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Immunoscreening of Lymphatic Filariid *Brugiamalayi* L3 cDNA library reveals novel Vaccine Candidates

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ABSTRACT

Immunoscreening of cDNA library has been the most preferred choice to pick up desired gene from a pool of genome.We have identified three novel immunodominant cDNA clones, BmL3-thioredoxin, BmL3-hydrolase and BmL3-Cytochrome P450 by immunoscreening of a larval stage cDNA library of *Brugiamalayi*. Currently there are no adulticidal drugs or vaccines available against worms that are causative agents of lymphatic filariasis. The identified clones can be potent vaccine candidates for developing prophylactic agents against the human filarial parasite. Nucleotide sequences reported in this paper are available in the GenBank™, EMBL, and DDBJ databases under the accession no: GenBank: EU684476.1, GenBank: EU519463, GenBank: EU668900

INTRODUCTION

Strategies to identify candidate vaccine antigens against filariasis have relied mainly on screening expression libraries with the sera of the putatively immune population (Freedman et al, 1989), differential screening of abundantly expressed mRNAs (Werner et al, 1989; Gregory et al, 2000), expressed sequence tags (EST) approach (Blaxter et al, 1996; Williams et al, 2000) and phage display method (Gnanasekar et al, 2004).Using these approaches few potential vaccine candidates have been identified and some of these have been reported to offer varying degrees of protection in animal models. Although there are effective drugs for the control of filarial transmission but an adulticidal drug is still lacking and developing a vaccine remains a promising strategy for mass control of this mosquito-borne infection in areas of end emicity. Immuno screening of cDNA library has been the most preferred choice to pick up desired gene from a pool of genome. Stage specific libraries derived from various filarial parasite species have been screened with the sera from infected human or animals or even animals immunized with filarial antigens or live parasites. Other than vaccine candidates, there is also an urgent need to identify and characterize novel drug targets to facilitate anti-filarial drug discovery.

Irradiated infective larvae have been reported to offer strong resistance to larval challenge in various animal models (Lucius et al, 1991). Since human filarial parasitic material is not available in sufficient amount, the construction of cDNA expression libraries and molecular cloning approaches are important methods for isolating and characterizing metabolic regulatory enzymes or protein antigens. Sera of putatively immune endemic normal patients have been known to differentially recognize antigen/s of L3 of B. *malayi* (Freedman et al, 1989; Nutman et al, 1991), therefore immuno screening of cDNA library of filarial infective larval stage appears to be an ideal choice.

Several group have generated cDNA libraries from specific life-cycle stages of filarial nematodes by reverse transcription of RNA followed by polymerase chain reaction (RT-PCR) using the conserved nematode spliced leader (SL1) and oligo (dT) as primers (Seeber et al, 1993; Martin, 1995; Devaney et al, 1996; Blaxter et al, 1996). More than 20 new high quality stage-specific cDNA libraries of B. *malayi* are available from most of the transition stages (Yenbutr and Scott, 1995; Blaxteret al, 1996)which have been distributed to over 100 research laboratories worldwide. So far more than 26000 expressed sequence tags (ESTs), derived from these cDNA libraries representative of the significant Brugia life-cycle stages (Williamset al, 2000; Parkinson et al, 2004; Williams, 2004) have been sequenced and submitted to dbEST, of which half are similar to genes already identified in other organisms and their functions can therefore be predicted, rest are unique to B. *malayi* and thus may be useful in the search for new targets for the development of drugs, vaccine and diagnostic tests. The recombinant proteins identified by immuno screening and tested for protective efficacy against filarial infections have not so far induced significant degree of immunity to challenge infection (Li et al, 1999; Peralta et al, 1999). There is still a need to identify more functional proteins/enzymes that can be used as vaccine candidate, drug-target or diagnostic proteins.

MATERIAL AND METHODS

Raising antiserum to irradiated L3

Aedes aegypti mosquitoes were fed on B. *malayi* infected micro filaraemic mastomys. Infective larvae were recovered from the gently crushed mosquitoes on day 9 ± 1 of infective feeding. The larvae were cleaned in Ringer's solution, exposed to Cobalt 60 irradiation at a dose of 25 krad and inoculated subcutaneously to mastomys (~100 L3 each) on three occasions at four week interval (Weil et al, 1992) and the sera of animals were checked for the development of high anti-L3 antibody titres.

