DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20160798

Retraction

With agreement from the corresponding author, the Editor-in-Chief retracts the article "Role of recombinant SXP/RAL-2 family protein *Wuchereria bancrofti* L_2 ($rWbL_2$) as vaccine candidate in lymphatic filariasis in mastomys", Andure D et al. Int J Res Med Sci. 2016 Apr;4(4):1140-1146, DOI: 10.18203/2320-6012.ijrms20160798, due to the duplication of figure from other previously published article. Figure 3 was reused from Figure 2 in the article "Immunization with Wuchereria bancrofti Glutathione-S-transferase Elicits a Mixed Th1/Th2 Type of Protective Immune Response Against Filarial Infection in Mastomys", Indian Journal of Clinical Biochemistry, First online: 09 February 2016, DOI: 10.1007/s12291-016-0556-y. The corresponding author Dr. Dhananjay Andure has published it on his own without informing the co-authors and his guide.

International Journal of Research in Medical Sciences
Andure D et al. Int J Res Med Sci. 2016 Apr;4(4):1140-1146
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20160798

Research Article

Role of recombinant SXP/RAL-2 family protein *Wuchereria bancrofti* L_2 ($rWbL_2$) as vaccine candidate in lymphatic filariasis in mastomys

Dhananjay Andure[1]*, M. V. R. Reddy[2], Kiran Pote[2], Ramchandra Padalkar[1], Vishal Khatri[2], Nitin Amdare[2]

[1]Department of Biochemistry, Padmashree Dr. Vithalrao Vikhe Patil Foundation's Medical College & Hospital, Ahmednagar - 414 111, Maharashtra, India
[2]Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram - 442 102, Maharashtra, India

Received: 04 February 2016
Revised: 05 February 2016
Accepted: 01 March 2016

***Correspondence:**
Dr. Dhananjay Andure,
E-mail: drdhananjayandure@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Lymphatic filariasis is caused mainly by three lymph dwelling parasites; *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. It remains main cause of clinical morbidity in tropical and sub-tropical countries.
Methods: In the present study, we evaluated the immunogenicity and efficacy of recombinant SXP/RAL-2 family protein, $rWbL_2$ to induce protective immune response against *B. malayi* infection. Mastomys were immunized intraperitoneally with $rWbL_2$ antigen and their ability to induce humoral response, cell mediated immune response and protective efficacy was evaluated.
Results: The mastomys, which are fully permissible rodents for *Brugia malayi* when immunized with $rWbL_2$ could induce 51.25% in situ cytotoxicity against *Brugia malayi* infective (L_3) larvae. We also seen that, it is characterized by higher levels of WbL_2- specific IgG1 and IgG2a antibodies and increased levels of IFN-γ, IL-4 and IL-10 cytokines production by the spleen cells.
Conclusions: The findings suggest that cellular as well as humoral immune response in the immunized mastomys and may represent effective vaccine candidate for filariasis.

Keywords: *Wuchereria bancrofti*, *Brugia malayi,* SXP/RAL-2 family protein WbL_2, Vaccine, Nematodes

Retracted

INTRODUCTION

Lymphatic filariasis is a major mosquito borne disease widely distributed through-out the tropical and subtropical regions of the world. It is caused by three lymph dwelling parasites: *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. It is estimated that there are approximately 120 million cases of lymphatic filariasis around the world and over one billion people are at risk of getting infected with this disorder.[1] It inflicts a considerable social and economic burden on many countries, specially developing and undeveloped countries of Asia, Africa, the western Pacific and the Americas. In India, filariasis has been recognized as a disease of National importance because of continuous spread of the disease and protracted sufferings and disability caused in the affected population. India alone contributes to 40% of the global disease burden.[2] To overcome socioeconomic burden of lymphatic filariasis, World Health Organization has initiated the 'global programme to eliminate lymphatic filariasis (GPELF) by 2020' to restrict the transmission of disease via mass drug

administration (MDA) and thus reducing the incidence of lymphatic filariasis.[3]

However, there are many restraints in the currently available tools i.e. specially MDA, as it requires several rounds of drug administrations, hence non-compliance and development of drug resistance to curative drugs may become a critical problem, thus affecting the eradication of disease from endemic and crowded areas.[4] The above limitations in mass drug delivery has been hindering to achieve the goal of eradication of transmission in the endemic and highly populated areas like India. Therefore, to tackle lymphatic filariasis, there is intense need of some preventive tools like developing vaccines against filariasis. Earlier studies also have shown the utilization of recombinant proteins as vaccine candidates to combat the lymphatic filariasis.[5-9]

