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Identification of *Brugia malayi* immunogens by an immunoproteomics approach

Sirichit Wongkamchai^a, Wararat Chiangjong^b, Supachok Sinchaikul^c, Shui-Tein Chen^c,
Wej Choochote^d, Visith Thongboonkerd^{b, e, *}

^aDepartment of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

^bMedical Proteomics Unit, Office for Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

^cInstitute of Biological Chemistry and Genomic Research Center, Academia Sinica, Taipei, Taiwan

^dDepartment of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

^eCenter for Research in Complex Systems Science, Mahidol University, Bangkok, Thailand

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ABSTRACT

Filariasis remains a health problem in tropical countries. Identification of immunogens from its causative organism would lead to development of a better diagnostic test, as well as vaccine discovery to effectively prevent this disease. We applied immunoproteomics to define potential immunogens of adult *Brugia malayi* that were recognized by IgM, IgG1 and IgG4 in sera of patients with four distinct clinical spectra of filariasis, including endemic asymptomatic, lymphangitis, elephantiasis and microfilaremia (n=5/group). Sera of healthy individuals (n=5) from non-endemic area served as the negative control. Brugian proteins were resolved by 2-DE and subjected to 2-D Western blot analysis probed with these sera. A total of 30 immunoreactive proteins recognized by IgM, IgG1 and IgG4 in sera from all four filarial groups were identified by Q-TOF MS and MS/MS analyses. Interestingly, only three immunogens were recognized by IgM in lymphangitis, elephantiasis and microfilaremia, but not in endemic asymptomatic group. IgG1 recognized 20 immunogens in endemic asymptomatic, lymphangitis and microfilaremia (mostly in endemic asymptomatic group), but not in elephantiasis, whereas IgG4 recognized 28 immunogens in all four filarial groups (mostly in microfilaremia). This large data set is an important resource for further development of a new diagnostic test and/or vaccine for filariasis.

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1. Introduction

Filariasis remains an important tropical disease. People, who live in endemic areas, are easily infected via mosquito-bite that transmits the causative parasite person-to-person. Patients with filariasis frequently have lymphatic invasion that causes a variety of temporary and permanent disabilities. It is now considered as one of the few infectious diseases that are potentially eliminable [1], and the World Health Organization (WHO) has taken a priority to reduce and finally eliminate lymphatic

filariasis by 2020 [2]. Hence, effective diagnostic test becomes crucial for the disease detection. Thick blood smear remains the routine laboratory method used for the diagnosis of lymphatic filariasis. This diagnostic method depends on the detection of microfilaria in peripheral blood (microfilaremia). Due to the diurnal variation of microfilaremic levels in most of endemic areas of lymphatic filariasis, this test requires specimen (blood) collection at night. Furthermore, this method is relatively insensitive and difficult to interpret precisely in the field survey. Serological diagnostic methods exhibit a better sensitivity as

* Corresponding author at: Siriraj Hospital, Mahidol University, 12th Floor Adulyadej Vikrom Building, 2 Prannok Road, Bangkoknoi, Bangkok 10700, Thailand. Tel./fax: +66 2 4184793.

E-mail addresses: thongboonkerd@dr.com, vthongbo@yahoo.com (V. Thongboonkerd).

compared to the thick blood smear, allowing the detection of subjects with prior infection but are currently non-microfilaremic (endemic asymptomatic). Serological methods also offer opportunity to examine daytime-collected blood samples [3]. Subsequent development of rapid immunochromatographic test (ICT) is a significant advance providing a reliable assay for detection of circulating antigens derived from *Wuchereria bancrofti* [4]. The desirable by-product of this breakthrough enables mapping of endemic areas for the mass drug administration program for eradication of filariasis. However, ICT is specific only for the detection of circulating antigens derived from *W. bancrofti* and is not applicable for the detection of *Brugia malayi*, another common etiologic species of filariasis. Apart from the still-imperfect diagnostic tests, there is no effective chemotherapy to completely eradicate lymphatic-dwelling adult filarial parasites that persist in the infected hosts for several years. In addition, there is no vaccine available for effective prevention of filariasis [5]. It is therefore crucial to develop a new diagnostic test that can detect all forms of filariasis and a vaccine that can effectively prevent the disease. Efforts towards these developments seem to be achievable through the characterizations of immune response in patients with filariasis and protective immunity in the exposed population [6].

