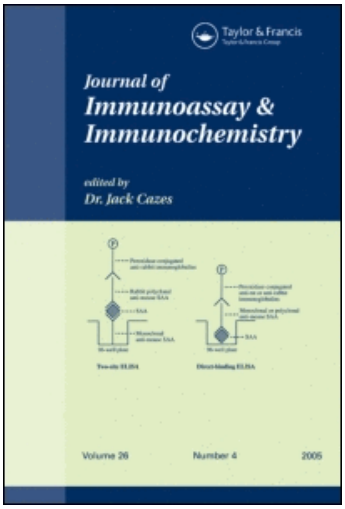

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Monoclonal Antibodies Generated Against Excretory/Secretory Antigens of Mammalian Stage Larvae of the Lymphatic Filarial Parasite *Wuchereria bancrofti*

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Abstract: Monoclonal antibodies (Mabs) against excretory/secretary (e/s) antigens of fourth stage (L4) larvae of *Wuchereria bancrofti* were raised and screened for their specificity and sensitivity and evaluated for their potential in detecting homologous e/s antigens in human blood samples. Five Mabs were obtained and, among them, Mab A7 showed high reactivity against e/s antigens of L4 and crude somatic antigens of microfilariae (mf) of *W. bancrofti*, and infective stage (L3) and adult stage larvae of *Brugia malayi*. It reacted strongly with sera of *Mastomys coucha* harbouring L4 stage of *B. malayi* moderately against sera of the animal having later stages of the parasite. But, it exhibited a low and negligible reactivity against the crude antigens of *Setaria cervi* and *Ascaris lumbricoides*, respectively.

Another Mab, A6, showed very high reactivity against mf antigens of *W. bancrofti* and *B. malayi* and a moderate reactivity against antigens of *S. cervi* and *A. lumbricoides*. The two Mabs were tested for their reactivity against filarial antigens in human sera, whose microfilaraemic status was determined by membrane filtration of 1 mL blood sample collected during night. When Mab A7 was tested, 7 out of 22 serum samples (32.0%) from amicrofilaraemic normal individuals from filariasis endemic areas showed positive reactions for filarial antigens, indicating the presence of early stage (L4) of the parasite in them. It also reacted with 84% (n = 19) mf positive samples and 11% of non endemic normal serum samples (n = 17). Mab A6 showed high reactivity with 86% (n = 26) of mf positive serum samples, but did not react with non-endemic normal serum samples (n = 17). The results, thus, indicate that the Mab A7 has potential in the detection of e/s antigens of L4 stage larvae of filarial parasites in humans, enabling early diagnosis of filariasis. Mab A6 could be

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used in the diagnosis of patent infection with microfilaraemia. Western blotting with Mab A7 reacted with the 29.0 kDa protein band of L4 e/s antigens of *W. bancrofti*.

Keywords: *Wuchereria bancrofti*, L4 stage, Excretory/secretory antigens, Monoclonal antibodies, Filarial specific antigens

INTRODUCTION

Nearly one billion people in 73 countries are at the risk of infection with filarial parasites that cause lymphatic filariasis, a disabling disease. In India, about 120 million people are either infected or with lymphatic pathology, accounting for 40% of the global burden of the disease.[1] Early detection of infection and treatment would prevent pathology and appearance of clinical manifestations.[2] The diagnosis based on the microscopic examination of finger prick thick blood smear for microfilaraemia has low sensitivity and is not accepted by the community because the blood has to be collected during night time.[3] Also, by the time the patent infection has developed, damage to lymphatics would have set in, in the form of lymphangiectasia.[4] Immunological tests, based on Mabs to filarial parasites have been developed recently[5,6] and are currently available commercially. However, they are not stage specific and cost effective. These test kits have been developed basically to detect microfilaraemia, although they test 30–40% of the endemic normal individuals (as detected by the conventional microscopic examination of blood smears) as positive for filarial antigens. This raises a question whether such individuals (Ag^+/mf^-) are false positives or they harbour single sex infection, have ultra-low level of parasites, or developing stages of parasites (pre-patent infections), which needs to be addressed. Gerusa et al.[7] have found live adult worms in scrotal areas of amicrofilaraemic but antigen positive individuals by using ultrasound technique. Itoh et al.,[8] while examining this issue, opined that the case of Ag^+/mf^- needs to be researched. Similarly, Rocha et al.[9] felt that the usefulness of the immunological tests needs to be supported by additional work. In the present investigation, we developed a monoclonal antibody against excretory/secretory (e/s) antigens of fourth stage larvae of *W. bancrofti* and investigated its potential in detecting e/s antigens of developing parasites *B. malayi*, in a rodent model and then *W. bancrofti* in a human host.