A nematode gene was identified that encodes a new member of the SXP/RAL-2 family protein. An SXP/RAL-2 family protein, WbL_2 is a *Wuchereria bancrofti* L_3 larval protein. The WbL_2 gene is specifically expressed in the subventral pharyngeal glands and the protein is most likely secreted.[10] Another member of SXP/RAL-2 family, *Ac*-16 an immuno-dominant surface antigen of *Ancylostoma caninum* has already been successfully tested as a vaccine candidate, induced significant protection against hookworm infection in dogs.[11] In the present study, we have assessed the immunogenicity and protective efficacy of WbL_2 in fully permissible filarial animal model *Mastomys coucha* and further characterized the humoral cellular and cytotoxic responses associated with the induced protection due to immunization.

METHODS

Experimental animals; mastomys and B. malayi parasites

Multi-mammate female mastomys (*Mastomys coucha)* (6-8 weeks of age) also known as swiss rats (Figure 1), bred and maintained in the committee for the purpose of control and supervision of experiments on animals, Government of India registered animal house facility of our institute, were used in this study. The animals were maintained under standard laboratory conditions with free access to animal chow and drinking water *ad libitum* and all the surgical procedures were performed under the strict aseptic conditions. All the experiments were approved by the institutional animal ethics committee.

B. malayi infective stage (L_3) larvae used in this study were obtained using Baermann's technique by the method described previously.[4,12] Four days old *Aedes aegypti* mosquitoes were fed with the blood of mastomys infected with *B. malayi* and dissected after two weeks to recover L_3 stage larvae.

Figure 1: Multi-mammate rats *Mastomys coucha,* is animal model of present study.

Recombinant protein Wuchereria bancrofti L_2 ($rWbL_2$)

$rWbL_2$ was expressed and purified. The *Escherichia coli* BL21 bacterial cells containing pRSET-B-WbL_2 construct was grown at 37°C to A600, 0.6 in LB medium containing suitable antibiotics. The expression of recombinant protein was induced by the addition of isopropyl β-D-1-thiogalactopyranoside (1 mM; Merck Millipore, Bengaluru, Karnataka, India). The recombinant protein was purified using a nickel affinity chromatography column (Thermo fisher scientific, Mumbai, Maharashtra, India) and the protein content was estimated using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Mumbai). Endotoxin contamination was checked by a quantitative LAL chromogenic endotoxin quantitation kit and the endotoxin content was found to be within permissible limits (Thermo Fisher Scientific, Mumbai).

Immunization of experimental animals with $rWbL_2$

Five mastomys (n = 5 in each group) each was divided under two groups, first one as: $rWbL_2$ group (mastomys immunized with $rWbL_2$ in alum adjuvant) and second one as alum control group (mastomys administered with alum alone). Immunization of mastomys with $rWbL_2$ consisted of three intraperitoneal doses of $rWbL_2$ (15 µg/dose in 200 µl of alum adjuvant) administered at 15 days intervals followed by one booster dose. Animals in alum control group received four doses of alum adjuvant only. Ten days after the final dose of immunization, the sera were collected from each mouse through caudal vein puncture and tested for the presence of anti-WbL_2 antibody levels.

Analysis of anti-WbL_2 antibody titres in the sera of mastomys

The levels of total anti-WbL_2 immunoglobulin (Ig)-G antibody and IgG antibody isotypes (IgG1, IgG2a, IgG3 and IgG4) were determined in the sera samples of mastomys using an indirect ELISA.[13,14] Immuno plates of 96 wells (Thermo Fisher Scientific, Mumbai) were coated with $rWbL_2$ protein (100 ng /100 µl / well) in carbonate-

Retracted

bicarbonate buffer (100 mM, pH 9.5) and incubated overnight at 4^0C. The wells were washed once with PBS/T (0.05 M PBS containing 0.05% of tween 20, pH 7.2) and blocked by BSA (2% in PBS, 300 µl/well) for 1 h at 37°C. After washing thrice, the optimally diluted sera samples (diluted in PBS) were added and incubated for 1 h at 37^0C. After washing the wells for five times, the bounded antibodies were detected by addition of HRP conjugated goat anti-mouse IgG (1:10000) or IgG1 (1:1000) or IgG2a (1:15000) or IgG3 (1:5000) or IgG4 (1:15000) antibodies (diluted in PBS; Thermo Fisher Scientific, Mumbai). After incubation of 15 minutes at 37°C, the colour developed was recorded by measuring absorbance at 450 nm using spectrophotometer (Biotek Mumbai, India).

