The Use of Monoclonal Antibody in the Detection of Circulating Antigen in Malayan Filariasis Cases
A Preliminary Report

By: Suryoko and Sri Sumarni

INTISARI

Suryoko dan Sri Sumarni – Penggunaan antibodi monoklonal untuk deteksi antigen pada penderita filariasis yang diinduksi 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 tertanam di sekitar endemik di lahan suaka. Diagnosis filariasis tampak menantang karena biasanya diperlukan berbagai aspek, seperti klinis, paraclinologis, dan imunologis, serta perlu kurangnya keterampilan penyelidik.

Dalam penelitian ini, digunakan teknik hibridoma, yaitu direaksikan antibodi monoklonal yang spesifik, teknik RIA untuk B. malayi dan muara memungkinkan deteksi antigen dalam serum ukuran filariasis.

Dengan teknik penentuan dosis dilakukan secara yang terkait dengan circulating antigen sebagai berikut: 75% pada serum kelenkup penderita sistematik-leadiliriaform, 40% serum kelenkup penderita sistematik-leadiliriaform, 35,0% serum kelenkup penderita sistematik-leadiliriaform, dan 19,6% serum kelenkup penderita sistematik-leadiliriaform.

Antibodi monoklonal yaitu serologi diagnosis filariasis tertanam pada yang sistematik-leadiliriaform.

Key Words: Brugia malayi – filariasis – filarial circulating antigen – antifilarial monoclonal antibodies – diagnosis accuracy

INTRODUCTION

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

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Diagnosis of filariasis is normally based on clinical, parasitological and immunological evidence. Although it is very easy to recognize classical ovipositing filarials of 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 microfilariae.

The importance of immunological tests in the diagnosis of filarial infection has been clearly described as detection of anti-filarial 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 & Jordan, 1982).

Frank (1946, cit. An 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 Malaysian filarisis cases.

MATERIALS AND METHODS

Sera

Sera were collected during epidemiological survey for Malaysian 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 Malaysian 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 anamnesticemic 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 microfilariaemia was detected by microscopic examination of stained filter-tipped of blood obtained intravenously.
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 g for 15 minutes at 4°C. The extraction buffer prepared before-hand was as follows:

- 1% cetyl trimethyl ammonium bromide
- 50 mM Hepes
- 100 mM glycine pH 7.2
- 1 mM ethylenediamine tetra acetic acid
- 0.2 mM α-d-erythrose-1-phosphate
- 0.05 mM L-isopeptide
- 0.025 mM p-nitro-phenylphosphate

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 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 (Bovine 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 was 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 shaken 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 microfilaria positive with microfilarial density, 1-230 mil/ml. 5 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 microfilariaemia. 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 in 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 microfilaria- (microfilarial density 965 mf/2 ml i. v. blood). There were 70.3% which were symptomless and microfilariaemia. 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.

<table>
<thead>
<tr>
<th>Donor groups</th>
<th>Surian</th>
<th></th>
<th>Krayan</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Positive</td>
<td>%</td>
<td>Number Positive</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>A. Clinical signs + Microfilaria +</td>
<td>4</td>
<td>3.3</td>
<td>2</td>
<td>3.7</td>
</tr>
<tr>
<td>B. Clinical signs + Microfilaria -</td>
<td>5</td>
<td>4.1</td>
<td>5</td>
<td>9.2</td>
</tr>
<tr>
<td>C. Clinical signs - Microfilaria +</td>
<td>10</td>
<td>8.2</td>
<td>9</td>
<td>16.6</td>
</tr>
<tr>
<td>D. Clinical signs - Microfilaria -</td>
<td>102</td>
<td>84.2</td>
<td>38</td>
<td>70.3</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>100</td>
<td>44</td>
<td>100</td>
</tr>
</tbody>
</table>

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 microfilariaemia contained circulating antigen, as did in 100% of sera from residence in Krayan. There were only 40-60% of sera from microfilaricemic patients with clinical signs had circulating antigen. Among sera from microfilaricemic patients without clinical signs living both endemic areas 88.3% showed circulating antigen. Only 19.6% of sera from microfilaricemic patients without clinical signs contained circulating antigen.

Diagnosis of filariasis is normally based on parasitological evidence including the demonstration and identification of circulating microfilariae recovered 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 (Moh, 1983).

By using dot-blot assay the circulating antigen which is present in the sera from microfilaricemic patients with or without clinical signs can be detected by monoclonal antibody. Therefore, this technique is a very sensitive immunological test for filarial
infections. There is no correlation between the amount of microfilariae and the level of circulating antigens in human sera.

<table>
<thead>
<tr>
<th>Donor groups</th>
<th>Origin</th>
<th>NO. pro/NO. bound</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Kuantan</td>
<td>34</td>
<td>75</td>
</tr>
<tr>
<td>B.</td>
<td>Kuantan</td>
<td>2/2</td>
<td>100</td>
</tr>
<tr>
<td>C.</td>
<td>Kuantan</td>
<td>2/5</td>
<td>40</td>
</tr>
<tr>
<td>D.</td>
<td>Kuantan</td>
<td>3/5</td>
<td>60</td>
</tr>
<tr>
<td>E.</td>
<td>Kuantan</td>
<td>1/3</td>
<td>33,3</td>
</tr>
</tbody>
</table>

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

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

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REFERENCES