IDENTIFICATION OF A LDL-RECEPTOR IN THE LYMPHATIC FILARIAL PARASITE *WUCHERERIA BANCROFTI*

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ABSTRACT

Little is known about host-parasite inter-relationship in the lymphatic filarial parasites. There is no information available about the ability of these parasites to acquire cholesterol, though it is known that in general, nematodes lack the ability to synthesise cholesterol *de novo*. In this study, we have shown that the filarial parasites also lack the ability to incorporate labelled acetate into cholesterol, indicating the absence of the machinery for cholesterol biosynthesis. We have further shown that they elaborate a 43 kDa surface receptor for acquiring LDL-bound cholesterol. We have shown by polymerase chain reaction the presence of a 860 bp fragment indicating the presence of the gene for LDL-related protein (LRP) in the human filarial parasite *Wuchereria bancrofti* in the genomic DNA. We have also shown that it is expressed as seen in the cDNA clones identified from an expression library.

KEY WORDS : *Wuchereria bancrofti*, LDL-receptor, LRP gene, polymerase chain reaction.

INTRODUCTION

Of the filarial parasites known to infect man, *Wuchereria bancrofti* and *Brugia malayi*, the lymphatic dwelling filariids and *Onchocerca volvulus*, the subcutaneous filariae are the three species that are responsible for most of the pathology associated with these infections.

The lymphatic filarial parasites are transmitted by mosquitoes. They go through a complex life cycle. The adult male and female worms, which develop from the infective larva,

reside in the lymph nodes and the adjacent lymphatics in the definitive host, man. The adult female worm releases the microfilariae which circulate in the blood. Mosquito serves as an intermediary host. These microfilariae, when ingested by a mosquito develop into infective larvae in the thoracic muscle of the mosquito. These infective larvae are injected into a second human host during a subsequent bite where it reaches the lymphatics and develop into adult worms. The cycle thus continues.

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The adult worm can survive the defense mechanisms of the host and survive for several

years. A complex relationship is established between the parasite and the host. The molecular mechanism(s) underlying the inter-relationship is not fully understood. Survival of the parasite would not only depend upon the availability of the required nutrients but also their adaptability to acquire and utilise other host molecules.

Cholesterol and long chain fatty acids are essential components for membrane construction. Most of the helminths lack the ability to synthesise *de novo* cholesterol (1). It is known that other blood dwelling parasites like the schistosomes (2) and trypanosomes (3) lack the enzymatic machinery for cholesterol biosynthesis and have to depend on the exogenous acquisition from the environment.

In the mammalian blood stream, cholesterol is not freely available but is found in the form of lipoprotein molecules (4). Schistosomes (5) and trypanosomes (6) have been shown to adapt by expressing on their surface, membrane receptors for acquiring cholesterol via LDL particles. *Schistosoma mansoni* expresses a 45 kDa doublet protein (7), *Schistosoma japonicum* a 43 kDa protein (8) and *Trypanosoma brucei brucei* a 145 kDa protein (9), all of which have been demonstrated to bind to radiolabelled LDL.

The ability of the filarial parasites to synthesise *de novo* cholesterol, or its ability to acquire cholesterol from the host has so far not been studied or reported. In this communication, we have shown that the worms were unable to incorporate ^{14}C -acetate into the cholesterol fraction. We present a preliminary report on the presence of a putative LDL receptor gene in the lymphatic filarial parasite *W. bancrofti*.

The adult worm resides in the lymphatics, hence it is almost impossible to isolate the adult worms. As there is no animal model for the *W. bancrofti*, and it is possible to get only the circulating microfilariae from patients and that too, from a night blood specimen, all the above work could not be done with *W. bancrofti*. Therefore some experiments were performed with the cattle filarial parasite *Setaria digitata*. Genomic DNA from *W. bancrofti* was isolated from circulating peripheral blood microfilariae from infected patients and used for polymerase chain reaction (PCR) amplification. Uptake studies were done with the cattle filarial parasite *S. digitata*.