In the present study, we applied an immunoproteomics approach to define potential immunogens of *B. malayi*. Briefly, proteins derived from adult *B. malayi* were resolved by two-dimensional gel electrophoresis (2-DE) and then subjected to 2-D Western blot analysis probed with sera collected from patients with four clinical entities of filariasis, including endemic asymptomatic, lymphangitis, elephantiasis and microfilaremia. Sera of healthy individuals from non-endemic area served as the negative control. The immunoreactive protein spots detected with anti-IgM, anti-IgG1 and anti-IgG4 antibodies conjugated with horseradish peroxidase were then identified by Q-TOF MS and MS/MS analyses.

2. Materials and methods

2.1. Human subjects and sample collection

A total of 25 human subjects were included in this study. Sera were collected from patients in the endemic area of filariasis in Thailand with various clinical entities, including endemic asymptomatic, lymphangitis, elephantiasis, and microfilaremia ($n=5$ for each group). Other five sera samples were collected from healthy individuals in the non-endemic area (Bangkok, Thailand) and used as the negative control. The study on human subjects was approved by the Institutional Ethical Committee.

2.2. Cultivation of adult stage of *B. malayi* and protein extraction

B. malayi was maintained in *Meriones unguiculatus* gerbils and then harvested from the infected gerbils using a method described by Ash and Riley [7]. The parasites were washed extensively with distilled water and then with 0.1 M phosphate buffer, pH 7.2. They were then ground in distilled water containing protease inhibitors (0.1 M PMSF, 0.1 M TPCK and 0.1 M EDTA) using a glass tissue grinder. The homogenate was

then sonicated in an ice box using an ultrasonic sonicator (MSE Scientific Instruments; Sussex, UK) at 20 kilocycle/second and 4 °C for 20 min. The pellet was resuspended in a lysis buffer containing 8 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate (CHAPS), 120 mM dithiothreitol (DTT), 2% (v/v) ampholytes (pH 3–10) and 40 mM Tris-base, and incubated at 4 °C for 30 min. Unsolubilized nuclei, cell debris, and particulate matters were removed by a centrifugation at 10,000 rpm and 4 °C for 5 min. Protein concentration was determined by the Bradford method using Bio-Rad protein assay (Bio-Rad Laboratories; Hercules, CA).

2.3. 2-DE

For each 2-D gel, Immobiline DryStrip (non linear pH gradient of 3–10, 7-cm-long; GE Healthcare, Uppsala, Sweden) was rehydrated overnight with an equal amount of 50 µg total protein that was premixed with a rehydration buffer containing 7 M urea, 2 M thiourea, 2% CHAPS, 2% (v/v) ampholytes (pH 3–10), 120 mM DTT, 40 mM Tris-base, and bromophenol blue (to make the final volume of 150 µl per strip). The first dimensional separation or isoelectric focusing (IEF) was performed in Ettan IPGphor III System (GE Healthcare) at 20 °C, using a stepwise mode to reach 9,083 Vh with limited current of 50 mA/strip. After completion of the IEF, the strip was first equilibrated for 15 min in an equilibration buffer containing 6 M urea, 130 mM DTT, 112 mM Tris-base, 4% SDS, 30% glycerol and 0.002% bromophenol blue, and then in another similar buffer that replaced DTT with 135 mM iodoacetamide, for further 15 min. The second dimensional separation was performed in 12% polyacrylamide gel using SE260 Mini-Vertical Electrophoresis Unit (GE Healthcare) at 150 V for approximately 2 h. The resolved protein spots were either stained with CBB-G250 (USB, Cleveland, OH, USA) or transferred onto a PVDF membrane for subsequent immunoblot analysis.