EXPERIMENTAL

Collection of Various Stages of Parasites Required for the Study

Microfilariae (mf) of *W. bancrofti* were obtained from the blood of mf positive patients residing in Pondicherry town, which is a known endemic

area for Bancroftian filariasis.[10] A survey for such mf positive cases was carried out by collecting blood smears from several hundred individuals during 21.00–23.00 hrs by the finger prick method and examining them for mf after Giemsa's staining. An additional amount of blood (about 5 mL) was drawn from high count (>100 mf/20 μL) mf carriers and the mf were separated through a Percoll-gradient centrifugation technique.[11] Microfilariae of *B. malayi* (sub-periodic strain) were lavaged out from experimentally infected mongolian gerbils (*Meriones unguiculatus*) obtained from the colony maintained at the Vector Control Research Centre (VCRC). Infective stage larvae (L3) of *W. bancrofti* were raised in the vector mosquito, *Culex quinquefasciatus*, employing an artificial membrane feeding technique[12] and those of *B. malayi* in *Aedes aegypti* (Liverpool strain) mosquitoes fed on experimentally infected mf positive multimammate rats (*M. coucha*). L3 larvae of either parasite were harvested on the 14th day post-infection using Bearman's funnel technique.[13] For raising fourth stage larvae of *W. bancrofti*, mongolian gerbils (immunosuppressed) were inoculated intraperitoneally with L3, and L4 stage larvae were harvested from the peritoneal cavities after 20 days.[14] L4, L5 or adult stage parasites of *B. malayi* were raised in mongolian gerbils through intra-peritonial (ip) inoculation of L3. Adults of *S. cervi* were collected in a sterile flask containing phosphate buffered saline [PBS (pH 7.4)] from peritoneal cavities of cattle slaughtered in a local abattoire. The adult *A. lumbricoides* were obtained by de-worming of infected children residing in Pondicherry. Children were given an oral single dose of 10 mL (400 mg) Albendazole I.P (Zentel) suspension and the worms were collected when expelled. The worms were washed several times with PBS and used for antigen preparation.

Preparation of Different Filarial, Non-Filarial Worm Antigens

Somatic antigens of mf, L3, and adult of *B. malayi*, mf and L3 of *W. bancrofti*, and adults of *S. cervi* and *A. lumbricoides* were prepared by the following method. Each stage of the parasite was homogenized in phosphate buffered saline (pH 7.4) in a tissue homogenizer (Top Syringes Ltd., Mumbai, India); the homogenate was sonicated at 13 Hg for 30 seconds in 8 cycles in a sonicator (Ultrasonic processer XL, Heat systems, Denmark) in the presence of protease inhibitors (2μL/mL containing EDTA-1M, EGTA-1M, NEM-1M, Pepstatin-1 mM, PMSF-0.33M, TPCK-0.1M). Sonicated homogenate was held overnight at 4°C and centrifuged at 5,000 rpm for 10 minutes in a refrigerated centrifuge (4°C) (Eppendorf, Germany). Protein concentration of the supernatant was estimated and stored at −20°C until further use.

E/s antigens of the L4 stage of *W. bancrofti* (Fig. 1) were obtained by maintaining the L4 larvae in modified Frank's medium supplemented with

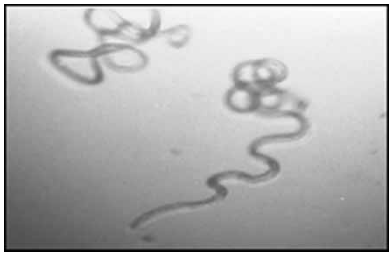

Figure 1. Live L4 of *Wuchereria bancrofti* in *in vitro* medium as observed under inverted phase contrast microscope × 100.