RESULTS

Uptake of ^{14}C -acetate by adult *Setaria digitata* worms.

To male and female worms maintained in RPMI 1640 medium was added 17,000,000 cpm (Expt. I) and 34,000,000 cpm (Expt. II) ^{14}C -sodium acetate (sp. activity 50-62 mCi/mmol, Amersham). After 24 hr. incubation, the worms were washed well with RPMI medium; lipids were extracted with chloroform:methanol (2:1 v/v), dried down (Table 1)

Table 1. Recovery of ^{14}C -acetate in the total lipid fraction.

Assay system	Recovery of the label in the total lipid fraction	
	Total cpm	% of total added label
Adult female worms	Expt. I : 38585	0.226
	Expt. II : 80705	0.237
Adult male worms	Expt. I : 36165	0.213
	Expt. II : 62630	0.184

The value in each experiment is the average of duplicate experiments.

and the extracted lipids were subjected to thin layer chromatography on silica gel G using hexane : diethyl ether : acetic acid (85:15:1.5). The spots were visualized by exposure to iodine vapour, scraped out and the radioactivity determined in a liquid scintillation counter (Fig.1).

Amplification by polymerase chain reaction (PCR) of an 860 bp product from *W.bancrofti* microfilariae using heterologous primers from *Caenorhabditis elegans*.

PCR amplification of a putative LD receptor gene sequence from the genomic DNA

Fig. 1 : Lane 1 represents the total label loaded for chromatography (taken as 100%) Lane 2: phospholipid, Lane 3: monoglyceride, Lane 4: cholesterol, Lane 5: diglyceride, Lane 6: triglyceride and Lane 7: cholesterol ester.

of *C.elegans* and *W.bancrofti* microfilariae were performed as follows: Genomic DNA was taken at concentration of 1ug/mL; the two primers used were :

Forward primer LRP1: AAC TCA ATG TGT TCT CCG TG

Reverse primer LRP2: GTT CTG TTA CCA TG

PCR was performed with 1µg of the template DNA and the cycle consisted of denaturation at 95°C for the first 5 min, with 30 cycles of annealing at 45°C for 1 min, extension at 72°C 1min, and denaturation at 95°C for 1 min; this was followed by a final extension at 72°C for 5 min. Fig. 2 shows the 860 bp product amplified from the genomic DNA of both *W.bancrofti* and *C.elegans*.

Fig. 2 : PCR amplification of the putative LRP sequence from genomic DNA : Lane 1: Molecular weight marker lambda DNA/Hind III cut, Lane 2 and 3: PCR product amplified from genomic DNA of *W.bancrofti* microfilariae and adult *C.elegans*. An 860 bp product was identified in both the lanes as the major product though a fainter higher molecular size product was also observed.

Identification of recombinant clones.

The λgt11 cDNA library of adult female *W.bancrofti* (kindly given by Dr. Nithyakalyani

Raghavan) was screened by using the 860 bp PCR product amplified from *C. elegans*. Non radioactive method of detection using the dUTP-digoxigenin (DIG) (Boehringer Mannheim) was used. Basically, the probe was labelled during PCR with dUTP-digoxigenin (1:1 mixture of dTTP:dUTP-digoxigenin used in the dNTP mix). Using XL-1 blue as the host, standard protocols (10) were followed for the preparation of the recombinant plaques on a nitrocellulose filter. They were incubated with the DIG labelled probe, followed by anti digoxigenin antibodies coupled to alkaline phosphatase. The nitrocellulose filters were incubated with AMPPD as the chemiluminescent substrate for 1h at 30°C and subjected to autoradiography by exposure to X-ray film (Kodak, X-Omat) overnight.

About 14 clones were identified. 7 of these were subjected to PCR, under conditions as mentioned above, using the LRP1 and LRP 2 primers (Fig.3). The amplified products were analysed on an agarose gel. The figure shows 760 bp products in two of the clones. The smaller size could be due to the fact that the template is cDNA clones as compared to the 860 bp product amplified from the genomic DNA.