2.4. 2-D Western blot analysis

Proteins resolved by 2-DE as described above were transferred onto a PVDF membrane using a semi-dry transfer system. After blotting, the membrane was blocked with 5% milk in PBS, pH 7.2 with 0.1% Tween-20 for 30 min and then incubated with the pooled sera of each group at 4 °C overnight. The membrane was washed three times with a washing buffer (containing 2% milk, 0.1% Tween-20 and PBS, pH 7.2) at room temperature and then incubated with mouse monoclonal anti-human IgM, anti-human IgG1 or anti-human IgG4 conjugated with horseradish peroxidase (Invitrogen; Camarillo, CA) diluted 1:10,000 at room temperature for 1 h. The immunoreactive protein spots were then visualized using enhanced chemiluminescence reagent (Pierce Biotechnology; Rockford, IL) followed by autoradiography.

2.5. Intensity analysis and matching of immunoreactive spots with protein spots visualized by CBB-G250 staining

Image Master 2D Platinum (GE Healthcare) software was used for matching and analysis of protein spots on 2-D gels. Parameters used for spot detection were (i) minimal area=10 pixels,

(ii) smooth factor=2.0, and (iii) saliency=2.0. A reference gel was created from an artificial gel combining all of the spots presenting in different gels into one image. The reference gel was then used for matching of corresponding protein spots across different gels.

2.6. In-gel tryptic digestion

All the immunoreactive protein spots detected by anti-IgM, anti-IgG1 and anti-IgG4 antibodies were excised from 2-D gels, washed twice with 200 μ l of 50% acetonitrile (ACN)/25 mM NH_4HCO_3 buffer (pH 8.0) at room temperature for 15 min, and then washed once with 200 μ l of 100% ACN. After washing, the solvent was removed, and the gel pieces were dried by a SpeedVac concentrator (Savant; Holbrook, NY) and rehydrated with 10 μ l of 1% (w/v) trypsin (Promega; Madison, WI) in 25 mM NH_4HCO_3 . After rehydration, the gel pieces were crushed and incubated at 37 °C for at least 16 h. Peptides were subsequently extracted twice with 50 μ l of 50% ACN/5% trifluoroacetic acid (TFA); the extracted solutions were then combined and dried with the SpeedVac concentrator. The peptide pellets were resuspended with 10 μ l of 0.1% TFA and purified using ZipTip_{C18} (Millipore; Bedford, MA). The peptide solution was drawn up and down in the ZipTip_{C18} ten times and then washed with 10 μ l of 0.1% formic acid by drawing up and expelling the washing solution three times. The peptides were finally eluted with 5 μ l of 75% ACN/0.1% formic acid.

2.7. Protein identification by Q-TOF MS and MS/MS analyses

The trypsinized samples were premixed 1:1 with the matrix solution containing 5 mg/ml α -cyano-4-hydroxycinnamic acid (CHCA) in 50% ACN, 0.1% (v/v) TFA and 2% (w/v) ammonium citrate, and deposited onto the 96-well MALDI target plate. The samples were analyzed by Q-TOF Ultima™ mass spectrometer (Micromass; Manchester, UK), which was fully automated with predefined probe motion pattern and the peak intensity threshold for switching over from MS survey scanning to MS/MS, and from one MS/MS to another. Within each sample well, parent ions that met the predefined criteria (any peak within the m/z 800–3,000 range with intensity above 10 count±include/exclude list) were selected for CID MS/MS using argon as the collision gas and a mass dependent ± 5 V rolling collision energy until the end of the probe pattern was reached. The MS and MS/MS data were extracted and outputted as the searchable .txt and .pkl files, respectively, for independent searches using the MASCOT search engine (<http://www.matrixscience.com>), assuming that peptides were monoisotopic. Fixed modification was carbamidomethylation at cysteine residues, whereas variable modification was oxidation at methionine residues. Only one missed trypsin cleavage was allowed, and peptide mass tolerances of 100 and 50 ppm were allowed for peptide mass fingerprinting and MS/MS ions search, respectively.

