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# The Use of Monoclonal Antibody in the Detection of Circulating Antigen in Malayan Filariasis Cases

## A Preliminary Report

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***INTISARI***

Soeyoko dan Sri Sumarni – *Penggunaan antibodi monoklonal untuk deteksi antigen pada penderita filariasis yang disebabkan B.malayi.*

Filariasis (penyakit kaki gajah) di Indonesia disebabkan oleh cacing filaria *Wuchereria bancrofti, Brugia malayi* dan *Brugia timori*. Dari ketiga spesies tersebut, *B. malayi* merupakan penyebab utama filariasis terutama di daerah endemik di luar Jawa. Diagnosis filariasis sampai saat ini masih didasarkan atas hasil pemeriksaan klinis, parasitologis dan imunologis konvensional, namun ketiga cara tersebut banyak kelemahannya.

Dalam era bioteknologi, dengan ditemukannya teknik hibridoma, dapat dihasilkan antibodi monoklonal yang spesifik terhadap filaria *B. malayi* dan mampu mengikat *circulating antigen* dalam serum penderita filariasis.

Dengan teknik pemeriksaan *dot-blot* didapatkan serum yang mengandung *circulating antigen* sebagai berikut: 75% pada serum kelompok penderita simtomatik-mikrofilaremia; 40% serum kelompok penderita simtomatik-amikrofilaremia; 88,8% serum kelompok penderita asimtomatik-mikrofilaremia; dan 19,6% serum kelompok penderita asimtomatik-amikrofilaremia.

Antibodi monoklonal dapat membantu diagnosis filariasis terutama pada yang asimtomatik-amikrofilaremia.

*Key Words:* *Brugia malayi* – filariasis – filarial circulating antigen – antifilarial monoclonal antibodies – dot-blot assay

## INTRODUCTION

*Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* are the causative agents of lymphatic filariasis in Indonesia (Partono, 1977), however in many endemic areas *Brugia malayi* is more profound.

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Diagnosis of filariasis is normally based upon clinical, parasitological and immunological evidence. Although it is very easy to recognize a classical obstructive filariasis or elephantiasis, many people who are infected do not show any signs and symptoms. Therefore, a laboratory confirmation is necessary.

Parasitological techniques involve the demonstration and identification of circulating microfilariae, which are normally prepared from the peripheral blood are taken at night. Unfortunately; in endemic areas some infected people do not show microfilaraemia.

The importance of immunological test in the diagnosis of filarial infection has been clearly described as detection of antifilaria antibodies in serum. However, most of the filarial antigen are not species specific. Therefore, cross reactivity among species may occur and species diagnosis may be less reliable.

The discovery of monoclonal antibody recently is expected to provide a firm scientific background in immunology, and add a new dimension to the efforts in developing a specific and sensitive immunological test for various stages of filarial infection (Haque *et al.*, 1982; Dissanayake & Ismail, 1982).

Frank (1946, *cit.* Au *et al.*, 1981) who first demonstrated the presence of a filarial circulating antigen in the sera from patients with *Wuchereria bancrofti* infection, suggested that the detection of antigen could be used for diagnosis.

In this report, a monoclonal antibody against *Brugia malayi* was produced, and field trial has been carried out to detect filarial circulating antigen in the sera from Malayan filariasis cases.

## MATERIALS AND METHODS

### Sera

Sera were collected during epidemiological survey for Malayan filariasis in the endemic areas of South and East Kalimantan Province, and from uninfected volunteers living in non-endemic area in Yogyakarta in the Central of Java. Sera were separated from blood sample and stored at -20°C until used.

Human Malayan filariasis sera were classified into 4 categories:

1. Sera from microfilaremic patients with clinical signs such as fever, edema of extremities, lymphangitis, lymphadenitis or elephantiasis.
2. Sera from amicrofilaremic patients with clinical signs.
3. Sera from microfilaremic patients without clinical signs.
4. Sera from individuals in endemic areas without clinical and parasitological evidence of filariasis.

The presence of microfilaremia was detected by microscopic examination of stained filtertipped of blood obtained intravenously.

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**Antigen**

Preparation of filarial antigen extract was carried out according to Freedman *et al.* (1988) with minor modification. *B. malayi* adult worms in extraction buffer were centrifuged at 6000 x for 15 minutes at 4°C. The extraction buffer prepared before-hand was as follows:

1% cetyl trimethyl amonium bromide
50 mM hepes
100 mM glycine pH 7.2
1 mM ethylene diamine tetra acetic acid
0.2 mM n-d-tesyl-1-lysine
0.05 mM leupeptine
0.025 mM p-nitro-guanidinobenzoate

Protein concentration in the supernatant was determined spectrophotometrically.

**Monoclonal antibody**

The monoclonal antibody used in this study was prepared by fusion of NS-1-myeloma cells with spleen cells from Balb-c mice sensitized with filarial antigen extract as previously described. Antibody producing hybrids were then cloned using a limiting dilution technique on feeder layer of mouse peritoneal macrophages, and were grown to large number *in vitro* or *in vivo* as an ascites tumours in mice.

