9-1-2014

Vaccination with a genetically modified Brugia malayi cysteine protease inhibitor-2 reduces adult parasite numbers and affects the fertility of female worms following a subcutaneous challenge of Mongolian gerbils (Meriones unguiculatus) with B. malayi infective larvae.

Sridhar Arumugam
Louisiana State University

Junfei Wei
Texas Children Hospital

Danielle Ward
Louisiana State University

David Abraham
Thomas Jefferson University

Sara Lustigman
New York Blood Center

See next page for additional authors

HHS Public Access

Author manuscript

Int J Parasitol. Author manuscript; available in PMC 2017 March 03.

Published in final edited form as:

Int J Parasitol. 2014 September ; 44(10): 675–679. doi:10.1016/j.ijpara.2014.05.003.

Vaccination with a genetically modified *Brugia malayi* cysteine protease inhibitor-2 reduces adult parasite numbers and affects the fertility of female worms following a subcutaneous challenge of Mongolian gerbils (*Meriones unguiculatus*) with *B. malayi* infective larvae

Sridhar Arumugam[a], **Junfei Wei**[b], **Danielle Ward**[a], **David Abraham**[c], **Sara Lustigman**[d], **Bin Zhan**[b], and **Thomas R. Klei**[a,*]

[a]Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Louisiana State University, 1909 Skip Bertman Drive, Baton Rouge, LA, 70803 USA

[b]Department of Pediatrics and Tropical Medicine, Baylor College of Medicine, Texas Children Hospital, 1102 Bates St., Ste.550, Houston, TX 77030, USA

[c]Department of Microbiology and Immunology, Thomas Jefferson University, 233 S. 10th Street, Philadelphia, PA 19107, USA

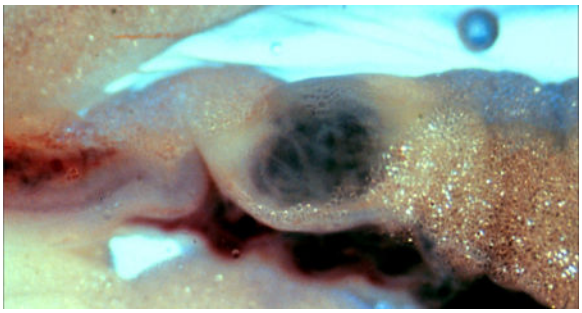
[d]Laboratory of Molecular Parasitology, Lindsley F. Kimball Research Institute, New York Blood Center, 310 E 67th St, New York, NY 10065, USA

Abstract

Vaccination of Mongolian gerbils with *Brugia malayi* cysteine protease inhibitor-2 in which the amino acid Asn66 was mutated to Lys66 (*Bm*-CPI-2M) resulted in reduced parasite numbers of 48.6% and 48.0 % at 42 and 90 days p.i. with *B. malayi* L3s. Fertility of female worms was also affected at 90 days p.i. In vitro killing of L3s observed in the presence of gerbil peritoneal exudate cells and anti-*Bm*-CPI-2M sera suggests antibody-dependent cell-mediated cytotoxicity as a putative protective mechanism. These observations suggest that *Bm*-CPI-2M is a promising prophylactic and anti-fecundity vaccine candidate.

Toc image

*Corresponding author. Tel.: +1 225-578-3363; fax: +1 225-578-9701. tklei1@lsu.edu.



Keywords

Brugia malayi cysteine protease inhibitor-2 (*Bm*-CPI-2); Filariasis; Vaccination; Fecundity; Antibody dependent cell-mediated cytotoxicity (ADCC)

Lymphatic filariasis and onchocerciasis, two major neglected tropical diseases, affect 150 million people worldwide with 1.39 billion people at risk (WHO, 2012). Mass drug administration (MDA) of antifilarial drugs has successfully reduced the transmission of these infectious agents and the disease burden in some endemic areas (WHO, 2012). However, continued MDA can potentially contribute to the development of drug resistance as reported in onchocerciasis (Osei-Atweneboana et al., 2011). Development and distribution of filarial vaccines is another feasible strategy for the global control and elimination of filariasis (Lustigman, 2012; Prichard et al., 2012). The vaccine candidates of *Onchocerca volvulus* and lymphatic filariae have been reviewed by Lustigman (2012). The level of protection reported ranges from 40% to 76%. Recently, a recombinant fusion protein of *Brugia malayi* α crystalline domain and C-terminal extension of heat shock protein 12.6 *(Bm*-HSPαc), *B. malayi* abundant larval transcript-2 (*Bm*-ALT-2) and *B. malayi* tetraspanin large extracellular loop (*Bm*-TSP LEL) showed more than 95% protection against *B. malayi* infection in mice (Dakshinamoorthy and Kalyanasundaram, 2013).