Antibody measurement by Enzyme-linked Immunosorbent Assay (ELISA)

The blood was collected just before the start of immunization and on day 7 of each immunizing dose. Serum antibody titre was assessed by ELISA using soluble somatic antigen of L3. ELISA strips (Nunc, Denmark) were coated overnight with the L3 antigen (1 µg/ml) in carbonate buffer (pH 9.6) at 4 0C. The strips were blocked with 1% gelatin in 1x PBS+Tween-20 (PBST) for 2 h at room temperature (RT). After washing the wells thrice with PBST, mastomys serum in the form of primary antibody raised against irradiated L3 was added at serial two-fold dilutions starting from 1:50 and kept for 90 min at room temperature (RT). The pre-immunized serum was added in the control antigen coated wells in the same manner. Washing of wells was done with PBST thrice and goat anti mouse-IgG-HRP (Sigma, USA) at 1: 10000 dilution (in 1% gelatin in PBS) was added to each well as secondary antibody and incubated for 90 min. at RT. Plates were washed again and finally the reaction was developed with buffer containing 12 mg of substrate Orthophenyldiamine (OPD, Sigma) and 10 µl H2O2 in PBS. The reaction was stopped with 2.5 N H2SO4. Absorbance was read at 492 nm in multiplate reader (Infinite M200, Tecan, Switzerland) to determine the IgG antibody titre at different time points.

Screening of L3 cDNA expression library

B. *malayi* L3cDNA library (SAW94WL-BmL3 library) constructed in the lambda UniZap XR vector (Stratagene) was received as a kind gift from Prof. S.A. Williams, Smith College, Northampton, Massachusetts, USA. The protocol of Stratagene and Maniatis (Sambrook et al; 1998) was used with some modifications for screening of library. One loop of stock E. coli bacteria (XL-1 Blue MRF' strain) was inoculated to 5 ml of 2.2% NZCYM medium (37 °C, 224 rpm) overnight (O/N). Sub-culture was prepared in 20 ml of fresh 2.2 % NZCYM medium containing 15 µl of 20 % maltose and incubated till OD600=0.5. The bacterial culture was pelleted at 3,000 rpm for

for 20 min at RT, pellet was suspended in 2 ml of 10 mM MgSO4 and kept in ice till use. For screening, 2 µl of stock cDNA library was diluted to10-4 and kept in ice till use. 30 µl of this diluted library was mixed in 300 µl of grown XL1 Blue bacterial culture and incubated in a water bath at 37 °C for 20 min for infection of bateriophage. 8 ml of top agar heated at 50 °C was mixed with bacteriophage infected bacterial cells. This mixture was overlaid slowly on to the bactoagar plates uniformly and plates were left till agar solidifies. The plates were then kept inverted in an incubator at 42 °C for 3-4 hrs till clear plaques appear. Nitrocellulose filter discs (Schleicher and Schull) soaked in 10 mM IPTG were dried and laid down onto the bactoagar plates containing visible plaques. Plates were incubated at 37 °C O/N in an inverted position. Plates were taken out and kept at 4 °C for 2 h in order to avoid sticking of agar to filter discs. Plates were asymmetrically marked for identification by using waterproof ink after incubation. Filters were carefully peeled off from the plates and immediately kept in washing buffer (1XPBS) while plates were stored at 4 °C for later use. Blocking was done with 10% skimmed milk in PBS for 4 h on a moving platform. Each membrane was suspended in mastomys hyper immune serum (1:100) in 1% blocking buffer and incubated O/N at 4 °C on a moving platform. Anti-mouse IgG-HRP labelled secondary antibody (1:1000) was added and membrane was agitated slowly on rotating platform for 2 h followed by washing with PBS. Filters were developed in DAB solution (10 mMTris-HCl (pH 7.5), 150 mMNaCl, 7.5 mg DAB (Sigma), 1.5 µl H2O2) till the signals appeared as dark brown spots on the membrane surface. The discs were matched with master plate in right orientation and positive plaques were picked up from the plate and suspended individually in 1 ml of SM buffer. Few drops of chloroform were added to the tubes and stored at 4 °C for secondary screening. Secondary and tertiary screenings were carried out in the same manner to purify the positive plaques. In-vivo excision of purified recombinant pBluescript phagemid from λ Uni Zap vector was carried out with the help of Ex Assist helper phage (Stratagen). XL-1 Blue MRF' bacterial cells suspended in $MgSO_4$ (as described above) and O/N grown SOLR cells in LB broth (Stratagene) with OD600= 1.0 were used for excision procedure. Briefly, 100 µl of XL1 Blue was mixed with 15 µl lysate, 5 µl of ExAssist helper phage and incubated at 37 °C for 20 min in a water bath. 2 ml of LB broth was added to this tube and culture was allowed to grow for next 3.5 h at 37 °C with constant stirring 224 rpm. The grown culture was heated at 70 °C for 20 min in a water bath and centrifuged at 10,000 rpm for 10 min at RT and supernatant containing phagemid was transferred to a fresh tube. For transformation in to SOLR cells, 200 µl of previously grown culture of SOLR cells was mixed with 100 µl of phagemid and incubated at 37 °C for 20 min in a water bath and the mixture was spread on the LB ampicilin (LB amp+) plate uniformly. Plates were incubated overnight at 37°C to obtain positive colonies. Plasmid was isolated from the positive colonies by kit as per manufacturer's protocol. The size of the B. *malayic* DNA inserts was determined by double digestion with Eco RI and XhoI restriction enzymes. Partial nucleotide sequencing of the 5′ and 3′ ends of the inserts using T3 and T7 universal primers was performed.