30% normal human serum at 37°C and 5% CO_2 in air in an incubator.[15] The culture supernatant was collected at regular intervals, centrifuged to remove debris, and the clear supernatant was stored at 70°C until further use, after mixing with protease inhibitors. The resulting antigen preparations were dialyzed overnight against PBS (2-3 changes) and, after estimating the protein content,[16] were stored at −70°C until further use.

Immunological Techniques

Generation of Hybridomas Against L4 e/s Antigens of *W. bancrofti* and Screening by ELISA

Standard protocols were followed for generating the hybridomas.[17] In view of the limited quantity of e/s antigens of L4 stages, BALB/c mice were immunized by direct sensitization of the spleen.[18] The spleens from the animals were removed after 4 days and used for fusion with Sp2/01-Ag14 myeloma cells. Hybridomas were screened for reactivity against L4 e/s antigens and the positive ones were cloned by a limiting dilution technique.

The monoclonal antibodies of clones were produced by inoculating the clones into the peritoneal cavity of pristane primed BALB/c mice (as ascites) and purified using Protein-G-column chromatography.

Monoclonal antibodies from the hybridoma culture supernatants and ascitic fluid were quantified by a direct Enzyme Linked Immunosorbant Assay (ELISA) procedure.[19] Briefly, e/s antigens (0.5 μg/well) were coated onto 96-well polystyrene plates and incubated o/n at 4°C. The hybridoma supernatants, without dilution or ascitic fluid (1:100), were reacted for 2 hrs after blocking the wells with 1% bovine serum albumin (BSA). Anti-mouse peroxidase conjugate was allowed to react with the antigen-antibody complex, followed by the addition of ø-phenylenediamine dihydrochloride (OPD) as a substrate. The optical density (O.D.) of the reaction product was read at 492 nm in an ELISA reader.

Cross Reactivity of Mabs

The reactivity of the clones obtained by single cell cloning were tested against antigens of mf, L3 and adult of *B. malayi*, mf, L3, and e/s antigen of L4 stage larvae of *W. bancrofti* and adult antigens of *S. cervi* and *A. lumbricoides* by direct-ELISA as discribed elsewhere. Clones A6, A7 were found to be highly reactive against their respective antigens and were taken for further processing.

Determination of Reactivity of Mabs of the Clones A6 and A7 with Various Stages of *B. malayi* Antigen in Animal Model

Initially, the potential of the two clones in detecting the L4 antigens was validated in a rodent model having defined stages of filarial parasites, as there was no system/tool available for the identification of human individuals with developmental stages of filarial parasites. Infective stage larvae of *B. malayi* were inoculated into the peritoneal cavity of mongolian gerbils, the blood samples were collected on 17th, 30th, 60th, and 120th days and tested for the reactivity of Mabs against antigen present in the serum samples.

Detection of Filarial Specific Antigens in Serum Samples

For quantifying bancroftian filarial antigens in human sera samples, a direct-ELISA protocol was employed.[19] Blood samples for sera were collected during the night time (21.00–23.00 hrs) from volunteers belonging to three groups: a) filariasis endemic normal individuals with no microfilariae in the blood (as determined by a membrane filtration technique on 1 mL venous blood); b) non-endemic normal individuals residing in the tribal villages (Nilgiri Hills) in Tamil Nadu; and also c) microfilaraemic cases in Pondicherry town. The sera were coated at 1/10 dilution (as determined by checkerboard titration) and incubated o/n at 4°C. Monoclonal antibodies were allowed to react for 2 hrs following the blocking of wells with 1% BSA. Anti-human IgG peroxidase conjugate was added to the wells followed by the substrate after another wash. After 20 minutes of incubation, the reaction was arrested and the O.D. was read at 492 nm. The cut-off O.D. value was determined based on the average O.D. of 3 non-endemic normal sera added with 2 standard deviations.