In parasitic nematodes, the endogenous native cysteine protease inhibitors (CPIs) or cystatins were shown to be involved in essential developmental processes and in the interactions of the parasite with the vector and the mammalian hosts (de Macedo Soares and de Macedo, 2007; Gregory and Maizels, 2008). In filarial nematodes, CPIs have been characterized in *B. malayi*, *O. volvulus*, *Litomosoides sigmodontis* and *Acanthocheilonema viteae* (Lustigman et al., 1992; Pfaff et al., 2002; Gregory and Maizels, 2008). Three CPIs, *Bm*-CPI-1, *Bm*-CPI-2 and *Bm*-CPI-3, have been identified in *B. malayi*. *Bm*-CPI-2 is secreted by adult female worms and is present in all life stages of the parasite, whereas *Bm*-CPI-1 and *Bm*-CPI-3 are found only in the L2 and L3 stages (Manoury et al., 2001; Murray et al., 2005; Gregory and Maizels, 2008). Interestingly, immunization of mice with the *O. volvulus* recombinant onchocystatin (*Ov*-CPI-2/Ov-7), when formulated with alum, significantly reduced *O. volvulus* larval survival by 49% and inhibited development of L3s to L4s in a *O. volvulus*- diffusion chamber model (Abraham et al, 2001; Lustigman et al., 2002).

In the *L. sigmodontis* mouse model, DNA vaccination of genetically modified *Ls*-CPI-2 with Asn66 mutated to Lys66 together with *L. sigmodontis* abundant larval transcript-1 (*Ls*-ALT-1) significantly increased their immunogenicity and reduced the number of developing adult worms and microfilaremia (Babayan et al., 2012). Recently, we reported that vaccination of gerbils with recombinant *Bm*-CPI-1 or -2 did not confer protection against *B. malayi* infection but rather altered the site of establishment of the developed adult worms, causing an increase in their residence in heart and lungs instead of lymphatic vessels (Arumugam et al., 2014). The reason for the lack of protective efficacy of the native *Bm*-CPIs vaccine is unclear, but it could be related to the known immunomodulatory property of cystatins, especially *Bm*-CPI-2. *Bm*-CPI-2 has an amino acid sequence motif, SND, which constitutes a distinct inhibitory site that is specific for asparaginyl endopeptidase (AEP) in antigen presenting cells (APC) (Manoury et al., 2001). This immunomodulatory property of native *Bm*-CPI-2 could have evoked a strong immune suppression, dampening the host immune response upon vaccination by inhibiting cysteine proteases of APC.

If the putative immunomodulatory properties of the native *Bm*-CPI-2 reduced its efficacy as an anti- *B. malayi* vaccine, it is possible that mutating it, as was done with *Ls*-CPI-2 (Babayan et al., 2012), would enhance its efficacy as a vaccine. This was tested by vaccinating gerbils with a recombinant protein containing a mutation at amino acid position 66, Asn66 to Lys66 (*Bm*-CPI-2M). Results indicate that vaccination of gerbils with *Bm*-CPI-2M reduces *B. malayi* parasite numbers and affects fertility of female worms. Furthermore, anti-*Bm*-CPI-2M sera in the presence of gerbil peritoneal exudate cells (PEC) efficiently killed L3s in vitro in an antibody-dependent cell-mediated cytotoxicity (ADCC) manner.