3. Results and discussion

We applied an immunoproteomics approach to identify potential immunogens of adult *B. malayi*, a causative parasite of

filariasis. Proteins extracted from *B. malayi* were resolved by 2-DE and subjected to 2-D Western blot analysis probed with pooled sera from five subjects in individual groups, including endemic asymptomatic, lymphangitis, elephantiasis and microfilaremia. The pooled sera from five healthy individuals in non-endemic area served as the negative control. Thereafter, the immunoreactive protein spots were detected by anti-IgM, anti-IgG1 and anti-IgG4 antibodies conjugated with horseradish peroxidase. Combination of 2-DE, immunoblotting and mass spectrometric analysis is a common immunoproteomics approach used for identification of novel antigens [8]. This approach has been proven for characterizing antigens derived from parasitic organisms, e.g. *Toxoplasma gondii*, *Fasciola hepatica*, *Schistosoma japonicum*, *Ascaris suum* and *Neospora caninum* [9]. Using this method, we identified a total of 30 immunoreactive protein spots that were recognized by sera of filarial patients regardless of clinical group and immunoglobulin type examined, whereas none was detected by the negative-control sera (Fig. 1).

Overall, IgM recognized smallest number of antigens, whereas IgG4 recognized greatest number of potential immunogens. Only three immunogens were recognized by IgM, including T-complex protein 1 theta subunit (detectable only in lymphangitis), chaperonin homolog HSP60, mitochondrial precursor (detectable only in elephantiasis) and enolase (detectable only in microfilaremia). There was no IgM-recognized immunogen found in endemic asymptomatic group. IgG1 recognized 20 immunogens in endemic asymptomatic, lymphangitis and microfilaremia (mostly in endemic asymptomatic group), but not in elephantiasis. IgG4 recognized 28 immunogens in all 4 filarial groups (mostly in microfilaremia) (Fig. 1). All of these immunoreactive protein spots were successfully identified by Q-TOF MS and MS/MS analyses. Their mass spectrometric data (identities, identification scores, sequence coverage, number of matched peptides, isoelectric point or pI, molecular weight or MW, etc.) are summarized in Table 1. Moreover, details of their quantitative data, in terms of degree of immune recognition as reflected by intensity volume of the immunoreactive spots recognized by IgM, IgG1 and IgG4, are provided in Table 2.

Indeed, proteomics has previously been applied to filariasis. Hewitson et al. [5] employed 2 parallel proteomic approaches, namely 2-DE and shotgun liquid chromatography (LC) coupled to MS/MS, and successfully identified 80 excretory–secretory proteins and 63 somatic proteins from adult *B. malayi*. In other studies, Moreno and Geary [10] performed 1-DE (SDS-PAGE) followed by LC-MS/MS and identified 228 excretory–secretory proteins derived from adult female, adult male and microfilarial *B. malayi*, whereas Bennuru et al. [11] identified 852 excretory–secretory proteins from various stages of *B. malayi* using microcapillary reverse-phase LC-MS/MS. However, none of the immunogenic proteins were characterized or identified in these studies. Weinkopff et al. [12] identified 18 unique proteins from adult *Brugia pahangi* using 2-DE followed by MALDI-TOF MS. From these 18 identified proteins, 7 were detected as potential immunogens of *B. pahangi*. Among these, only galectin was consistent with our data. The differences might reflect differential species of filarial parasite used in these two studies. In the present study, we have identified, for the first time, potential immunogens of *B. malayi* that could be

