% of the mf fluoresced with the anti-IgG3 reagent and only at level 1; 49% of the mf fluoresced with the IgG1 reagent and 60% fluoresced with the IgG4 reagent, mostly at level 1.

These results demonstrate that the mf of periodic *B. malayi* from people, like those of *B. pahangi* from cats, are coated with Ig but not host albumin. Our results contrast with those of Mania and Kar (1994), who found albumin on *B. malayi* mf from people in the Balasore district of Orissa State, India. Kar (personal communication) suggests that this parasite is morphologically different from that occuring in Kerala, and this may account for the discrepancy. The mf of the Keralan periodic *B. malayi* differ from the mf of *Wuchereriat bancrofti,* which are coated in albumin and Ig. Kar et al. 1993 found with *W. bancrofti* that 60% of mf had IgG on their surface, with 52% being positive for IgG4 and 39% being positive for IgG1.

Most *B. malayi* and *W. bancrofti* patients with chronic pathology possess antibodies to the mf sheath (Wong and Guest 1969; Piessens et al. 1980; Ravindran et al. 1990). In our study, every patient with chronic pathology was positive for anti-sheath antibody, with the highest titres being found in those patients with Grade III oedema. The percentage of mf reacting with each subclass was similar to the pattern observed with *W. bancrofti* (Ravindran et al. 1990).

Acknowledgements We thank the Principal and Superintendent of T-D Medical College and Hospital, Alleppey, for their support and encouragement. We gratefully acknowledge the assistance of Dr. T. Lesitha, Mercy George, and K.B. Zaina, E. Nazeer and S. Beegum Shamna as well as S. Sindh and all of the other staff of the Filariasis Research Unit. We also thank Dr. C.P. Ramachandran of the World Health Organization for his help in setting up this project.

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