The L3s were harvested from infected mosquitoes, *Aedes aegypti*, using the previously described technique (Klei et al., 1990). The Institutional Animal Care and Use Committee (IACUC) at Louisiana State University (LSU), USA approved the animal experimental protocols. DNA encoding *Bm*-CPI-2 (GenBank accession number **AF015263**), minus the signal peptides, with the immunomodulatory active Asn66 mutated to Lys66 (*Bm*-CPI-2M) was synthesized by GenScript, USA and recombinant protein was expressed using the pET41a expression vector and the BL21 (DE3) *Escherichia coli* strain (Novagen, USA) as described previously (Zhan et al., 2002). Gerbils were immunized three times at 2 week intervals. *Bm*-CPI-2M was formulated with alum (Rehydragel LV, General Chemical, NJ, USA). Performed twice, both experiments consisted of 10, 8 week old male gerbils in each of the two groups; one group was vaccinated with 25 μg of *Bm*-CPI-2M absorbed to 320 μg of alum in tris buffered saline (TBS) buffer and one group was vaccinated with 320 μg of alum in TBS buffer alone. Sera were collected 1 week prior to the start of each experiment and 1 week following the third immunization. Two weeks after second boost, gerbils were challenged with 100 L3s of *B. malayi* s.c. in the medial surface of the left leg. In the first and second *Bm*-CPI-2M vaccination experiments, necropsies were performed 42 and 90 days p.i., respectively. In the first experiment, necropsy at day 42 p.i. was performed to investigate the impact of *Bm*-CPI-2M vaccination on the development of L3s to adult male and female worms. In the second experiment, necropsy at day 90 p.i. not only allowed for the data of the first experiment to be confirmed but also made it possible to test the effect of the vaccine on

the fertility of female worms. Fertility was determined by measuring the levels of microfilariae (MF) in gerbil blood and by performing embryograms of female worms. The total number of worms recovered was recorded, male and female worms were differentiated and tissue location was noted upon harvest from heart, lung and various lymphatic organs as previously described (Arumugam et al., 2014). Female worm length was measured under an Olympus MVX10 microscope using the Olympus DP2-BSW software. Statistical significance was determined by a Mann–Whitney U Test using GraphPad Prism version 4.03. $P \leq 0.05$ represents a statistically significant difference between vaccinated and alum adjuvant control groups.

In the first experiment, a significant reduction (48.6%) in worm recovery was observed at 42 days p.i. in the *Bm*-CPI-2M vaccinated group compared with controls (Fig. 1A). The reduction in worm numbers was seen in both the heart and lung sites and in the lymphatic organs of vaccinated animals (Fig. 1B, C).

A significant reduction (48.0%) in worm recovery was also observed in vaccinated animals in the second experiment when necropsies were performed at 90 days p.i. (Fig. 1D). In this experiment, one gerbil in the control group died of unknown causes prior to challenge and thus the group contained only nine animals. As in the first experiment, a reduction in worm numbers was seen in both the heart and lung sites and in the lymphatic organs of vaccinated animals (Fig. 1E, F). However, the results of two experiments differ in that the statistically significant reduction in numbers of worms was seen in the lymphatic organs in the second experiment compared with the larger reduction that was seen in the heart and lungs in animals in the first experiment. The reasons for these statistical differences between tissue sites are unclear but may relate to the timing of the necropsies and subsequent movement of parasites.

We analyzed sex ratios of worms recovered in both of the experiments. While there was a very slight decrease in the female:male ratio in *Bm*-CPI-2M vaccinated gerbils this was not statistically significant (Supplementary Fig. S1A, B).

Antigen-specific IgG responses were determined by ELISA following the method previously described (Arumugam et al., 2014). In both experiments, a strong antigen-specific IgG response with a final end point titer of up to 1:2,048,000 was seen in sera of vaccinated animals taken post-vaccination prior to L3 challenge. The level of antigen-specific IgG did not correlate with the level of protection seen. The IgG levels were the same between a non-protected vaccinated gerbil with a worm count of 24 compared with a protected vaccinated gerbil with a worm count of 1. The final end point titer was 1: 2,048,000 for both gerbils and there was no antigen-specific IgG in the alum control or pre-immune gerbil serum (Supplementary Fig. S2).

The number of circulating MF was measured in the second experiment at 90 days p.i. in 20 μl of blood from control and vaccinated gerbils at the time of necropsy. Only three gerbils were microfilareamic at 90 day p.i. Two of these were in the control group and one in the vaccinated group. There was no statistical difference in MF levels seen (Supplementary Fig. S3). To further investigate the impact of *Bm*-CPI-2M vaccination on fertility of female