Determination of Reactivity of Mabs Against *W. bancrofti* mf and L4 e/s Antigens Through Antigen Profile and Immunoblotting

SDS-PAGE Profiles of the Antigens

The reactivity of MAbs against mf antigens *of W. bancrofti*, and e/s antigens of its L4 was analysed by an immunoblotting technique. The somatic extracts

of *W. bancrofti* mf and L4 e/s antigens were separated on SDS-PAGE using Mini-Protean® Electrophoresis Cell (Bio-Rad Laboratories, USA) according to the method of Laemmli[20] with some modifications. This was carried out using 10% (w/v) non-gradient slab gel (8 × 7 cm vertical gel) with 0.8% stacking gel. The antigens were diluted with sample buffer (1:4) containing SDS: 10%, glycerol: 10%, bromophenol blue: 0.05%, and mercaptoethanol: 5% in 0.5 M Tris HCl buffer (pH 6.8). The samples were boiled in a water bath for 5 minutes, and loaded into the wells (10 μg protein per well in 80 μL of sample buffer), along with known molecular weight markers (low and medium weight marker proteins ranging from 6.5 kDa to 97.4 kDa, Genei Pvt Ltd-Bangalore, India). Electrophoresis was performed at 100 V in a Tris glycine buffer system. When the bromophenol dye reached 1 cm from the bottom of the gel, electrophoresis was stopped, and the gel was removed and stained for 30 minutes. The gel was rinsed with distilled water and destained.

Western Blot Analysis

In order to determine the location or the presence of specific proteins in the SDS-PAGE, the protein patterns were electro-transferred to a nitrocellulose membrane (NCM) as described by Towbin et al.[21] After the electrophoresis was complete, the gel was incubated for 10–15 minutes in transfer buffer to eliminate swelling. In the meantime, NCM was cut to the desired size and incubated for 5–10 minutes in transfer buffer. The NCM was overlaid onto the gel (avoiding air bubbles), sandwiched between filter paper sheets, and then between fibre pads. The transfer was carried out at 30 V/90 mA overnight at 4°C using a Mini Transblot® Electrophoretic Transfer Cell (Bio–Rad Laboratories, USA). After the transfer was complete, the molecular weight marker lane was cut and stained with 1% amido black for 5 minutes and destained with 7% acetic acid with several changes of the destainer until the background stain was eliminated.

RESULTS

From three fusions carried out, a total of 10 hybridomas were obtained and, upon screening against L4 e/s antigens of *W. bancrofti*, 5 were found to be positive. The hybridomas were cloned by a limiting dilution which yielded 5 clones (A1, A4, A6, A7, and A9) that reacted highly against L4 stage e/s antigens. These clones were tested for cross-reactivity against crude somatic antigens of mf, L3, adult stages of *B. malayi* and somatic antigens of mf, e/s antigens of L4 stage larvae of *W. bancrofti*, as well as adults of *S. cervi* and *A. lumbricoides*. Among the 5 clones, clone A7 showed high reactivity (O.D-0.27) against e/s antigens of L4 and crude antigens of mf stage of *W. bancrofti*, and L3 and adult stages of *B. malayi*. But, it exhibited very low (O.D-0.016) and no reactivity (0.000), respectively, against *S. cervi* and

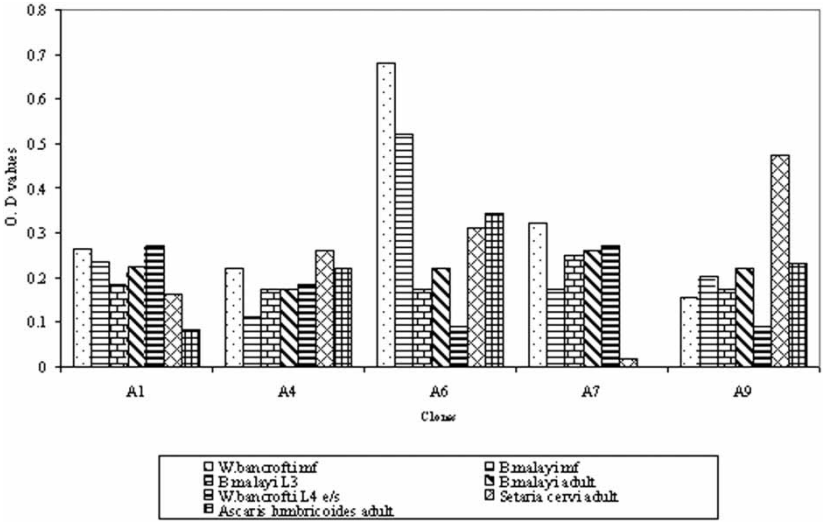

Figure 2. Cross reactivity of l4 specific Mabs against different antigens.