Fig. 3: PCR amplification of the isolated clones. Lanes 1-7 represent 7 different clones. In lanes 3 and 4, a 760 bp product (indicated by arrow) is identified.

Identification of the LDL-receptor in protein extracts: SDS-PAGE analysis and Western Blotting studies.

S. digitata worms were maintained alive for 24 hr. in the presence of RPMI 1640 alone, RPMI 1640 and 10% fetal calf serum (FCS, lipoprotein deficient-Sigma Chemical Company), RPMI 1640 and 20% cattle serum (obtained from slaughter house), RPMI 1640 and LDL from human plasma (Sigma Chemical Company) and RPMI 1640 and 20% human serum respectively.

Protein extracts of these worms were prepared by subjecting to ultrasonication 8 times at 30s duration with 30s of cooling intervals; this was followed by centrifugation at 3000 rpm to remove all cell debris. 75 µg of supernatant protein was subjected to SDS-PAGE (11) followed by transfer to nitrocellulose membrane (12).

Fig. 4A shows the proteins stained with

Fig. 4A. SDS-PAGE analysis: Lane M: Molecular weight markers. The others lanes represent protein extract of the worms preincubated in RPMI containing added LDL (Lane 1), 20% human serum (Lane 2), 10% FCS (Lane 3), 20% cattle serum (Lane 4) and RPMI alone (Lane 5).

Coomassie blue. The blot (Fig. 4B) was developed by incubating first with goat anti-human betalipoprotein antibody (Sigma), followed by rabbit anti-goat IgG-alkaline phosphatase conjugate ; the colour was developed with BCIP (5 - bromo, 4 - chloro, 3 - indolyl phosphate) and NBT (nitro blue tetrazolium) as the substrate. Another identical blot (Fig. 4C) was subjected to an initial incubation with LDL, followed by the rest of the procedure as above.

Fig. 4B: Western blot analysis. Lane 1: RPMI plus cattle serum, Lane 2 : RPMI plus human serum, Lane 3: RPMI plus FCS, Lane 4: RPMI alone and Lane 5: RPMI plus LDL.

Fig. 4C: The blot was subjected to prior incubation with LDL . Lane 1: RPMI alone, Lane 2: RPMI plus cattle serum, Lane 3: RPMI plus FCS, Lane 4: RPMI plus human serum, RPMI plus LDL.

DISCUSSION

Nematodes, in general have been shown to lack *de novo* synthesis of sterols. Other parasites like the schistosomes and trypanosomes also lack the machinery for the same.

From the uptake studies, it is seen that very little ^{14}C -acetate gets incorporated into the lipid fraction in general and the cholesterol fraction in particular. The inability of these parasites to synthesise cholesterol from acetate would probably necessitate them to elaborate mechanisms for acquiring it from the environment. Other blood dwelling parasites like the trypanosomes and schistosomes have been shown to acquire cholesterol from the circulating lipoprotein by elaborating suitable receptors for LDL.

In the free living *C.elegans*, the gene for an LDL Related Protein (LRP) has been identified and completely sequenced. Using primers containing sequences derived from heterologous LRP gene sequence, evidence is being presented in this study for the presence of LRP like sequence in the filarial parasites. Amplification of a 860 bp product from the genomic DNA and a 760 bp product from the cDNA library of *W.bancrofti* adult female worm is the first report about putative lipoprotein receptor in these parasites and it needs to be investigated in detail to understand the complex host-parasite interaction in human lymphatic filariasis.

Our results also indicate the presence of a 45 kDa protein in the cattle filarial parasite and it will be interesting to see if the human filarial parasite also expressed a similar protein.

This is preliminary data identifying the presence of a receptor and its gene for LDL in the filarial parasite. Further work needs to be done to strengthen the role of the 45 kDA protein as a receptor and also to show its expression in the pathogenic *W.bancrofti* and *Brugia malayi*. The results probably could form the basis for a detailed investigation of the filarial parasite-host interrelationship.

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