worms, embryograms were performed to enumerate the progeny in individual female worms harvested from control and vaccinated gerbils at 90 days p.i. The worms used for these counts were previously fixed in 70% ethanol containing 30% glycerin. This fixation likely accounts for the slight distortions seen in the recovered developmental stages (Fig. 2A). Nonetheless, differentiation and counting of stages was easily accomplished. The morphology of eggs, early embryos, late embryos, pretzel stage MF and stretched MF are shown in Fig. 2A. Single female worms were transferred to a 1.5 ml tube containing 200 μl of PBS. A disposable pestle was used to gently crush the worms and 300 μl of PBS were used to wash the worm debris from the pestle into the same tube. The tube was centrifuged at 400 *g* for 2 min and 450 μl of PBS was carefully removed without disturbing the pellet. The pellet with worm content in the remaining 50 μl was mixed well and 10 μl was added to the hemocytometer. The total number of eggs, embryos, pretzel stage MF and stretched MF (Ziewer et al., 2012) was counted. Early and late stage embryos were grouped together as embryos for the purposes of counting. At least two female worms per gerbil were analyzed from each group except for one gerbil in the vaccinated group from which only one female worm was harvested. Embryogram data demonstrated that there was no reduction in the number of eggs/female worm between control and vaccinated gerbils (Fig. 2B). In contrast, there was a significant reduction in the number of embryos, pretzel stage MF and stretched MF per female worm harvested from vaccinated gerbils. (Fig. 2C–E).

Parasite naïve male gerbils were injected with 3 ml of 3% (w/v) of thioglycolate i.p. to recruit PECs used in in vitro killing assays. Five days after injection, PECs were harvested from the peritoneal cavity and suspended in RPMI supplemented with 10% FCS and penicillin-streptomycin (Sigma, USA). The serum used in the assays was from *Bm*-CPI-2M immunized gerbils with an end point titer of anti-*Bm*-CPI-2M specific IgG antibodies of 1:2,048,000. A control serum was from gerbils vaccinated with alum alone. In vitro killing assays were performed twice. Assays were performed in 24-well culture plates containing 1 ml of RPMI medium and 10 L3s were added per well (Chandrasekhar et al., 1985). An initial dilution assay was performed in replicates in the presence of PECs using serum dilutions of 1:20, 1:40, 1:80 and 1:100 from gerbils vaccinated with alum only, anti-*Bm*-CPI-1, anti-*Bm*-CPI-2 or anti-*Bm*-CPI-2M sera. Sera used were recovered following the second vaccination boost. The plate was incubated for 48 h at 37°C with 5% CO_2. After 48 h, the survival of L3s was recorded based on their motility and the integrity of their internal tissues. Those that were completely immotile with granulated, irregular tissues within the body were considered dead.

No L3 killing was observed in the presence of 2×10^5 PEC in any of the dilutions of control sera. However, L3 killing of more than 50% was observed in the 1:20 dilution of anti-*Bm*-CPI-2M sera. This decreased to 15% in the 1:40 dilution and was not seen at higher dilutions. In vitro killing assays with sera from gerbils immunized with the native *Bm*-CPI-1 or- 2 at 1:20 dilution in the presence of PECs induced only 0–20% L3 killing and no killing was observed above 1:40 dilution of native *Bm*-CPI-1 or- 2 sera (Supplementary Table S1). No killing was observed in RPMI media controls or when L3s were cultured either in the presence of PEC alone or 1:20 dilutions of alum/*Bm*-CPI-1 or- 2/*Bm*-CPI-2M serum alone (Supplementary Table S1).

The initial killing assay performed with different serum dilutions determined that 1:20 dilution of anti-*Bm*-CPI-2M serum in the presence of 2×10^5 PECs was able to kill more than 50% of cultured *B. malayi* L3s. The L3 killing assay was repeated with 1:20 anti-*Bm*-CPI-2M compared with sera from control gerbils vaccinated with alum. Ten L3s were placed in each well corresponding to the following five treatment groups: i) RPMI media; ii) 2×10^5 PECs; iii) 1:20 dilution of anti-*Bm*-CPI-2M sera; iv) 2×10^5 PECs plus 1:20 dilution of control sera; and v) 2×10^5 PECs plus 1:20 dilution of anti-*Bm*-CPI-2M sera. In groups i to iv, 100% of cultured L3s survived (Supplementary Movie S1, living L3 cultured with PECs plus control serum), while 60% of L3s were killed in group v, when they were cultured with PECs and anti-*Bm*-CPI-2M antibodies (Supplementary Video S2, Supplementary Movie S3 - higher magnification). Notably, the remaining L3s incubated with PECs plus anti-*Bm*-CPI-2M sera were counted as living but were sluggish and contained granules within their bodies (Supplementary Movie S4). Adherence of PECs to the surface of L3s was observed on the dead or dying larvae (Fig. 3B, Supplementary Movies S2–S4), whereas no adherence of PECs was observed in L3s incubated with PECs plus sera from the alum control group (Fig. 3A, Supplementary Movie S1).