A. lumbricoides antigens (Fig. 2). Another clone, A6, showed very high reactivity against *W. bancrofti* and *B. malayi* microfilarial antigens (O.D – 0.68 and 0.53), which also exhibited a moderate reactivity against other antigens, including those of *S. cervi* and *A. lumbricoides*. All of the other three clones reacted almost uniformly with filarial, as well as other, antigens tested (O.D. value ranging from 0.10 to 0.45). Thus, clones A6 and A7 generated against L4 stage e/s antigens showed high specificity against filarial antigens and were investigated further with reference to their potential in detecting the microfilarial and e/s antigens of L4 stages, respectively.

Reactivity of Clones A6 and A7 Against Early Stages of *B. malayi* Antigen in an Animal Model

Initially, the potential of the Mabs of the two clones, A6 and A7, in detecting the L4 antigens was validated in a rodent model, *M. unguiculatus*, having defined stages of *B. malayi*, as there was no system/tools available for the identification of human individuals with different developmental stages of filarial parasites. In order to carryout this study, the infective stage larvae of the *B. malayi* were inoculated into the peritoneal cavity of gerbils, the blood samples collected at different time points by tail puncture, sera separated and tested for the presence of parasite antigens by direct-ELISA by employing purified Mabs of clones A6 and A7. The reactivities of the clones with serum samples from animals having different stages of the *B. malayi* are shown in Fig. 3. Clone A7 showed the highest reactivity (O.D - 1.93) with the sera of the animal

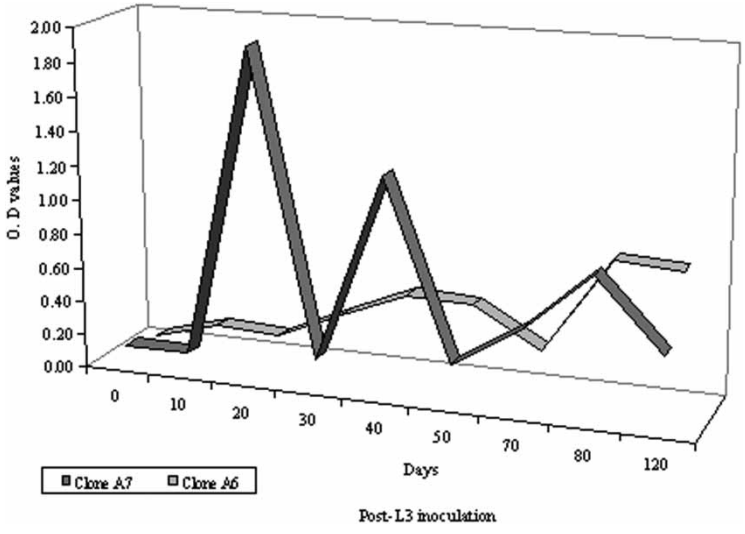

Figure 3. Reactivity of Mabs A6 and A7 against antigens of *Brugia malayi* in serum samples of *Mastomys coucha* collected during 0–120 days post-L3 inoculation.

collected on 20th day post-L3 inoculation, which corresponds with the development of inoculated L3 to L4 stage. This clone also showed a moderate reactivity (O.D – 0.83 – 1.27) against sera collected on time points corresponding to a stage later than L4 (adult) and poor reactivity against sera collected on 120th day when the patent infection was established and mf were produced by the female adult parasite. On the other hand, clone A6 showed high reactivity (O.D – 0.8) against sera collected on the 80th and 120th days from animals with adult and mf stages of the parasite, and moderate activity against adult stage, while it exhibited negligible activity against L4 stage antigens, i.e., against the sera collected on 20th day (from L4 positive animal). This pattern of activity almost reflected that obtained from cross reactivity studies against antigens of different stages mentioned in an earlier section. The results, thus, showed that the Mab of clone A7 has the potential to detect the antigens of L4 stage larvae. Therefore, these Mabs were tested for their potential in detecting the infection in human serum samples in a preliminary analysis. For this, sera from individuals whose microfilaraemic status was determined by membrane filtration of 1 mL of night blood sample, was used.

