BIOTECHNOLOGY OF THE FILARIA OF INDONESIA

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ABSTRACT

More than 90 million people were currently infected with lymphatic filariasis and two-third of them lived in China, India and Indonesia. Filariasis is endemic throughout the entire Indonesian Archipelago. More than 20 million people lived in endemic areas and 3-4 million people were estimated to have the infection. Control measures have reduced the prevalence of infections in some areas, but the disease remained a public health problem in many outer islands of Indonesia. Recent development in monoclonal antibodies and recombinant technology of DNA have made it possible to apply these new tools in the studies of filariasis, and three groups in Indonesia are currently using these new technology. The studies with the Imperial College of London will be presented by Dr. Rick Maizels. Collaboration with the New England Biolabs and Smith College involve the use of a stage and species-specific monoclonal antibody against the infective larvae of *Brugia malayi*, a double blind comparison of conventional methods and DNA probes for the diagnosis of brugian filariasis, and phylogentic studies of the brugian parasites. The ELISA using the monoclonal antibody has been adapted for field use in Jakarta. It is simple to use, does not cross-react with the infective stage of *Brugia pahangi*, but does so with the infective stage of the non-sympatric *Brugia timori*. The reagent is useful to acurately monitor the progress of control programs in endemic areas of brugian filariasis. The oligonucleotide DNA probes for *B.malayi* and *B.pahangi* were both qualitatively and quantitatively comparable to the conventional methods for the diagnosis of brugian parasites in cats and man. Sequencing data of the repeated DNA sequences of various brugian parasites indicated their homologies and divergences. The anthropophilic strain of *B.malayi* and *B.timori* showed similarity in their biological characteristics and repeated DNA sequences and they are phylogenically probably closely related. One isolate of *B.malayi* from Tanjung Pinang showed closer homology to the repeated DNA sequences of *B.pahangi* than to that of *B.malayi*. Studies on the repeated DNA sequences of different isolates of brugian parasites are essential before DNA probes can be widely used in field studies.

INTRODUCTION

Lymphatic filariasis remains a public health problem in many parts of the world. According to a recent WHO estimate, more than 90 million people were currently infected with the parasites, and two-third of them lived in China, India and Indonesia (WHO Expert Committee Meeting, Geneva, 1983). Filariasis is endemic throughout the Indonesian Archipelago. More than 20 million people are at risk of acquiring the infection, and 3-4 million people were estimated to be infected. Control measures have reduced the prevalence of infection in some economic development areas, but the disease

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remains a public health problem in many outer islands of Indonesia. Recent advances in immunology and recombinant DNA technology have made it possible to apply these new tools in the studies of filariasis. Several groups are currently involved in biotechnology research in Indonesia and three are working on filariasis: Dr. Kurniawan's, Dr. Nurhajati's and our group. Our project is a collaborative research effort involving the laboratories of Dr. Rick Maizel of Imperial College, London, Dr. Steven Williams of Smith College, USA, Drs. Larry Mc Reynolds, Mario Philipp and Clotilde Carlow of New England Biolabs, USA, and the Department of Parasitology, Faculty of Medicine, University of Indonesia.

IMMUNOLOGICAL STUDIES

A range of surface, secreted and somatic antigens from filarial parasites have been studied in order to analyse the response of humans infected with these parasites, and to develop reliable diagnostic and prophylactic agents. Diagnostic procedures are being developed by two techniques. Firstly, detection of host antibody is carried out using selected specific parasite antigens in the form of recombinant peptides from a filarial DNA library. Secondly, measurement of parasite antigen released into host serum during infection is performed by an ELISA antigen capture assay, using a monoclonal antibody (Table 1). In addition, a longer term objective is to isolate molecules which may stimulate protective immune responses against filarial parasites. A major focus has been a parasite surface glycoprotein known to be closely conserved between adult worms of *Brugia malayi, Brugia timori* and *Wuchereria bancrofti*. The antigen has been cloned from a cDNA library, and its primary sequence established. In addition to being a constant feature of the adult surface, it is expressed by developing larvae and represents an attractive target for vaccine production. Finally, we are trying to dissect the genesis of pathological reactions, by comparing the immune responses of individuals of differing clinical status to certain defined parasite antigens in an attempt to correlate disease development with particular categories of immune response in infected patients.