DNA sequence analysis

The open reading frame (ORF) of cDNA clones was deduced using ORF Finder at National Centre for Biotechnology Information (NCBI; Bethesda, Md). Sequences were compared with nucleotide and protein sequences available in non-redundant databases and B. malayi draft genome using NCBI basic local alignment search tool BLAST 2. Comparison with expressed sequence tag (EST) was performed using tBLASTn (NCBI) and NemaBLAST (Washington University BLAST, version 2) and multiple sequence alignment by CLUSTAL-W algorithm.

RESULTS

Antibody titre measurement by ELISA

The susceptible rodent host Mastomys that were inoculated with irradiated L3 generated high titres of specific IgG antibodies as compared to control group. The antibody titre was still higher than that of control animals even at 1:6400 dilution. None of the animal serum from control groups revealed any non-specific reactivity with L3 antigen (Figure 1).

Immunoscreening of larval stage cDNA Library

About 2 x 10^5 recombinant phages were screened with the B. malayi L3 resistant mouse serum. In primary screening five immunoreactive clones were identified (Figure 2). Of these, only three showed strong immunoreactivity and therefore were individually purified by secondary and tertiary screening. In-vivo excision of each purified clone in pBluescriptphagemid from λ UniZap vector was performed with the help of ExAssist helper phage (Stratagene). The excised clones were transformed into SOLR strain of E coli cells. Positive colonies on LB amp+ plates were grown in LB broth for plasmid isolation. The insert size of the clones was determined by double digestion of plasmid with Eco RI and Xho I. Sequencing and homology matching (using BLASTx at NCBI) of the three immunodominant clones demonstrated homology with B. malayithioredoxin peroxidase, epoxide hydrolase and cytochrome p450.

Deduced Nucleotide sequence of identified clones

Clone 1:

Hydrolase, 439 bp partial CDS Accession No. GenBank: EU668900

GGCACGAGTGATTTCTCATACGGTTTTAACAGTAAATATCTCAAATATG
TGGCTAATTATTGGCTTAACAAATATAATTGGAAATATCATGAAGGCATT
ATAAACACTTTACCTCAATTCACTACTGAAATTGAAGGCCTTAAGATCC
ATTTCATTCATGCTAAACCGATGCATAATAATTACGAAGTTATCGTTCCA
CTGCTCATACTGCATGGTTGGCCCGGAAATGTGTTCGAATTTCTTAAAA
TCATTCCTCTGCTGGTGGATCCAATTCAGCAGATTGGTTCTGATATTAG
TATAACCTTTGAAATAATTGCACCATCAATCCCCGGATTCGGTTGGTCT
GCTGCACCAATAAAAAAAGGTGAGATGAAAGATTAATGAAATATTAAG
AAAGTTACTTTCACTTTTTTAAATATTACTTCCACTT
TTGAAGCAAAAAAAAAAAAAAAAA