Adherence of PECs to the surface of L3s followed by killing of L3s in the presence of anti-*Bm*-CPI-2M sera suggests that ADCC is a potential mechanism of immune response induced by the vaccination that resulted in a reduction in the number of adult worms.

We did not detect any evidence of adult killing at the time of necropsy. There was no attachment of cells or formation of granulomas around the adult worms in any group and all recovered worms were alive. There was no significant difference in the length of female worms recovered from alum control groups and *Bm*-CPI-2M vaccinated gerbils (data not shown).

While there is no direct evidence of effect of vaccination on adult worm viability and growth, *Bm*-CPI-2M vaccination did affect the fertility of female worms, significantly reducing the numbers of developmental stages in female worms. Similarly, vaccination-induced reduced fecundity of *L. sigmodontis* worms was observed when mice were vaccinated with *Ls*-CPI-2M or with *L. sigmodontis* MF (Babayan et al., 2012; Ziewer et al., 2012). We also previously reported that vaccination of gerbil *Brugia pahangi* excretory/secretory (ES) products reduced the fecundity of female worms in gerbils (Zipperer et al., 2013). In *Caenorhabditis elegans*, cystatin-like inhibitor CPI-2a deletion mutants showed that CPI-2a has an essential regulatory role during oogenesis and fertilization (Hashmi et al., 2006). It is possible that *Bm*-CPI-2 could have a similar role in the embryogenesis of *B. malayi*. Alteration of *Bm*-CPI-2 function in female worms by vaccination could explain the results seen in these experiments.

In the current experiments, *Bm*-CPI-2 mutated to alter its immunomodulatory properties proved to reduce adult worm numbers and reduce fecundity. While we did not test the immunomodulatory activity of mutant *Bm*-CPI-2, the results suggest that this alteration increased the vaccine efficacy of this molecule. Comparison of the immunomodulatory activities of native and mutant *Bm*-CPI-2 might further explain its relationship to the enhancement of conferred protective immunity. Our findings suggest that *Bm*-CPI-2M is a

potential anti-filarial vaccine candidate but further investigations are necessary to explore the role of immunomodulation in vaccinology and protective immunity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by the National Institutes of Health (NIH), USA -AI07831401. We thank Julie Woody and Jackie Macaluso for their help with this study.

References

Abraham D, Leon O, Leon S, Lustigman S. Development of a recombinant antigen vaccine against infection with the filarial worm *Onchocerca volvulus*. Infect Immun. 2001; 69:262–270. [PubMed: 11119514]

Arumugam S, Zhan B, Abraham D, Ward D, Lustigman S, Klei TR. Vaccination with recombinant *Brugia malayi* cystatin proteins alters worm migration, homing and final niche selection following a subcutaneous challenge of Mongolian gerbils (*Meriones unguiculatus*) with *B. malayi* infective larvae. Parasit Vectors. 2014; 7:43. [PubMed: 24450869]

Babayan SA, Luo H, Gray N, Taylor DW, Allen JE. Deletion of parasite immune modulatory sequences combined with immune activating signals enhances vaccine mediated protection against filarial nematodes. PLoS Negl Trop Dis. 2012; 6:e1968. [PubMed: 23301106]

Chandrasekhar R, Rao UR, Subramanyam D. Serum dependent cell mediated reactions to *Brugia pahangi* infective larvae. Parasite Immunol. 1985; 7:633–642. [PubMed: 4094785]

Dakshinamoorthy G, Kalyanasundaram R. Evaluating the efficacy of r*Bm*HATαc as a multivalent vaccine against lymphatic filariasis in experimental animals and optimizing the adjuvant formulation. Vaccine. 2013; 32:19–25. [PubMed: 24211167]

de Macedo Soares MF, de Macedo MS. Modulation of anaphylaxis by helminth-derived products in animal models. Curr Allergy Asthma Rep. 2007; 7:56–61. [PubMed: 17504662]

Gregory WF, Maizels RM. Cystatins from filarial parasites: evolution, adaptation and function in the host-parasite relationship. Int J Biochem Cell Biol. 2008; 40:1389–1398. [PubMed: 18249028]

Hashmi S, Zhang J, Oksov Y, Ji Q, Lustigman S. The *Caenorhabditis elegans* CPI-2a cystatin-like inhibitor has an essential regulatory role during oogenesis and fertilization. J Biol Chem. 2006; 281:28415–28429. [PubMed: 16857685]