Depletion of anti WbL_2 antibodies from sera

Anti-WbL_2 antibodies in the sera of immunized mastomys were depleted by passing the sera over $rWbL_2$ coupled to Cobalt IMAC resin (Thermo Fisher Scientific, Mumbai). 1 mg of his-tagged $rWbL_2$ was coupled to the resin, washed and incubated overnight at 4^oC with about 200 ml of neat sera. Supernatant was collected by centrifuging the resin mixture for 2 min at 750 rpm. Depletion of anti-WbL_2 antibodies were confirmed using an ELISA as described above.

In vitro antibody dependent cellular cytotoxicity assay (ADCC)

The cytotoxic effect of anti-$rWbL_2$ antibodies against *B. malayi* L_3 larvae was determined by *in vitro* cytotoxicity assay by the method described previously.[7,15,16] Pooled sera samples from immunized mastomys before and after depletion of anti-WbL_2 antibodies were used in this assay. The ADCC assay was performed by adding about 20 L_3 larvae of *B. malayi* to a suspension of peritoneal exudates cells (PEC; 2 x 10^5 cells / well in 100 µl of RPMI medium) collected from normal mastomys. Sera samples (50 µl) were added to respective wells and the final volume of each well was adjusted to 200 µl by addition of RPMI medium in 96 well tissue culture plate (Thermo Fisher Scientific, Mumbai). After incubation (48 h at 37˚C in 5% CO_2), larval viability was determined under a light microscope. The larvae were considered as dead if they appeared limpid and straight with no movements. Percentage of cytotoxicity was expressed as the ratio of number of immobile or dead larvae to the number of larvae recovered within the experimental period.

In situ cytotoxicity against L_3 larvae in immunized mastomys

In situ cytotoxic response against *B. malayi* L_3 in immunized and control groups of mastomys was analysed by micropore chamber technique as described previously.[17] Briefly, micropore chambers (Diffusion chamber with hole; Millipore India, Bangalore, Karnataka, India) containing about 20 live and infective larvae in RPMI-1640 medium were implanted into the peritoneal cavity of experimental mastomys under the effect of anaesthesia (ketamine). After 48h of implantation, the mastomys were killed and the chambers were taken out from the peritoneum, washed in normal saline and the contents were removed onto a glass slide and examined microscopically for cell adherence and cytotoxicity. The percentage of cytotoxicity was expressed as mentioned above.

Assessment of splenocytes proliferation and cytokine analysis in in vitro culture of splenocytes

The spleens were aseptically removed from the mastomys and minced in RPMI 1640 medium (supplemented with 80 µg/ml gentamicin, 25 mM HEPES, 2 mM glutamine and 10% foetal calf serum), pelleted and resuspended in erythrocyte lysis buffer (0.1% ammonium hydrochloride). Cells (0.2×10^6 cells / well in 200 µl of RPMI media) were plated in triplicates in 96 well flat bottom tissue culture plate (Thermo Fisher Scientific, Mumbai). The cells were then stimulated with $rWbL_2$ (1 µg / well / 200 µl) or Concanavalin A (1 µg / well / 200 µl) (Con A; Sigma-Aldrich, Mumbai). Wells with media alone served as un-stimulated controls. After incubation (48 h at 37^0C in 5% CO_2), cell proliferation was measured using cell titre 96 aqueous non-radioactive cell proliferation kit (MTS assay; Promega, New Delhi). Cell proliferation expressed in terms of stimulation index (SI) was calculated by dividing geometric mean, GM absorbance of the cells stimulated by antigen/mitogen by the absorbance (GM) of the unstimulated cells.

Similar sets of cell cultures were placed in 24 well tissue culture plates and after the incubation for 72 h (at 37^0C in 5% CO_2), culture supernatants were collected in separate micro-centrifuge tubes by centrifugation for the estimation of the release of interleukin (IL)-4, IL-10 and interferon (IFN)-γ cytokines using ELISA kits from Invitrogen (Mumbai) as per the manufacturer's instructions.

Statistical analysis

The statistical analysis was performed using SPSS 21.0 (IBM, India) software. The data were checked for normality assumptions. Comparison between independent means was analysed by Student's *t* test. P values ≤0.05 were considered to be significant.