Detection of L4 Stage e/s Specifc Antigens in Serum Samples of Humans by the Clones A6, A7

The result showed that the Mab A6 detected 22 individuals as positives for circulating filarial antigens (CFA) out of 26 individuals who were found to be

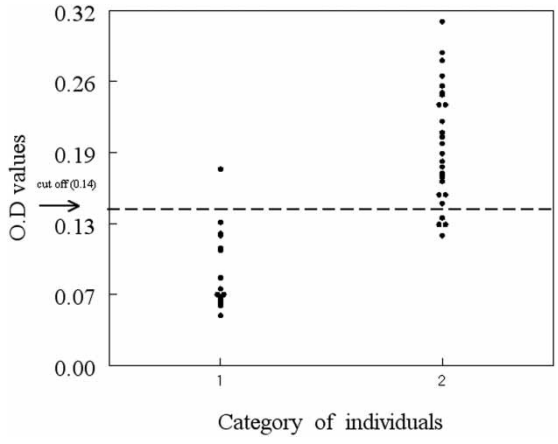

Figure 4. Reactivity of Mab A6 with *Wuchereria bancrofti* antigen in human serum sample 1-NEN, 2-Mf carrier.

microfilaraemic by the membrane filtration technique. It also detected one of the 15 non-endemic normal sera as positive (Fig. 4). Hence, the assay using Mab A6 exibited 93.3% specificity and 84.6% sensitivity. The positive and the negative predictive values for this assay were 95.7% and 77.8%, respectively.

When Mab A7 was used in the assay, 7 out of 22 serum samples (32.0%) from amicrofilaraemic normal individuals from a filariasis endemic area were found to be positive for L4 antigens. Four of them had higher levels of antigens (O.D–0.074–0.098), while the remaining 3 had medium levels (O.D–0.055–0.061). Of 17 serum samples from non-endemic normal

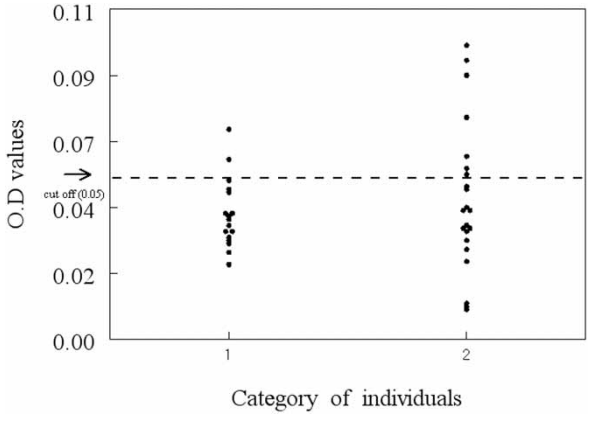

Figure 5. Reactivity of A7 with *Wuchereria bancrofti* antigen in human serum sample 1-NEN, 2-EN.

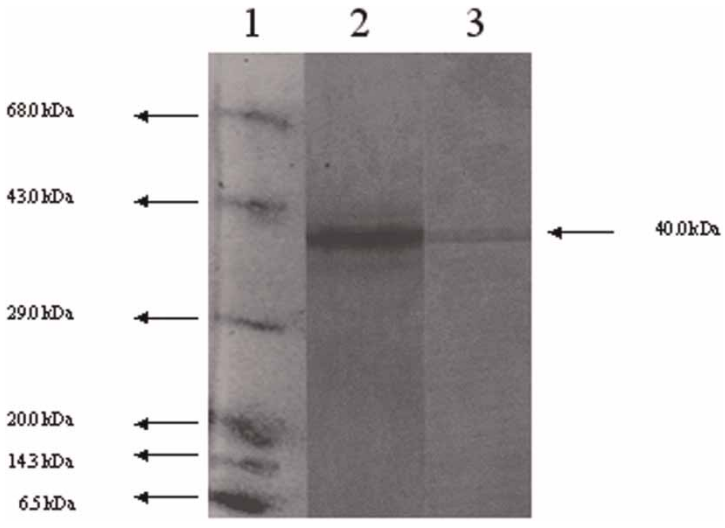

Figure 6. SDS-PAGE profile of L4 e/s antigens and their reactivities with Mab A7 in immunoblot analysis. 1-Molecular weight marker, 2-SDS-PAGE profile of L4 stage e/s antigen, 3-Antigen band recognized by Mab A7.