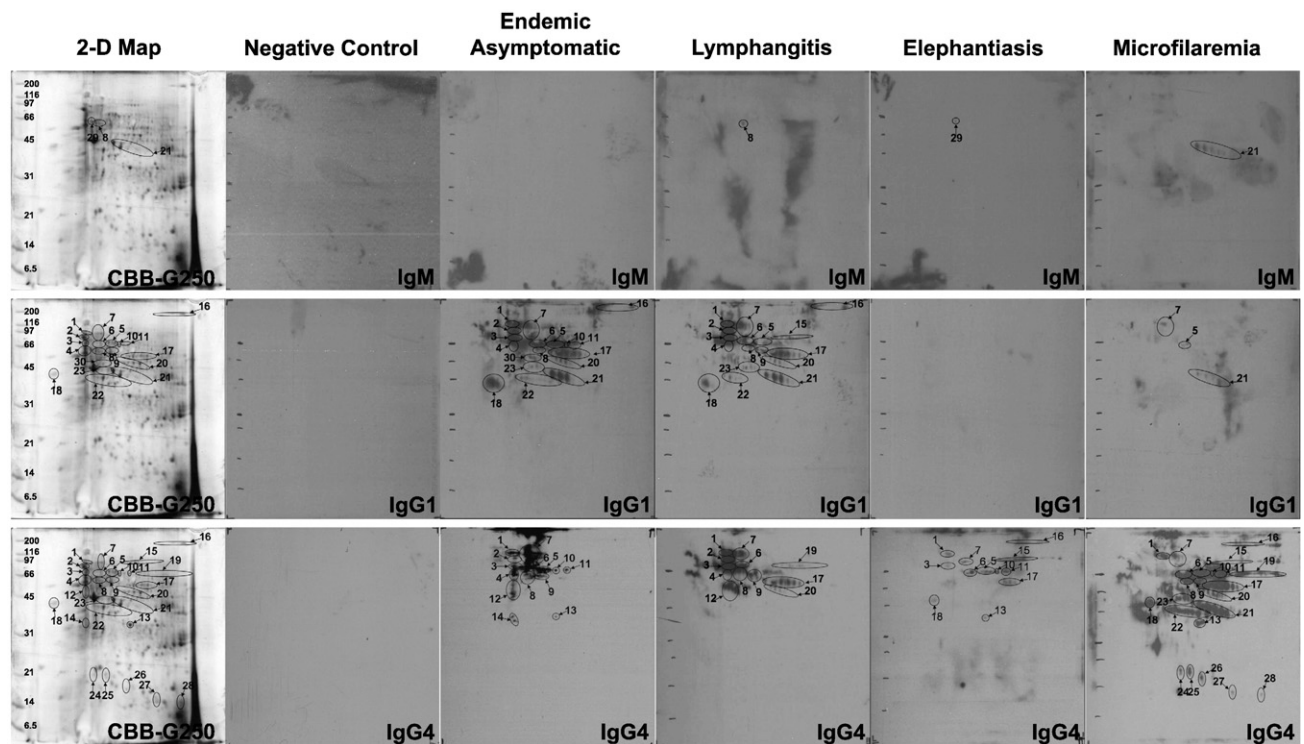


Fig. 1 – 2-D proteome map of all immunoreactive proteins. Proteins derived from adult *B. malayi* (with an equal amount of 50 μ g) were resolved in 2-DE and were then either stained with CBB-G250 dye (first column) or subjected to 2-D Western blot analysis (other columns). For the latter, the proteins transferred onto PVDF membranes were probed with pooled sera from patients with different clinical spectra of filariasis, including endemic asymptomatic, lymphangitis, elephantiasis and microfilaremia (sera from healthy individuals in non-endemic area served as the negative control). Immunoreactive protein spots were then detected using horseradish peroxidase-conjugated secondary antibodies against human IgM (first row), IgG1 (second row) and IgG4 (third row). All these immunoreactive proteins were then identified by Q-TOF MS and MS/MS analyses (see also [Table 1](#)). The quantitative data of immunoreactivity of these potential immunogens are summarized in [Table 2](#).

recognized by antibodies in sera of patients with different clinical spectra of filariasis. Moreover, another dimension of our data was that we also characterized type of antibodies that recognized such immunogens, including IgM, IgG1 and IgG4.