Clone 2:

Thoiredoxin, 680 bp partial CDS Accession No. GenBank: EU519463

GGCACGAGGAGAAGCGGAAACAACAATTTTTGCTAGATCAGCAAACT
CCTTTACAAAGCTCAGAAATGGCTGATTTACTTGCTAATATCAATTTGA
AGAAAGCTGATGGTACAGTAAAGAAAGGAAGTGATGCACTGGCTAAC
AAAAAAGTTGTGGCATTATATTTCTCAGCACATTGGTGCCCACCGTGC
CGACAATTTCACCGATCCTAAAAGAATTTTATGAAGAAGTAGACGATG
ACCAATTTGAAATAGTATTTGTCTCATTGGACCATTCCGAGGAAGATTT
GAATAACTATGTGAAAGAATCACATGGCAATTGGTATTACGTTCCTTTT
GGTTCCAGTGAAATCGAAAAACTAAAAAATAAGTATGAAGTTGCCGG
AATTCCTATGCTTATTGTGATTAAATCCGATGGTAACGTTATTACCAAAA
ATGGCCGAGCTGATGTTTCGGGCAAAGCACCACCGCAAACGCTTTCA
AGCTGGTTAGCAGCAGCGTAGTGCGGATTGAACGAAATAATCTATTGG
TATTTCTACTATCACTTTCTATTATTTCGAATATTCAAAAGAATTTTTCTT
ATCATATCGTTATCATATCTTTATGGTATTAGAATGGAAATTTTTGGCTC
AGCGTTCGTGTAATTATTTATTTGCTGTGCTGATAAAAAACACTTTTTT
AAAAAAAAAAAAAAAAAAA

Clone 3:

Cytochrome P450, 647 bp partial CDS Accession no: GenBank: EU684476.1

GAGAAGAATCCGGAATATTTCAAGGAAAAGCTGCAAAGAGGATATTG
ATAAAAGCGGTTGCTTCCCAACGGTGGACGGAGTTACGTACCACCCT
GGTGATGGGCCTTCGCTAATTGGATAATGTTGTGAATGAAACCCTTCG
CAAGTACCCTCCCGATCGGGAGTTTAAATCGCGTCCCCGACATCGGAC
TACACGGTTCCCGGGACGAAGCACGTCCTCCCGAAGCAAACGATGAT
AACCATACCGATCTATGCGTTGCATCACGATCCGGACTTTTACCTGGAT
CCGGACAACTTCGATCCGGATCGATTCCTCCCAGAAGCGGCCCAAGC
TCGGCATCCGTACCCCTTCATACCGTTCGGGGAAGGTCCCCGCAATTG
CATCGGAATGCGCTTCGGCCTAATGCAAACCAAAATCGGGTTGATCAC
ATTGTTGCGCAACTTCAGATTTTCACCTTCAGCGAAGACCCCCGATAA
GATTGTATTCGATGTGAAATCGTTCGTTCTGTCGCCGGACGGTGGAAA
TTATCTACGGTACGATAAGATATGACTTATTTGGGTTTAGATAGTAAGGT
TGTACTCTAAGGACGCCGCACCTAATCGGAGGCGGTTCAGTTCAGTAT
TAAAATAACGCAATAAATCACCCAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAA

DISCUSSION

Immuno screening of cDNA expression libraries and phage display libraries using patients' sera especially from endemic normal or monoclonal antibodies (Gnanasekar, 2004) has remained one of the most sought after approaches to search for filarial vaccine candidates (Rao, 2000). In the present study, three cDNA clones were isolated by immuno screening of L3 stage cDNA library of B. *malayi*. The hyper immune serum used for immuno screening of L3 library in the present study was raised against Co60 irradiated infective larvae of B. malayi.