**Dot-blot assay**

Sera to be tested were put on a nitrocellulose paper which have been fixed in dot-blot apparatus. The nitrocellulose papers were then blocked with 2% BSA (Bovin Serum Albumin), 0.2% Tween-20 in PBS (Phosphate Buffer Saline), and were incubated at room temperature for 1 hour. The papers were then washed three times with TBS (Tris Buffer Saline) containing 0.05% Tween-20 (TBS-T) before the ascites fluid containing monoclonal antibody were added. After incubation at room temperature for 1.5 hours, the nitrocellulose were washed three times with TBS-T, and the unbound antibody was removed. Alkaline phosphatase conjugated goat anti-mouse antibody was then added and incubated at room temperature for 1.5 hours. Last washing was carried out three times with TBS-T. Substrate was added, and then shaked for 10 minutes or until the color developed.

## RESULTS AND DISCUSSION

The clinical and parasitological information of sera donors living in endemic filariasis in Surian village, South Kalimantan, are shown in TABLE 1. The blood examination revealed that 14 persons (11.4%) were microfilaremia positive with microfilarial density, 1-230 mf/2 ml i. v. blood, Nine persons (7.4%) had clinical symptoms of filariasis such as fever, lymphadenitis, edema extremities and elephantiasis, and 102 persons (84.2%) were without clinical manifestation and amicrofilaremia. The results showed that the prevalence of filariasis in Surian was low (11.5%). This is

considered, that Surian is a new village of 10 years old. The inhabitants, who are migrants, have contracted the infection at their own places before migration.

Observation in Krayan village, East Kalimantan showed: 7 persons (12.9%) with clinical symptoms of filariasis, and 11 persons (20.3%) were microfilaremia (microfilarial density 945 mf/2 ml i. v. blood). There were 70.3% which were symptomless and amicrofilaremia. The prevalence of filariasis in Krayan was higher than in Surian, because Krayan is an old village and inhabited by indigenous people (TABLE 1).

TABLE 1. – Clinical and parasitological information of sera donors living in the endemic filariasis in Surian village, South Kalimantan and in Krayan village, East Kalimantan.

| Donor groups | Surian | | Krayan | |
|---|---|---|---|---|
| | Number Positive | % | Number Positive | % |
| A. Clinical signs: + Microfilaria : + | 4 | 3,3 | 2 | 3,7 |
| B. Clinical signs: + Microfilaria : – | 5 | 4,1 | 5 | 9,2 |
| C. Clinical signs: – Microfilaria : + | 10 | 8,2 | 9 | 16,6 |
| D. Clinical signs: – Microfilaria : – | 102 | 84,2 | 38 | 70,3 |
| Total | 121 | | 54 | |

The circulating antigen in sera donors living in this endemic filariasis area, was detected by a monoclonal antibody using dot-blot assay and summarized in TABLE 2. An amount of 75% of sera from donors living in Surian with clinical symptoms and microfilaremia contained circulating antigen, as did in 100% of sera from residence in Krayan. There were only 40-60% of sera from amicrofilaremic patients with clinical signs had circulating antigen. Among sera from microfilaremic patients without clinical signs living both endemic areas 88.8% showed circulating antigen. Only 19.6% of sera from amicrofilaremic patients without clinical signs contained circulating antigen.

Diagnosis of filariasis is normally based on parasitological evidence including the demonstration and identification of circulating microfilariae prepared from the peripheral blood. This method, however, has several limitations. Clinical signs of filariasis such as fever, lymphangitis, and lymphadenitis, without laboratory confirmation do not confirm the infection, since many people who are infected do not show signs and symptoms (Mak, 1983).

By using dot-blot assay the circulating antigen which is present in the sera from amicrofilaremic patients with or without clinical signs can be detected by monoclonal antibody. Therefore, this technique is a very sensitive immunological test for filarial

infection. There is no correlation between the amount of microfilaremia and the level of circulating antigen in human sera.

TABLE 2. – Circulating antigen detected with monoclonal antibody using dot-blot assay

| Donor groups | Origin | NO. pos/NO. tested | % |
|---|---|---|---|
| A. | Surian | 3/4 | 75 |
| | Krayan | 2/2 | 100 |
| B. | Surian | 2/5 | 40 |
| | Krayan | 3/5 | 60 |
| C. | Surian | 9/10 | 90 |
| | Krayan | 8/9 | 88,8 |
| D. | Surian | 20/102 | 19,6 |
| | Krayan | 14/47 | 29,6 |

A,B,C, and D see TABLE 1.

## CONCLUSION

By using the dot-blot assay the circulating antigen which is present in sera from amicrofilaremic patients with or without clinical signs can be detected by monoclonal antibody, and this technique is a very sensitive immunological test for filarial infecton.

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