Among the potential immunogens observed in our present study, actin 1 was identified as multiple isoforms, most likely due to post-translational modifications or cleavage ([Table 1](#)). Two of the three isoforms of actin 1 (spots #22, 23) were detected by IgG1 in endemic asymptomatic and lymphangitis, whereas all the three forms (spots #22–24) were detected by IgG4 in microfilaremia ([Fig. 1](#) and [Table 2](#)). Actin 1 is a highly conserved protein that is involved in cell motility and is ubiquitously expressed in all eukaryotic cells. Our data were consistent with those reported in a previous study by Sritharan and Piessens [13], demonstrating that actin of *B. malayi* contains a developmentally regulated antigenic epitope. Similarly, two forms of enolase (spots #20, 21) were detected by IgG1 in endemic asymptomatic and lymphangitis, and by IgG4 in microfilaremia ([Fig. 1](#) and [Table 2](#)). Enolase has been previously identified in excretory–secretory products of *B. malayi* [5], and been shown as an important antigen in many clinically relevant microbes [14]. These findings strengthen its role as a potential immunogen of *B. malayi*.

Leucine aminopeptidase (spot #6) was a target protein that induced IgG4 response across all clinical spectra of brugian

filariasis, and IgG1 response in endemic asymptomatic and lymphangitis ([Fig. 1](#) and [Table 2](#)). This aminopeptidase is a phosphorylcholine-containing glycoprotein posing an anti-inflammatory property [15], and has been detected also in excretory–secretory products of *B. malayi* in a previous study [5]. Galectin (spot #13) was identified as a target protein of IgG4 immune response in endemic asymptomatic, elephantiasis and microfilaremic groups ([Fig. 1](#) and [Table 2](#)). Strongest immunoreactivity against galectin was observed in microfilaremia. Note that this protein was not detected in lymphangitis group. In a previous proteomic study, galectin has been detected as the major component of *B. malayi* secretome [5]. Galectins are atypical secreted proteins that have been identified from a number of filarial parasites [16,17]. Accumulation of galectins occurs at endothelial interfaces by binding to extracellular proteins and promoting endothelial proliferation [18]. Our data were consistent with those reported by Hewitson et al. [5] demonstrating that galectin Bm1 24940, a protein found in *B. malayi* excretory–secretory products, could bind to IgG with the greatest extent in microfilaremic sera. These data also underscore the significant roles of leucine aminopeptidase and galectin as potential immunogens of *B. malayi*.

In addition, our data also revealed differential responses of human antibodies in different clinical spectra of brugian

Table 1 – Identification of immunoreactive proteins derived from *B. malayi*, detected by IgM, IgG1 and IgG4 in sera of patients with different clinical spectra of filariasis.