RESULTS

High titres of anti-WbL_2 antibody and isotype responses in the sera of immunized mastomys

To evaluate the immunoprophylactic effect of $rWbL_2$, sera from mastomys immunized with $rWbL_2$ was checked for the total IgG antibody levels by ELISA. Mastomys immunized with $rWbL_2$ developed high levels of anti-

Retracted

rWbL$_2$ IgG antibodies in their sera. The sera of immunized animals showed positivity for anti-rWbL$_2$ IgG antibodies even at the highest dilution tested indicating that the end titres of anti-WbL$_2$ antibodies in their sera were >10,000 (Figure 2a).

The isotype profile in the sera of mastomys immunized with rWbL$_2$ showed significantly elevated levels of IgG1 ($p<0.05$) and IgG2a ($p<0.05$) isotype of antibodies as compared to the levels in the sera of control group of mastomys (Figure 2b). Whereas, no significant change was observed in the levels of IgG2b and IgG3 antibodies among the vaccinated group of mastomys (Figure 2b).

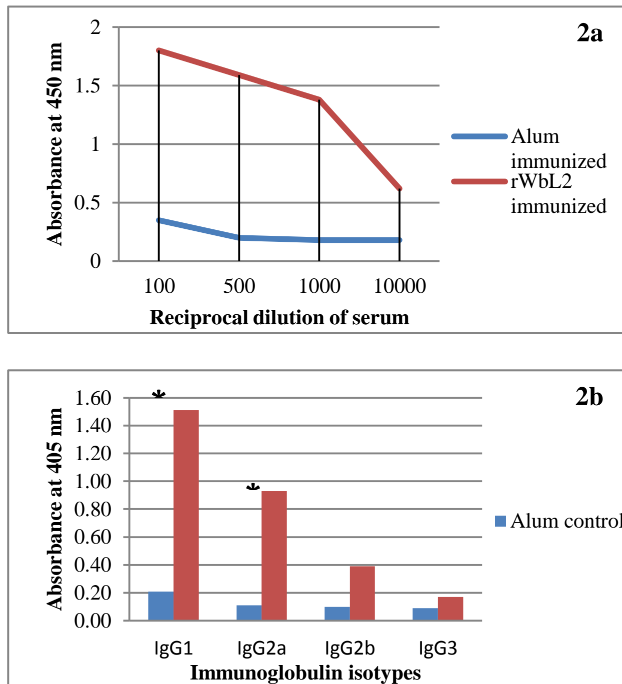

Figure 2: Anti-rWbL$_2$ antibody titres in mastomys immunized with rWbL$_2$; (a) Anti-WbL$_2$IgG antibody titre in mastomys immunized with rWbL$_2$. Each data point indicates mean of data from five animals; (b) Levels of anti-WbL$_2$ IgG isotypes in the sera of mastomys immunized with rWbL$_2$. Bars shown are mean±SD of data of different groups of animals (n=5 in each group). *p< 0.05 in comparison with alum group as analysed by Student's t test.

Antibody dependent cellular cytotoxicity (ADCC) induced against infective larvae of B. malayi parasite by the sera of immunized mastomys

Sera from the immunized mastomys promoted the adherence of PEC to the infective (L_3) larvae thereby inducing the cell mediated cytotoxicity of filarial parasite. Serum from rWbL$_2$ immunized mastomys demonstrated significant ($p<0.05$) cell mediated cytotoxicity (53.89%) against L_3 compared to the cytotoxicity induced by serum from control group of mastomys administered with alum alone (12.93%) (Table 1). While, the depletion of antibodies from the sera of immunized animals was found to decrease the cytotoxicity (21.2%) significantly ($p<0.05$) induced by the antibodies (Table 1).

Table 1: In vitro antibody dependent cellular cytotoxicity induced against *B. malayi* L_3 by pooled sera of mastomys immunized with rwbL$_2$.

Number of *B. malayi* L_3 recovered followed by treatment with sera of different groups of mastomys immunized with			
	Alum	rWbL$_2$	Anti-WbL$_2$ Ab depleted sera
Live L_3 larvae	18	11	16
	19	10	15
	17	9	13
Dead L_3 larvae	3	12	5
	2	11	4
	3	12	3
% cytotoxicity (Mean ± SD)	12.93± 2.97	53.89± 2.81*	21.2± 2.53

Peritoneal exudate cells (PEC) were incubated with the pooled sera from the mastomys administered with rWbL$_2$/alum /anti-WbL$_2$ Ab depleted sera and L_3 larvae in vitro. The total live and dead worms were counted in each well after 48h. The data shown is the number of larvae recovered from three different sets of experiments. *$p<0.05$ in comparison with Alum and anti-WbL$_2$ Ab depleted groups as analysed by Student's *t* test.