FIELD TESTING OF FILARIAL SPECIFIC DNA PROBES

A DNA probe to detect Brugian parasites has been developed by the New England Biolabs[1]. Based upon the region of divergence of the *Hha* I repeat of *B.malayi (Bm)* and *B.pahangi (Bp)*, a 29-mer *Bm* specific and a 21-mer *Bp* specific oligonucleotide probes were developed[2] (Table 2). These probes were radiolabeled with P32 and used in a dot blot assay to detect brugian microfilariae from donors in South Kalimantan, where *Bp* had been reported to occur in humans[3]. The tests were blindly performed using *Bm* and *Bp* from experimentally infected animals as controls, and the results compared with those obtained by parasitological examination of microfilariae. Two hundred human blood samples were tested by both methods, and the agreement was 97 % (Table 3). An additional 64 cat blood samples were also blindly tested by both methods and the agreement was 95 % (Table 4). The densities of microfilariae (Mf) in these samples were stratified into different levels, ranging from 0-49 to over 1000 per unit of blood. The degrees of agreement by both methods ranged from 0-100%, averaging 82 % (Table 5).

PRELIMINARY FIELD TESTING TO NONRADIOACTIVE DNA PROBES

A variety of non-radioactive DNA probes have been constructed and tested under laboratory conditions. Most of the effort was concentrated on the development of biotinylated oligonucleotide probes. The initial radio-labeled *Bm* specific 29-mer oligonucleotide and the *Bp* specific 21-mer oligonucleotide DNA probes were able to detect as little as 0.1 ng of *Brugia* DNA (approximately equal to 1 mf) and are species-specific. When these probes were labeled with six biotin residues attached via uridine nucleotides to the 5' end, the sensitivity was reduced to about 1-2 ng. To improve the sensitivity, two new oligonucleotide probes were synthesized based upon the *Hha* I repeat region of divergence: a 45-mer *Bm*-specific and a 44-mer *Bp*-specific DNA probe. They were labeled with 45 biotinylated uridine residues added to the 5' end. The biotinylated uridine residues were alternated in the chain with non-biotinylated thymidine residues, which served as spacers between the biotinylated uridine residues, and allow for more efficient binding of the biotin moieties by the avidin or streptavidin detector molecules. Thus, these new probes are 135 and 134 nucleotides in length. The sensitivity of detection of these probes was in the range of 0.1 ng and each probe still retained its species specificity.

The new probes were field tested on 43 human and 14 cat blood samples, collected from microfilaraemic donors on the island of Rampang, near Tanjung Pinang. A part of each sample was amplified by the polymerase chain reaction (PCR) and compared with the unamplified samples using both radioactive and biotinylated DNA probes. All the results were subsequently compared with the conventional detection system of microfilariae. The PCR amplified samples were accurately identified by both the radioactive and biotinylated probes. The samples that were not PCR amplified were accurately identified only after long exposures (more than one week) by the radioactive probes. The biotinylated probes were not sensitive enough to accurately identify the non PCR amplified samples.

CLONING AND CHARACTERIZATION OF THE *Hha* I REPEATS FROM BRUGIAN FILARIA

Studies on the evolution of filarial parasites in humans and animals were also done. Consensus sequences of the *Hha* I repeats of several isolates of brugian parasites have been determined by our group. The difference between the *Hha* I repeats of the zoophilic and the anthropophilic strain of *B.malayi* is small, and so is the difference between the repeats of *B.malayi* and *B.timori*. These data will be computer-analysed to determine whether it is possible to construct a strain-specific malayan or a timorian specific DNA probe.

There is one strain of *B.malayi* that we have isolated from a cat from Tanjung Pinang, which hybridized in a unique way to the DNA probes. The microfilariae are identified as *B.malayi* by morphological criteria (Purnomo, unpublished data) but the DNA of this isolate only hybridized to the *Bp*-specific DNA probe. Nine repeats of this parasite were sequenced in their entirety and the data computer-analysed. The analysis confirmed the results of the DNA hybridization studies, indicating similarity between the repeats of this isolate and that of *B.pahangi*. In order to investigate this problem further, a field trip to Tanjung Pinang was recently organized to

collect microfilariae from a number of infected humans and cats. These samples were currently being evaluated using our DNA probes and the polymerase chain reaction (PCR).