The aim of the current study was to identify immuno dominant proteins via immuno screening of cDNA library in an attempt to search for vaccine candidate. In the absence of satisfactory chemotherapeutic measures, identification and purification of functionally important antigens or genes possessing crucial role in parasite development and survival seems to be the foremost step to look for an alternative filarial control method. The criterion for selection of such gene is that it should not show homology to that of mammalian counterpart. In primary screening five clones were picked up, of which only three clones could be repeatedly purified. After sequencing, these clones were identified as partial sequences of B. *malayi* thioredoxin, epoxide hydrolase and cytochrome P450 enzymes. Thioredoxins belongs to family of small redox proteins that undergo NADPH-dependent reduction by thioredoxin reductase. This result in reducing equivalents that are used by cells in a wide spectrum of biological activities including maintaining reduced forms of the enzymes providing protection against oxygen radicals induced damage, the regulation of transcription factor activity and inhibition of apoptosis. Thioredoxins has also been shown to have a possible role in host immunomodulation (Kunchithapautham K, 2003). Epoxide hydrolases are a class of enzymes important in the detoxification of xenotoxic compounds. These enzymes are located in many organs and tissues and are responsible for controlling physiological signaling molecules. The cytochrome P450s are a large family of drug-metabolising enzymes present in almost all living organisms. They were thought to be absent in the parasitic nematodes but Roz Lainga et al (2015) have shown them to be present in various stages of Haemonchus contortus. The genome of Caenorhabditis elegans has been shown to encode more than 80 cytohrome p450s (Menzel, 2005). These enzymes catalyses a wide range of reactions, they are involved in the biosynthesis and catabolism of retinoids, steroids, prostaglandins and fatty acids. They also help in detoxification of drugs and insecticides from the body. All the three identified clones are partial sequences of the respective proteins. Cloning and characterization of the three full length proteins would be of utmost importance in the search of novel vaccine candidates.

CONCLUSION

The three immunoreactive B. *malayi* cDNA clones thioredoxin, epoxide hydrolase and cytochrome P450 that have been identified by immuno screenig using serum raised against irradiated L3 present us with novel vaccine candidates. It would be worthy enough to evaluate these proteins for their prophylactic efficacy