individuals, 2 of them were found to be positive for L4 antigens (Fig. 5). The assay using Mab A7 has a specificity of 88% and an 84% sensitivity. Thus, the results show that Mab A7 is able to detect antigens of developing filarial parasites in humans in endemic normal individuals, who were found to be amicrofilaraemic by membrane filtration of 1 mL of blood collected during the night. This indicates that these individuals had antigens of developing filarial parasites in their blood and, therefore, harbor developing filarial parasites in them.

The reactivity of the Mab A7 with the *W. bancrofti* L4 e/s antigens, against which it was raised, was determined by Western blotting. Crude mf of *W. bancrofti* were used for testing. Prior to this, the protein profile of the antigen preparation was generated. The SDS-PAGE profile of the antigen is presented in Fig. 6. The L4 e/s antigens showed 3 protein bands (1 major, 2 minor) of molecular weights ranging from 35 to 43 kDa. These bands were transferred onto the nitrocellulose membrane by a Western blotting technique. On this membrane, Mab A7 was reacted. The immunoblot analysis showed that Mab A7 reacted with the 40.0 kDa protein band of *W. bancrofti* L4 e/s antigens (Fig. 6).

DISCUSSION

India contributes to about 40% of the global burden of the filariasis[1] and recently has become a signatory to the filariasis elimination campaign. The

fact that the elimination programme has already been launched in the country, calls for rapid assessment of filariasis and delimitation of endemic areas. Early detection of infection and treatment would prevent pathology and appearance of clinical manifestations.[2] The conventional diagnosis based on the microscopic examination of finger-prick thick-blood smear for microfilaraemia has low sensitivity and is not acceptable to the community because of the inconvenient night blood collection.[3] Highly sensitive and rapid diagnostic techniques, especially those that can utilize day blood samples, are central towards achieving these tasks. Immunological kits, available commercially,[5,6] are expensive, as these need to be imported and are useful for detection of infection in microfilaraemia. Therefore, there is a need to develop an indigenous test that is cheaper, rapid, and especially one that can diagnose the disease in its earlier stage.

In an attempt to develop such immunological tests, Mabs against e/s antigens of the L4 stage larvae of *W. bancrofti* were raised and investigated for their reactivity against filarial parasite antigens. Two Mabs (A6 and A7) were identified which exhibited high reactivity against antigens of microfilarial/adult and fourth stage larvae of *W. bancrofti.* Antibodies against nematodes are known to exhibit cross reactivity against other filarial nematodes,[22] as well as the intestinal helminthes such as *Ascaris* sp. Similarly, cross-reactive antigens/antibodies between the bovine filarial parasite, *S. cervi*, and human filarial parasite, *B. malayi*, have been identified in immunoblotting analysis using hyper-immune rabbit sera.[23] In a tropical filarial endemic area, the chances of encountering antigens of zoonotic/intestinal helminthes are more and, hence, Mabs selected based on their reactivity against filarial antigens were tested against homologous (various stages of *B. malayi* and *W. bancrofti*) and heterologous (adults of *S. cervi* and *A. lumbricoides*) antigens. Mabs A6 and A7 were identified as having high reactivity against *W. bancrofti* antigens and least cross reactivity against adult antigens of *S. cervi* and *A. lumbricoides.* A7 was more specific to e/s antigens of 4th stage larvae of *W. bancrofti*, whereas, A6 to mf antigens of *W. bancrofti* as well as *B. malayi.*