FIELD TESTING OF A STAGE AND SPECIES SPECIFIC MONOCLONAL ANTIBODY

A stage and species specific monoclonal antibody to detect a unique surface epitope of *B.malayi* infective larvae has been developed by Mario Philipp's group at the New England Biolabs[4]. An ELISA field assay was subsequently developed to identify infective larvae of *B.malayi* in vector mosquitoes. The sensitivity of detection of this assay was in the range of 70 to 80% if the monoclonal antibody was incubated with the parasites for 1 hour in our laboratory in Jakarta, slightly lower than that obtained at the base laboratory. However, by increasing the incubation time to two hours, the sensitivity was increased to more than 90 % . The monoclonal antibody did not cross-react with the infective larvae of *B.pahangi*, but did so with the non-sympatric L3s of *B.timori*. The reagent was field tested in South Kalimantan in an area endemic with *B.malayi* and enzootic with *B.pahangi*, where DEC control measures against brugian parasites in humans have been repeatedly applied. More than 3000 potential vectors were dissected for filarial larvae. Only 131 infective larvae were recovered and of these only a small fraction of them (10) were identified as *B.malayi* (Table 6). This is a good example of how the use of modern technology can help endemic countries to cost-effectively control brugian filariasis. If the tool has not been made available for field use, the infective rates in vector mosquitoes would have been incorrectly estimated by a factor of more than 10 fold, which may probably result in a wrong decision making at the control level of filariasis, and consequently a waste in control efforts.

REFERENCES

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Table 1. Decreasing circulating antigen among villagers in Karakuak, Flores, after DEC treatments.

Year	Individuals tested	Antigen-Positive	Antigen-Negative
1982	130	21 (16.2%)	109 (83.8%)
1988	130	2 (1.5%)	128 (98.5%)

Circulating antigen levels were determined in a two-site ELISA using monoclonal antibody to the phosphoryl-choline epitope. The decrease in circulating antigen levels paralleled the decrease in microfilaria and disease rates.

Table 2. Comparison between the repeated DNA sequences of *B.malayi* and *B.pahangi*

B.malayi

0 322

93 % HOMOLOGY 72 % HOMOLOGY

B.pahangi

0 322

B. malayi 29 mer probe

AAGCT - TATTTTGAACCTAATTGACTATGTTACGTGCATTGTACCAGTGCTGGTCGTATATTGTGTT

* ** ** * * *** * ** * * * * * *

TAGCAATATTTTGAAGTTAATTCATTATGTTTTATGCATTGCACTGGGGCGGGTCATATATGGT - TG

209 275

B.pahangi 21 mer probe

* = Mismatch

Table 3. Detection of brugian mf in human blood

No. Tested	*B.malayi* *Par	Probe	*B.pahangi* Par	Probe	% Agree
200	200	193	0	1	97
20 Bp	0	0	20	20	100
37 Bm	37	37	0	0	100

* PAR = Parasitology

Table 4. Detection of microfilariae in cat blood

No. Tested	*B.malayi*		*B.pahangi*		*D.repens*		
	*Par	Probe	Par	Probe	Par	Probe	% Agree
64	3	5	37	36	22	NA	95
20 Bp	0	0	20	20	0	NA	100
37 Bm	37	37	0	0	0	NA	100

* Par = Parasitology

Table 5. Quantitative Determination of mf Densities

Density	Human blood			Cat blood SK			Cat blood Lab		
	Par	Probe	%	Par	Probe	%	Par	Probe	%
1 - 49	135	130	96	21	15	71	23	17	74
50 - 99	35	35	100	5	7	71	16	9	56
100-499	28	30	93	13	16	81	8	23	35
500-999	2	5	40	3	4	75	2	4	50
> 1000	0	0	-	0	0	-	8	4	50
Total	200	200		42	42	57	57		

Par = Parasitology
SK = South Kalimantan
Lab = Laboratory

Table 6. Identification of *Bm* L3 by Monoclonal Antibody

Number of wild caught mosquitoes	6446
Number of mosquitoes dissected	3492
Number of infective larvae isolated	131
Number of larvae identified by Mab	10

QUESTIONS AND ANSWERS :

1. Question: The monoclonal antibody you use in detecting L_3 is specific and highly sensitive. At this point, what further improvements and steps would you be taking before it could be implemented in a wider range/other areas.

   Answer : It is ready for use for the filariasis control programme to accurately define the problem of Brugian filariasis, to monitor control efforts and to decide when to stop control measures.

2. Question: - What is the origin of your cDNA library, (page 12).
   - Nucleic acid sequence of the T.Pinang strain showed several mismatches. How about the Amino Acid sequences ?

   Answer : - Any stage, but we use microfilariae.
   - No information on the amino acid sequences, because the repeats are a noncoding region.

3. Question: Is the present knowledge enough to predict that B. pahangi would not infect man, since it has a distinct pathway.

   Answer : We cannot confirm the infection of B.pahangi in man in our recent studies. The evidence presented by Palmiery of B.pahangi infection in man is not tight. Biotechnology can certainly resolve this problem in the future.