In situ cytotoxicity induced against infective larvae of B. malayi (L_3)

Micropore chamber method was used to evaluate the immunoprophylactic efficacy of rWbL$_2$. The microscopic observation of chambers implanted in the peritoneum of immunized mastomys showed the migration of hosts' immune cells into the chambers leading to their adherence and killing of the L_3 larvae within 48 h of their implantation. Results showed that, the antibodies in the rWbL$_2$ immunized mastomys were capable of inducing the significant ($p<0.05$) cytotoxicity (51.25%) compared to the control group of mastomys (7.79%) (Table 2).

Effect on the splenocytes proliferation and cytokine analysis

Cellular response was analysed to check the ability of the antigen protein of present study to stimulate lymphocytes from mastomys immunized with rWbL$_2$ after re-stimulation with the same protein. There was significant ($p<0.05$) proliferative response of spleen cells from animals immunized with rWbL$_2$ (stimulation index i.e. SI of 1.92±0.04) as compared with the control group of mastomys treated with alum (1.07±0.03) (Figure 4a).

The release of inflammatory cytokine, ifn-γ, was high in the culture supernatants of splenocytes from mastomys immunized with rwbl$_2$ (mean±sd value of 36.7±4.48

Retracted

pg/ml) suggesting cell mediated immune response (Figure 4b). Additionally, the release of anti-inflammatory cytokines of humoral immune response IL-4 (70.2±11.75 pg/ml) was increased significantly ($p<0.005$) and at the same time IL-10 was also fairly raised (49.92±4.75 pg/ml) in response to $rWBL_2$ in the mastomys immunized with $rwbl_2$ suggesting the $rWBL_2$ induced cell mediated as well as humoral immune response (Figure 4c).

Table 2: In situ cytotoxicity assay against *B. malayi* L_3.

Groups of mastomys (n=5) immunized with	L_3 larvae recovered			% cytotoxicity (Mean±SD)
	Live	Dead	Total	
Alum	13	1	14	7.14
	15	2	17	11.76
	15	1	16	6.25
	13	1	14	7.14
	14	1	15	6.66
				7.79±2.25
$rWbL_2$	7	8	15	53.33
	8	7	15	46.66
	7	8	15	53.33
	8	9	17	52.94
	7	7	14	50
				51.25±2.92*

Mastomys immunized with $rWBL_2$/alum were challenged intraperitoneally with *b. Malayi* L_3. After 48h, live L_3 were recovered from the micropore chambers implanted in to the peritoneal cavity of mastomys immunized with $rWBL_2$. *$p<0.05$ in comparison with alum group as analysed by student's *t* test.

Figure 3: Light micrographs of L_3 larvae of *B. malayi* recovered from cultures after in vitro ADCC assay; (a) L_3 incubated with the pooled sera sample from mastomys administered with alum adjuvant and peritoneal exudate cells (PEC). There are no cells adhered to the larva and the larva was active; (b) L_3 incubated with the pooled sera sample from mastomys immunized with $rWbL_2$ and PEC. The cells are observed to be adhered throughout the surface of larvae causing death of the larvae.