Spot no.	Protein name	NCBI ID	Identified by	Identification scores (MS, MS/MS)	%Cov (MS, MS/MS)	No. of matched peptides (MS, MS/MS)	pI	MW (kDa)
1	Endoplasmin precursor	gi 170589273	MS, MS/MS	89, 109	25, 6	17, 3	5.13	90.97
2	Heat shock protein 90	gi 19855062	MS, MS/MS	169, 172	36, 6	23, 4	4.98	83.01
3	Vacuolar ATP synthase catalytic subunit A, osteoclast isoform	gi 170593817	MS, MS/MS	174, 98	41, 5	21, 2	5.10	68.45
4	Tubulin alpha chain	gi 170588855	MS, MS/MS	153, 226	43, 16	17, 5	5.01	50.39
5	Heat shock protein 70	gi 6941872	MS, MS/MS	121, 86	32, 4	18, 2	5.40	70.55
6	Leucyl aminopeptidase	gi 170596311	MS, MS/MS	87, 42	29, 2	10, 1	5.41	45.53
7	Acyl-Coenzyme A oxidase 3, pristanoyl	gi 157073994	MS	94, NA	24, NA	11, NA	8.17	78.68
8	T-complex protein 1 theta subunit	gi 170592683	MS/MS	NA, 53	NA, 5	NA, 2	5.32	600.75
9	Cytoplasmic intermediate filament protein	gi 170596673	MS/MS	NA, 43	NA, 6	NA, 2	5.35	37.43
10	ATP-dependent helicase HrpA	gi 153886738	MS	96, NA	20, NA	19, NA	8.89	150.13
11	Hypothetical protein CHGG_00235	gi 116179214	MS	82, NA	12, NA	20, NA	8.98	223.69
12	Actin 1	gi 170581695	MS, MS/MS	86, 57	33, 7	11, 2	5.30	42.12
13	Galectin	gi 170583137	MS, MS/MS	160, 89	49, 13	17, 3	5.96	31.93
14	Actin 1	gi 170581695	MS/MS	NA, 113	NA, 11	NA, 3	5.30	42.12
15	Carbohydrate phosphorylase	gi 170579084	MS, MS/MS	235, 118	39, 4	31, 3	6.06	96.68
16	Collagen alpha 2(IV) chain precursor	gi 170579429	MS/MS	NA, 95	NA, 1	NA, 2	9.02	168.51
17	Independent phosphoglycerate mutase isoform 1	gi 37681504	MS, MS/MS	141, 104	33, 6	20, 3	6.27	57.97
18	Hypothetical protein MGL_1413	gi 164660214	MS	86, NA	49, NA	8, NA	7.85	19.55
19	Transketolase	gi 170576173	MS, MS/MS	94, 78	24, 7	13, 3	6.12	68.08
20	Enolase	gi 170582775	MS, MS/MS	266, 169	70, 18	28, 5	5.98	47.83
21	Enolase	gi 170582775	MS, MS/MS	90, 26	41, 4	12, 1	5.98	47.83
22	Actin 1	gi 170581695	MS, MS/MS	96, 128	35, 15	14, 4	5.30	42.12
23	Actin 1	gi 170581695	MS, MS/MS	125, 163	37, 12	12, 3	5.30	42.12
24	Actin 1	gi 170581695	MS/MS	NA, 50	NA, 7	NA, 2	5.30	42.12
25	Immunoglobulin heavy chain variable region	gi 112696951	MS	57, NA	51, NA	5, NA	8.91	11.20
26	Putative sigma-54 interacting transcription antiterminator	gi 126699898	MS	74, NA	16, NA	14, NA	5.47	111.54
27	Thymidine kinase	gi 148980443	MS	91, NA	42, NA	8, NA	5.75	22.05
28	Hypothetical protein	gi 118084358	MS	86, NA	14, NA	20, NA	5.89	195.32
29	Chaperonin homolog HSP60, mitochondrial precursor	gi 170576770	MS/MS	NA, 33	NA, 5	NA, 1	4.81	30.54
30	Predicted protein	gi 156362061	MS	87, NA	52, NA	9, NA	9.42	30.32

NCBI=National Center for Biotechnology Information.

%Cov=%Sequence coverage [(number of the matched residues/total number of residues in the entire sequence) × 100%].

NA=not applicable.

Table 2 – Quantitative data of immunoreactivity of potential immunogens detected by IgM, IgG1 and IgG4 in sera of patients with different clinical spectra of filariasis.