Although these two antibodies reacted with e/s antigens of L4 and mf antigens isolated from parasites, the efficacy of these antibodies to detect various stage specific antigens present in the host-serum had to be studied. For this purpose, serum samples collected from an animal model, *M. coucha* experimentally infected with *B. malayi* (sub-periodic strain) and having defined stages of this parasite, was chosen. This system was chosen also because *M. coucha* inoculated with *B. malayi* L3 and amicrofilaraemic is known to simulate amicrofilaraemic asymptomatic human filariasis in an endemic area.[24] Mab A7 detected antigens in the sera of animals having L4 or adult stage larvae of parasite. But, the reactivity was higher with animals having L4 than those with adult stage of the parasite. As it showed high reactivity with samples collected on the 20th day post-inoculation, when the developmental stage of the parasite is L4, the results reveal that

antibodies from clone A7 have potential of detecting L4 stage e/s antigens of the parasite in humans. The pre-patent period of *B. malayi* in *M. coucha* was found to be 90–120 days post-inoculation of L3 and the development of L3 to L4 takes 15 to 30 days and L4 to adult up to 90 days. This indicates that mab A7 detected the antigens of L4 stage larvae, as L3 develops to L4 stage in 20 days of inoculation into the animals. On the other hand, Mab A6 showed high reactivity against serum samples from animals the 80th day post-L3 inoculation, i.e., when the L3 inoculated would have developed into adult stage, while it exhibited negligible activity against L4 stage antigens.

When the Mab A7 was tested against the serum samples from human subjects who were amicrofilaraemic and asymptomatic, it detected a significant proportion of them as antigen positives, thus indicating that they may be harboring developing filarial parasites (L4) in their body. These people are called endemic normals, as they appear to be free from infection,[25] but may harbor undetectable parasitaemia. Of 17 non-endemic normal samples, two of the samples reacted positive for filarial antigens, which could be the lesser specificity of the assay, or it is possible that these two individuals visited the nearby areas which are very close to their residence known to be endemic for filariasis (Coimbatore, Mettupalayam-Tamil Nadu, India).

With respect to Mab A6, 86% of ($n = 30$) microfilarae positive samples reacted with it. None of the serum samples from non-endemic normals ($n = 21$) reacted with this Mab. This indicates that the Mab from A6 has high sensitivity and specificity in detecting the mf positive persons and, therefore, could be used for detecting patent infection of *W. bancrofti* in humans. Mab A6 exhibited 93.3% specificity and 84.6% sensitivity, while Mab A7 had a specificity of 88% and 84% sensitivity.

Mabs have been developed earlier against various filarial antigens by different workers. Wongkamachai et al.[25] developed a Mab (17E10) that recognized 37 to 200 kDa antigens of adult *B. malayi.* Kirithika et al.[26] reported another Mab of 38.0 kDa against mf antigens of *B. malayi*. Similarly Parab et al.[27] developed an Mab against the L3 stage of *B. malayi* which recognized antigens of 80, 67, 52, and 36 kDa of the infective larvae. The Mabs developed by Lala Dhas and Kaleysa Raj[28] reacted with the 90 kDa protein of the *W. bancrofti* microfilariae antigens. The Mab A7 developed by us recognized a 40.0 kDa e/s antigen of the L4 stage of *W. bancrofti i*n Western blot analysis.

The two Mabs had slightly lesser specificity, compared to the membrane filtration technique, as they showed antigen positivity in a few serum samples from non-endemic normals. The two Mabs also exhibited a slightly lesser sensitivity compared to the membrane filtration technique, mainly because they did not detect samples with very low mf count (≤ 3). With respect to the very low mf count, the lack of sensitivity has been reported also for the two commercially available kits, viz., the ICT card test and the Og4C3 (Trop-Bio) ELISA test.[2,9] Thus, the immuno-assays developed in the present investigation have shown potential in the diagnosis of filarial infection in humans.

However, the diagnostic potential of these assays needs to be evaluated on a larger number of samples.

CONCLUSION

The results of the present study show that the Mab A7 has potential towards the early diagnosis of filariasis. Similarly, the Mab A6 detected microfilaraemic cases and, hence, could also be used for detecting patent infections. However, the specificities of assays based on these Mabs need to be improved and validated using larger numbers of samples before being used in routine diagnosis/surveillance.

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