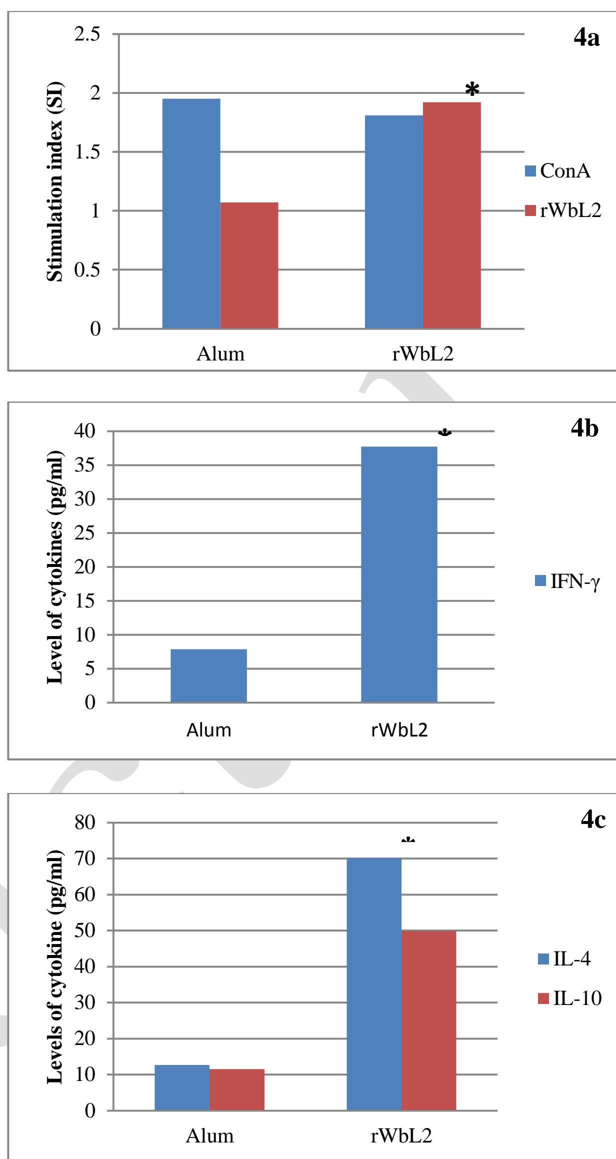

Figure 4: Effect of the immunization of mastomys with $rWbL_2$ on splenocytes proliferation and cytokines profile; (a) Splenocytes from mastomys administered with $rWbL_2$/alum were cultured in vitro and re-stimulated with either ConA (concanavalin A is a mitogen) or $rWbL_2$ and the effect on the splenocytes proliferation was checked by MTS assay after 48h. Each bar represents mean stimulation index (SI)±SD. The levels of cytokines; (b) IFN-γ; (c) IL-4, IL-10 were measured in the culture supernatants of splenocytes after 72h using ELISA. Each bar represents Mean±SD. n=5 mastomys per group; *$p<0.05$ in comparison with alum group as analyzed by Student's t test.

DISCUSSION

An SXP/RAL-2 family protein, WbL_2 is a *Wuchereria bancrofti* L_3 stage specific protein. By differential display, a nematode gene was identified that encodes a new member of the SXP/RAL-2 protein family. The gene

which is required for SXP/RAL-2 is specially expressed in the subventral pharyngeal glands few nematodes and the protein is most likely secreted.[18] The similarity of WbL_2 to other proteins from the SXP/RAL-2 family, such as from *Ancylostoma caninum (Ac*-16), *Ascaris suum* (*As*14), *Acanthocheilonema viteae* (*Av*-RAL-2), *Setaria digitata* (*Sd*-SXP-1), *Brugia malayi* (*Bm*-SXP-1), and *Onchocerca volvulus* (*Ov*17 and P36991) suggests that WbL_2 might share some biological properties with these (apparently) nematode-specific proteins.[19] While little is known of the functions of these proteins, some of them were identified as targets for nematode vaccines due to the protection they conferred upon immunization.

The member of SXP/RAL-2 family, *Ac*-16 an immunodominant surface antigen from the hookworm *Ancylostoma caninum* induced significant protection in dogs.[11] The *O. volvulus* homologue, Ov17/*Ov*-RAL-2, induced protective immunity of 51 to 60% in mice against an L_3 challenge.[19] Moreover, the *Ascaris* homologue (*As*14) belongs to SXP/RAL-2 family of proteins has been shown to confer protective immunity against *A. suum* infection in mice. [20] SXP/RAL-2 proteins have also been identified as potential antimicrofilarial vaccines with the finding that gerbils immunized with recombinant *B.malayi* SXP protein showed significantly reduced worm burdens after challenge. [21]

Thus, *W.bancrofti* SXP/RAL-2 family protein $rWbL_2$ can be important protein to be explored for prophylactic studies in lymphatic filariasis. In the present study, the vaccine potential of $rWbL_2$ was assessed in another rodent model that is fully permissible for *B. malayi* infection and further we have also characterized the humoral, cellular and cytokine responses associated with the protection induced by $rWbL_2$. The mastomys which are fully permissible for *B. malayi* when vaccinated with $rWbL_2$ developed significant titres of anti-WbL_2 antibodies. The immunoglobulin antibody isotype profile for the $rWbL_2$-immunized mastomys showed predominance of both IgG1 and IgG2a antibodies which is suggestive of a both cell mediated and humoral immune response. Increased IgG1 and IgG2a isotypes in rodents have the ability to fix complement and bind to protein antigens and have been shown to participate in ADCC reactions against invading pathogens.[22,23]