Spot no.	Protein name	Intensity volume ($\times 10^4$ pixels)														
		Healthy control			Endemic asymptomatic			Lymphangitis			Elephantiasis			Microfilaremia		
		IgM	IgG1	IgG4	IgM	IgG1	IgG4	IgM	IgG1	IgG4	IgM	IgG1	IgG4	IgM	IgG1	IgG4
1	Endoplasmin precursor	0	0	0	0	5.08	5.17	0	11.39	10.18	0	0	0.87	0	0	7.99
2	Heat shock protein 90	0	0	0	0	5.74	1.80	0	6.94	4.53	0	0	0	0	0	0
3	Vacuolar ATP synthase catalytic subunit A, osteoclast isoform	0	0	0	0	4.12	4.81	0	2.36	3.38	0	0	0.55	0	0	0
4	Tubulin alpha chain	0	0	0	0	1.96	3.95	0	2.77	1.88	0	0	0	0	0	0
5	Heat shock protein 70	0	0	0	0	6.56	14.11	0	4.92	0	0	0	3.85	0	1.27	13.45
6	Leucyl aminopeptidase	0	0	0	0	6.46	6.65	0	8.10	7.97	0	0	2.28	0	0	16.27
7	Acyl-Coenzyme A oxidase 3, pristanoyl	0	0	0	0	8.63	35.78	0	8.38	7.99	0	0	1.22	0	1.78	7.68
8	T-complex protein 1 theta subunit	0	0	0	0	8.97	4.09	0.77	5.75	7.25	0	0	0	0	0	4.45
9	Cytoplasmic intermediate filament protein	0	0	0	0	0	6.05	0	2.92	8.05	0	0	0	0	0	6.19
10	ATP-dependent helicase HrpA	0	0	0	0	1.41	1.04	0	0	0	0	0	0.43	0	0	0.33
11	Hypothetical protein CHGG_00235	0	0	0	0	5.69	1.11	0	0	0	0	0	1.13	0	0	12.17
12	Actin 1	0	0	0	0	0	9.97	0	0	15.69	0	0	0	0	0	0
13	Galectin	0	0	0	0	0	0.54	0	0	0	0	0	0.37	0	0	5.56
14	Actin 1	0	0	0	0	0	1.93	0	0	0	0	0	0	0	0	0
15	Carbohydrate phosphorylase	0	0	0	0	0	0	0	7.53	0	0	0	4.53	0	0	10.31
16	Collagen alpha 2(IV) chain precursor	0	0	0	0	14.55	0	0	10.37	0	0	0	4.65	0	0	8.25
17	Independent phosphoglycerate mutase isoform 1	0	0	0	0	15.58	0	0	16.98	15.81	0	0	4.16	0	0	41.58
18	Hypothetical protein MGL_1413	0	0	0	0	12.46	0	0	5.52	0	0	0	1.68	0	0	6.45
19	Transketolase	0	0	0	0	0	0	0	0	3.41	0	0	0	0	0	24.22
20	Enolase	0	0	0	0	6.55	0	0	3.88	2.94	0	0	0	0	0	23.44
21	Enolase	0	0	0	0	20.20	0	0	16.53	0	0	0	8.76	10.92	48.37	48.37
22	Actin 1	0	0	0	0	20.68	0	0	3.51	0	0	0	0	0	0	53.36
23	Actin 1	0	0	0	0	1.75	0	0	2.13	0	0	0	0	0	0	7.50
24	Actin 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.42
25	Immunoglobulin heavy chain variable region	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.50
26	Putative sigma-54 interacting transcription antiterminator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.94
27	Thymidine kinase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.55
28	Hypothetical protein	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.71
29	Chaperonin homolog HSP60	0	0	0	0	0	0	0	0	0	0.31	0	0	0	0	0
30	Predicted protein	0	0	0	0	3.44	0	0	0	0	0	0	0	0	0	0

filariasis. Among these, IgG4 was the strongest immune response, particularly in microfilaremic subjects, as compared to IgM and IgG1 (Fig. 1 and Table 2). Microfilaremic subjects are the infected individuals who are mostly asymptomatic, despite the presence of circulating microfilaria in the peripheral blood [19]. Its asymptomatic state is perhaps a result of suppression of both Th1 and Th2 responses. This down-regulation of the host immune responses is characterized by impaired proliferation of T cells, increased production of the regulatory cytokine IL-10, and high levels of IgG4 [20]. Thus, the immunoreactive proteins detected by IgG4 may be directed to the discovery of a vaccine for prevention of filariasis, a new therapeutic target, and a novel diagnostic test. On the other hand, filaria-specific IgG1 was far more prominent in endemic asymptomatic and lymphangitis. It is likely that antibody and humoral mechanisms initiated by IgG1 may contribute to the pathology through antibody-dependent cell-mediated cytotoxicity (ADCC) or immune complex formation [21]. Nevertheless, IgG1 is similar to IgG4 in some aspects, e.g. inability to activate complement. The latter may

explain why antifilarial IgG1 was also dominantly detected in endemic asymptomatic group.

In summary, we report herein a total of 30 potential immunogens of *B. malayi* identified by an immunoproteomics approach. This large data set is an important resource for further development of a new diagnostic test and/or vaccine for filariasis.

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