Klei TR, McVay CS, Dennis VA, Coleman SU, Enright FM, Casey HW. *Brugia pahangi*: Effects of duration of infection and parasite burden on lymphatic lesion severity, granulomatous hypersensitivity, and immune responses in jirds (*Meriones unguiculatus*). Exp Parasitol. 1990; 71:393–405. [PubMed: 2226701]

Lustigman S, Brotman B, Huima T, Prince AM, McKerrow JH. Molecular cloning and characterization of onchocystatin, a cysteine proteinase inhibitor of *Onchocerca volvulus*. J Biol Chem. 1992; 267:17339–17346. [PubMed: 1512269]

Lustigman S, James ER, Tawe W, Abraham D. Towards a recombinant antigen vaccine against *Onchocerca volvulus*. Trends Parasitol. 2002; 18:135–141. [PubMed: 11854092]

Lustigman, S. Current approaches to the development of a vaccine against filarial nematodes. In: Lamb, TJ., editor. Immunity to Parasitic Infection. John Wiley & Sons, Ltd; Chichester, UK: 2012. p. 459-470.

Manoury B, Gregory WF, Maizels RM, Watts C. *Bm*-CPI-2, a cystatin homolog secreted by the filarial parasite *Brugia malayi*, inhibits class II MHC-restricted antigen processing. Curr Biol. 2001; 11:447–451. [PubMed: 11301256]

Murray J, Manoury B, Balic A, Watts C, Maizels RM. *Bm*-CPI-2, a cystatin from *Brugia malayi* nematode parasites, differs from *Caenorhabditis elegans* cystatins in a specific site mediating

inhibition of the antigen-processing enzyme AEP. Mol Biochem Parasitol. 2005; 139:197–203. [PubMed: 15664654]

Osei-Atweneboana MY, Awadzi K, Attah SK, Boakye DA, Gyapong JO, Prichard RK. Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. PLoS Negl Trop Dis. 2011; 5:e998. [PubMed: 21468315]

Pfaff AW, Schulz-Key H, Soboslay PT, Taylor DW, MacLennan K, Hoffmann WH. *Litomosoides sigmodontis* cystatin acts as an immunomodulator during experimental filariasis. Int J Parasitol. 2002; 32:171–178. [PubMed: 11812494]

Prichard RK, Basáñez MG, Boatin BA, McCarthy JS, García HH, Yang GJ, Sripa B, Lustigman S. A research agenda for helminth diseases of humans: intervention for control and elimination. PLoS Negl Trop Dis. 2012; 6:e1549. [PubMed: 22545163]

WHO. Global programme to eliminate lymphatic filariasis: progress report in 2011. Wkly Epidemiol Rec. 2012; 87:346–356. [PubMed: 22977953]

Zhan B, Hotez JP, Wang Y, Hawdon MJ. A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of protease. Mol Biochem Parasitol. 2002; 120:291–296. [PubMed: 11897134]

Zipperer GR, Arumugam S, Chirgwin SR, Coleman SU, Shakya KP, Klei TR. *Brugia pahangi*: Immunization with early L3 ES alters parasite migration, fecundity and lymphatic lesion formation in gerbils (*Meriones unguiculatus*). Exp Parasitol. 2013; 135:446–455. [PubMed: 23981910]

Ziewer S, Hübner MP, Dubben B, Hoffmann WH, Bain O, Martin C, Hoerauf A, Specht S. Immunization with *L. sigmodontis* microfilariae reduces peripheral microfilaraemia after challenge infection by inhibition of filarial embryogenesis. PLoS Negl Trop Dis. 2012; 6:e1558. [PubMed: 22413031]

Highlights

- *Brugia malayi* cysteine protease inhibitor-2 (*Bm*-CPI-2) can modulate the host immune response.
- Vaccination with *Bm*-CPI-2 does not protect against *B. malayi* infection in gerbils.
- Amino acid Asn66 in *Bm*-CPI-2 is involved in immunomodulation of host immune response.
- Amino acid Asn66 was mutated to Lys66 in *Bm*-CPI-2 (*Bm*-CPI-2M).
- *Bm*-CPI-2M vaccination reduces adult *B. malayi* numbers and its fecundity in gerbils.