ADCC is one of the principal immunological mechanisms working behind the dis-appearance of circulating filarial parasites and their clearance. [15,16,24] Here in, we observed that the sera from mastomys immunized with $rWbL_2$ promoted adherence of peritoneal exudate cells to L_3 larvae and induced significant killing of parasite (53.89% of cytotoxicity against L_3). Whereas, depletion of these antibodies from the sera of immunized mastomys reverted the killing activity of parasite. These results indicate that, anti-WbL_2 antibodies may be playing a crucial role against the filarial infection.

The results observed in the ADCC experiment were replicated in the *in situ* micropore chamber experiment. Mastomys vaccinated with $rWbL_2$ induced significantly higher cytotoxicity of 51.25%. The chambers retrieved from the immunized mastomys showed high cellular infiltration. These results suggest that $rWbL_2$ is the promising vaccine candidate against filarial infection. T cells are also important in inducing the antibody meditated protection against the parasite. We observed significantly higher levels of WbL_2 cytokine IFN-γ in the immunized animals. The immunized mastomys showed both cell mediated and humoral immune response associated with the significant increase in cytokine IL-4 and another cytokine IL-10 was also found to be farely high, which is in correlation with the predominance of IgG1 and IgG2a antibody isotypes as seen in the antibody analysis.

CONCLUSION

Thus, considering result of present study it shows the protective immune response elicited by the rWbL2 protein against filarial infection which is found to be associated with the increased IFN-γ production along with the presence of both Th1 and Th2 protective immune responses. Taken together, rWbL2 could be a promising vaccine candidate against lymphatic filariasis infection or it can serve as one of the important vaccine candidate in cocktail vaccines in filariasis.

ACKNOWLEDGEMENTS

The financial support by Department of Biotechnology (DBT) through research project (Project Id. BT/PR/4988/INF/22/155/2012) and FIST grant from Department of Science and Technology (Project Id. SR/FST/LSI-470/2010) both from Ministry of Science & Technology, Government of India is gratefully acknowledged.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. World Health Organization's GPELF progress report 2000–2009 and Strategic plan 2010–2020; 1-78.
2. Ramaiah KD, Das PK, Michael E, Guyatt H. The economic burden of Lymphatic filariasis in India. Parasitol Today. 2000;16(6):251-3.
3. Hotez PJ. Mass drug administration and integrated control for the world's high-prevalence neglected tropical diseases. Clin Pharmacol Ther. 2009;85:659-64.
4. Anugraha G, Madhumathi J, Prince PR, Jeya Prita PJ, Khatri VK, Amdare NP, et al. Chimeric Epitope

Retracted

Vaccine from Multistage Antigens for Lymphatic Filariasis. Scand J Immunol. 2015;82(4):380-9.