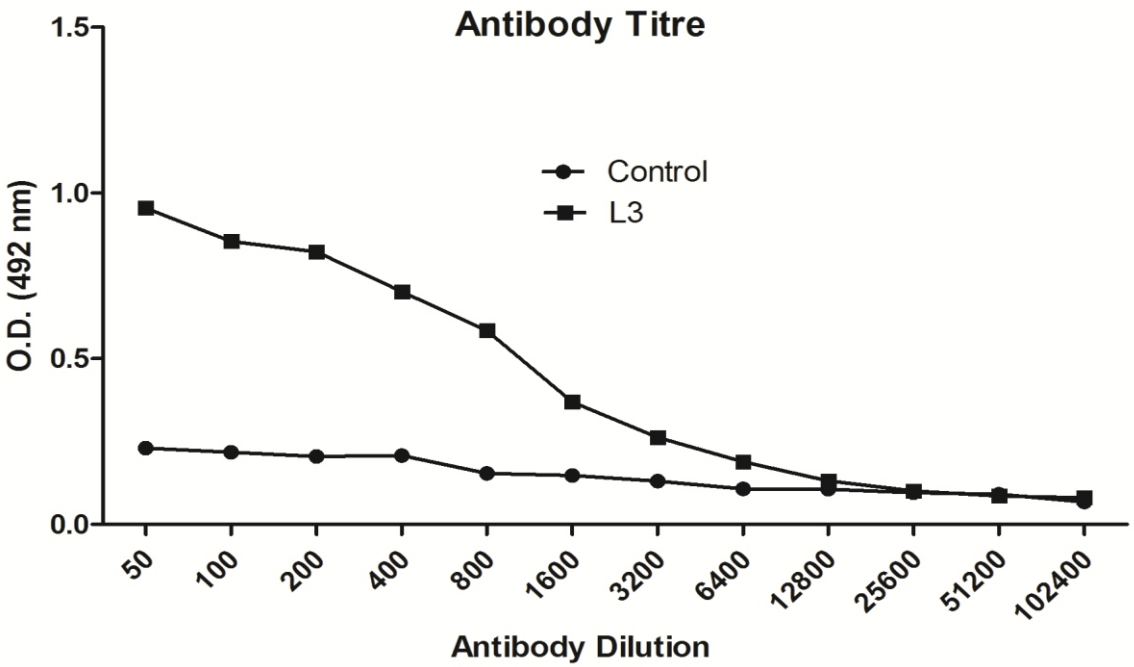

Figure 1: L3 specific IgGantibodies were detected by ELISA in the sera of mastomys inoculated with irradiated L3. Elevated IgG level was maintained in the L3 inoculated group and a high antibody titre was obtained in the serum from experimental group. Each point represents meanO.D. value taken at 492 nm obtained with pooled sera of five experimental animals.

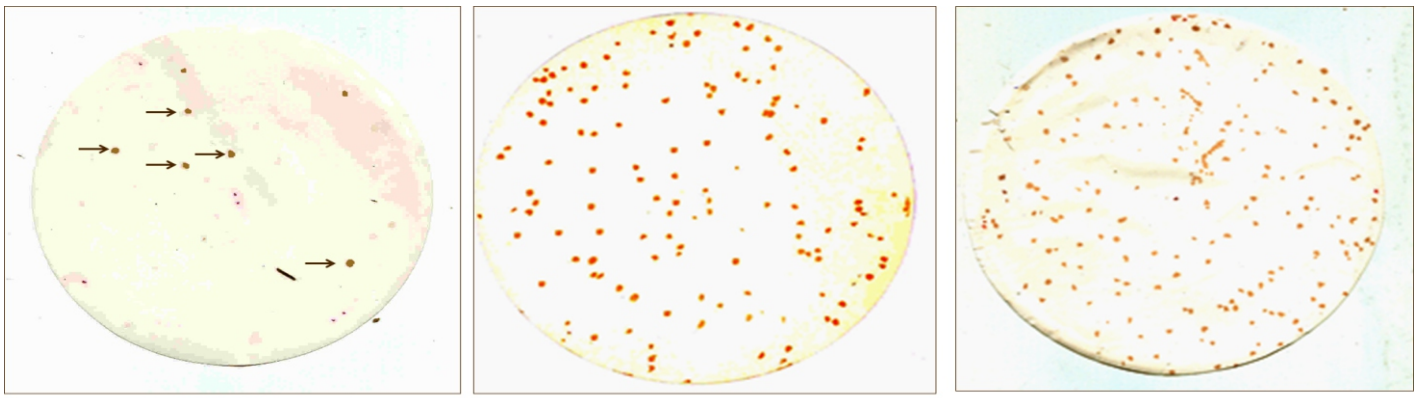

Figure 2: Immunoscreening of Larval cDNA library of Brugiamalayi. Arrows denote the positive plaques picked up for further purification. Five immunoreactive plaques were observed which were then individually purified by secondary and tertiary screening for characterization.

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Disclosure statement

No potential conflict of interest was reported by the author.