5. Anand SB, Murugan V, Prabhu PR, Anandharaman V, Reddy MV, Kaliraj P. Comparison of immunogenicity, protective efficacy of single and cocktail DNA vaccine of Brugia malayi abundant larval transcript (ALT-2) and thioredoxin peroxidase (TPX) in mice. Acta Trop. 2008;107:106-12.
6. Vanam U, Pandey V, Prabhu PR, Dakshinamurthy G, Reddy MV, Kaliraj P. Evaluation of immunoprophylactic efficacy of Brugia malayi transglutaminase (BmTGA) in single and multiple antigen vaccination with BmALT-2 and BmTPX for human lymphatic filariasis. Am J Trop Med Hyg. 2009;80:319-24.
7. Veerapathran A, Dakshinamoorthy G, Gnanasekar M, Reddy MV, Kalyanasundaram R. Evaluation of Wuchereria bancrofti GST as a Vaccine Candidate for Lymphatic Filariasis. PLoS Negl Trop Dis. 2009;3(6):e457.
8. Anand SB, Kodumudi KN, Reddy MV, Kaliraj P. A combination of two Brugia malayi filarial vaccine candidate antigens (BmALT-2 and BmVAH) enhances immune responses and protection in jirds. J Helminthol. 2011;85:442-52.
9. Dakshinamoorthy G, Samykutty AK, Munirathinam G, Reddy MV, Kalyanasundaram R. Multivalent fusion protein vaccine for lymphatic filariasis. Vaccine. 2013;31:1616-22.
10. Tytgat T, Vercauteren I, Vanholme B, De Meutter J, Vanhoutte I, Gheysen G, et al..An SXP/RAL2 protein produced by the subventral pharyngeal glands in the plant parasitic root-knot nematode Meloidogyne incognita. Parasitol Res. 2005;95:50-4.
11. Fujiwara RT, Zhan B, Mendez S, Loukas A, Bueno LL, Wang Y, et al. Reduction of Worm Fecundity and Canine Host Blood Loss Mediates Protection against Hookworm Infection Elicited by Vaccination with Recombinant Ac-16. Clinical and vaccine immunology. 2007;14(3):281-7.
12. Suzuki T, Seregey IG. Mass dissection technique for determining infectivity rate of filariasis vector. Jpn J Exp Med. 1979;49(2):117-21.
13. Reddy MVR, Alli R, Devi KK, Narayan R, Harikrishnan R, Cheirmaraj K, Harinath BC. Comparative evaluation of microtitre plate peroxidase & stick penicillinase enzyme immunoassay for detection of filarial antibodies using *B.malayi* microfilarial excretory secretory antigen. J Parasitic Disease. 1996;20:173-6.
14. Dalai SK, Das D, Kar SK. Seteria digitata adult 14- to 20-kDa antigens induce differential Th-1/Th-2 cytokine responses in the lymphocytes of endemic normals and asymptomatic microfilariae carriers in bancroftian filariasis. J Clin Immunol. 1998;18: 114-23.
15. Chandrashekar R, Rao UR, Parab PB, Subrahmanyam D. *Brugia malayi*: serum dependent cell-mediated reactions to microfilariae. Southeast Asian J Trop Med Public Health. 1985;16(1):15-21.
16. Chandrashekar R, Rao UR, Subrahmanyam D. Serum dependent cell-mediated immune reactions to Brugia pahangi infective larvae. Parasite Immunol. 1985;7(6):633-41.
17. Weiss N, Tanner M. Studies on Dipetalonema viteae (Filarioidea) 3. Antibody-dependent cell-mediated destruction of microfilariae in vivo. Tropenmed Parasitol. 1979;30(1):73-80.
18. Rao KVN, Eswaran M, Ravi V, Gnanasekhar B, Narayanan RB, Kaliraj P. The Wuchereria bancrofti orthologue of Brugia malayi SXP-1 & the diagnosis of bancroftian filariasis. Mol Biochem Parasitol. 2000;107:71-80.
19. Lustigman S, MacDonald AJ, Abraham D. CD4+-dependent immunity to Onchocerca volvulus third-stage larvae in humans and the mouse vaccination model: common ground and distinctions. Int. J. Parasitol. 2003;33:1161-71.
20. Tsuji N, Suzuki K, Kasuga-Aoki H, Matsumoto Y, Arakawa T, Ishiwata K, et al. Intranasal immunization with recombinant Ascaris suum 14-kilodalton antigen coupled with cholera toxin B subunit induces protective immunity to A. suum infection in mice. Infect Immun. 2001;69:7285-92.
21. Wang SH, Zheng HJ, Dissanayake S, Cheng WF, Tao ZH, Lin SZ, Piessens WF. Evaluation of recombinant chitinase and SXP1 antigens as antimicrofilarial vaccines. Am J Trop Med Hyg. 1997;56:474-81.
22. Akiyama Y, Lubeck MD, Steplewski Z, Koprowski H. Induction of mouse IgG2a- and IgG3-dependent cellular cytotoxicity in human monocytic cells (U937) by immune interferon. Cancer Res. 1984;44:5127-31.
23. Lawrence RA. Immunity to filarial nematodes. Vet Parasitol. 2001;100:33-44.
24. Mehta K, Subrahmanyam D, Sindhu RK. Immunogenicity of homogenates of the developmental stages of Litomosoides carinii in albino rats. Acta Trop. 1981;38(3):319-24.

Cite this article as: Andure D, Reddy MVR, Pote K, Padalkar R, Khatri V, Amdare N. Role of recombinant SXP/RAL-2 family protein *Wuchereria bancrofti* L_2 ($rWbL_2$) as vaccine candidate in lymphatic filariasis in mastomys. Int J Res Med Sci 2016:4:1140-6.

Retracted