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REFERENCES

1. Blaxter, M.L., Raghavan, N., Ghosh, I., Guiliano, D., Lu, W., Williams, S.A., Slatko, B., Scott, A.L., (1996). Genes expressed in Brugia malayi infective third stage larvae. MolBiochemParasitol 77, 77-93.
2. Devaney, E., Martin, S.A.M., Thompson, F.J., (1996). Stage-specific gene expression in lymphatic filarial nematodes.Parasitol Today 12, 418-424.
3. Devaney, E., Osborne, J., (2000). The third-stage larva (L3) of Brugia: its role in immune modulation and protective immunity. Microbes Infect 2, 1363-1371.
4. Freedman, D.O., Nutman, T.B., Ottesen, E.A., (1989).Protective immunity in bancroftianfilariasis.Selective recognition of a 43-kD larval stage antigen by infection-free individuals in an endemic area. J Clin Invest 83, 14-22.
5. Gnanasekar, M., Rao, K.V.N., He, Yi-Xun., Mishra, P.K., Nutman, T.B., Kaliraj, P., Ramaswamy, K., (2004). Novel phage display based subtractive screening to identify vaccine candidates of Brugia malayi. Infect Immun 72, 4707-4715.
6. Gregory, W.F., Atmadja, A.K., Allen, J.E., Maizels, R.M., (2000). The abundant larval transcript-1 and -2 genes of Brugia malayi encode stage-specific candidate vaccine antigens for filariasis. Infect Immun 68, 4174-4179.
7. Kunchithapautham K, Padmavathi B, Narayanan RB, Kaliraj P, Scott AL., (2003). Thioredoxin from Brugiamalayi: defining a 16-kilodalton class of thioredoxins from nematodes. Infect Immun. Jul;71(7):4119-26.
8. Li, B.W., Zhang, S.R., Curtis, K.C., Weil, G., (1999). Immune responses to Brugiamalayiparamyosin in rodents after DNA vaccination. Vaccine 18, 76-81.
9. Lucius, R., Textor, G., Kern, A., Kirsten, C., (1991). Acanthocheilonemaviteae: vaccination of jirds with irradiation attenuated stage-3 larvae and with exported larval antigens. Exp Parasitol 73, 184–196.
10. Martin, S.A.M., Thompson, F.J., Devaney, E., (1995). The construction of spliced leader cDNA libraries from the filarial nematode Brugiapahangi.MolBiochemParasitol 70, 241-245.
11. Nutman, T.B., (1991). Experimental infection of humans with filariae.Reviews in Infectious Diseases 13, 1018.
12. Parkinson, J., Mitreva, M., Whitton, C., Thomson, M., Daub, J., (2004). A transcriptomic analysis of the phylum Nematoda. Nat Genet 36, 1259–1267.
13. Peralta, M.E., Schmitz, K.A., Rajan, T.V., (1999). Failure of highly immunogenic filarial proteins to provide host-protective immunity. Exp Parasitol 91, 334–340.
14. R. Menzel, M. Rodel, J. Kulas, C.E. Steinberg., (2005). CYP35: xenobiotically induced gene expression in the nematode Caenorhabditiselegans. Arch. Biochem. Biophys., 438, 93–102.
15. Rao, U.R., Salinas, G., Mehta, K., Klei, T.R., (2000). Identification and localization of glutathione-s-transferase as a potential target enzyme in Brugia species.Parasitol Res 86, 908–915.
16. RozLainga, David J. Bartleyb, Alison A. Morrisonb, Andrew Rezansoffc, Axel Martinellid, Steven T. Lainga, John S. Gilleardc., (2015). The cytochrome P450 family in the parasitic nematode Haemonchuscontortus. International Journal for Parasitology 45 (4), 243–251.
17. Seeber, F., Pogonka, T., Lucius, R., (1993) .PCR-mediated cDNA synthesis from minute amounts of filarial L3-RNA. Trop. Med Parasitol, 44:57-59.
18. Weil, G.J., Li, B.W., Liftis, F., Chandrashekar, R., (1992). Brugia malayi: antibody responses to larval antigens in infected and immunized jirds. ExpParasitol 74, 315-323.
19. Werner, C., Higashi, G.I., Yates, J.A., Rajan, T.V., (1989). Differential recognition of two cloned Brugia malayi antigens by antibody class. Mol Biochem Parasitol 35, 209-218.
20. Williams, S.A., Lizotte-Waniewski, M.R., Foster, J., Guiliano, D., Daub, J., Scott, A.L., Slatko, B., Blaxter, M.L., (2000). The filarial genome project: analysis of the nuclear, mitochondrial and endosymbiont genomes of Brugia malayi. Int J Parasitol 10, 411-419.
21. Williams, S.A., Lizotte-Waniewski, M.R., Foster, J., Guiliano, D., Daub, J., Scott, A.L., Slatko, B., Blaxter, M.L., (2000). The filarial genome project: analysis of the nuclear, mitochondrial and endosymbiont genomes of Brugia malayi. Int J Parasitol 10, 411-419.
22. Yenbutr, P., and Scott, A.L., (1995). Molecular cloning of a serine proteinase inhibitor from Brugia malayi.InfImmun 63, 1745-1753.