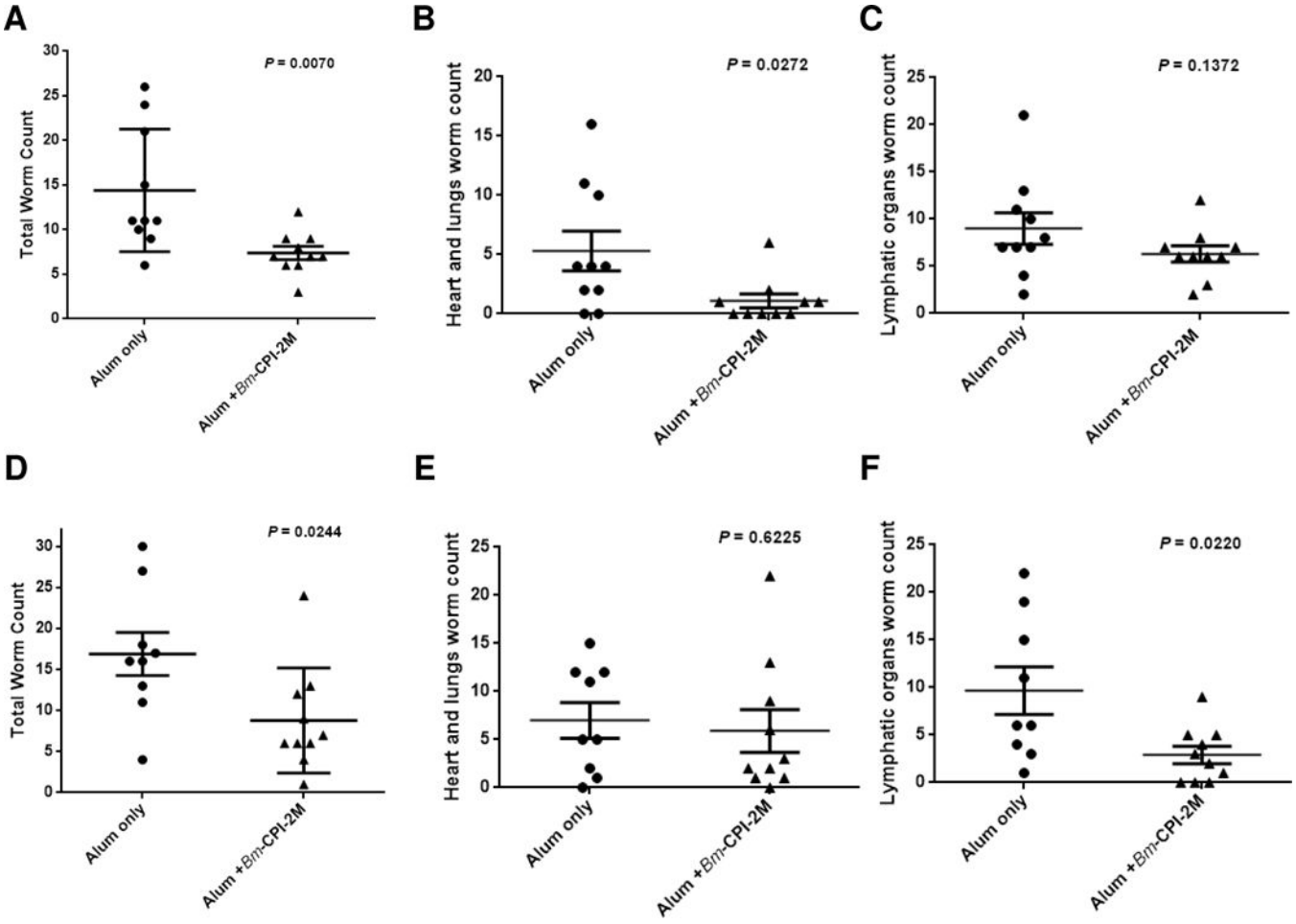


Fig. 1.

Vaccination with recombinant *Brugia malayi Bm*-CPI-2M protein confers protection against *B malayi* L3 infection in Mongolian gerbils. (A) Experiment 1, total number of recovered adult worms (42 days p.i.). (B) Distribution of worms in heart and lungs in experiment 1. (C) Distribution of worms in lymphatic organs in experiment 1. (D) Experiment 2, total number of recovered adult worms (90 days p.i.). (E) Distribution of worms in heart and lungs in experiment 2. (F) Distribution of worms in lymphatic organs in experiment 2. The lines represent the mean with S.Ds.

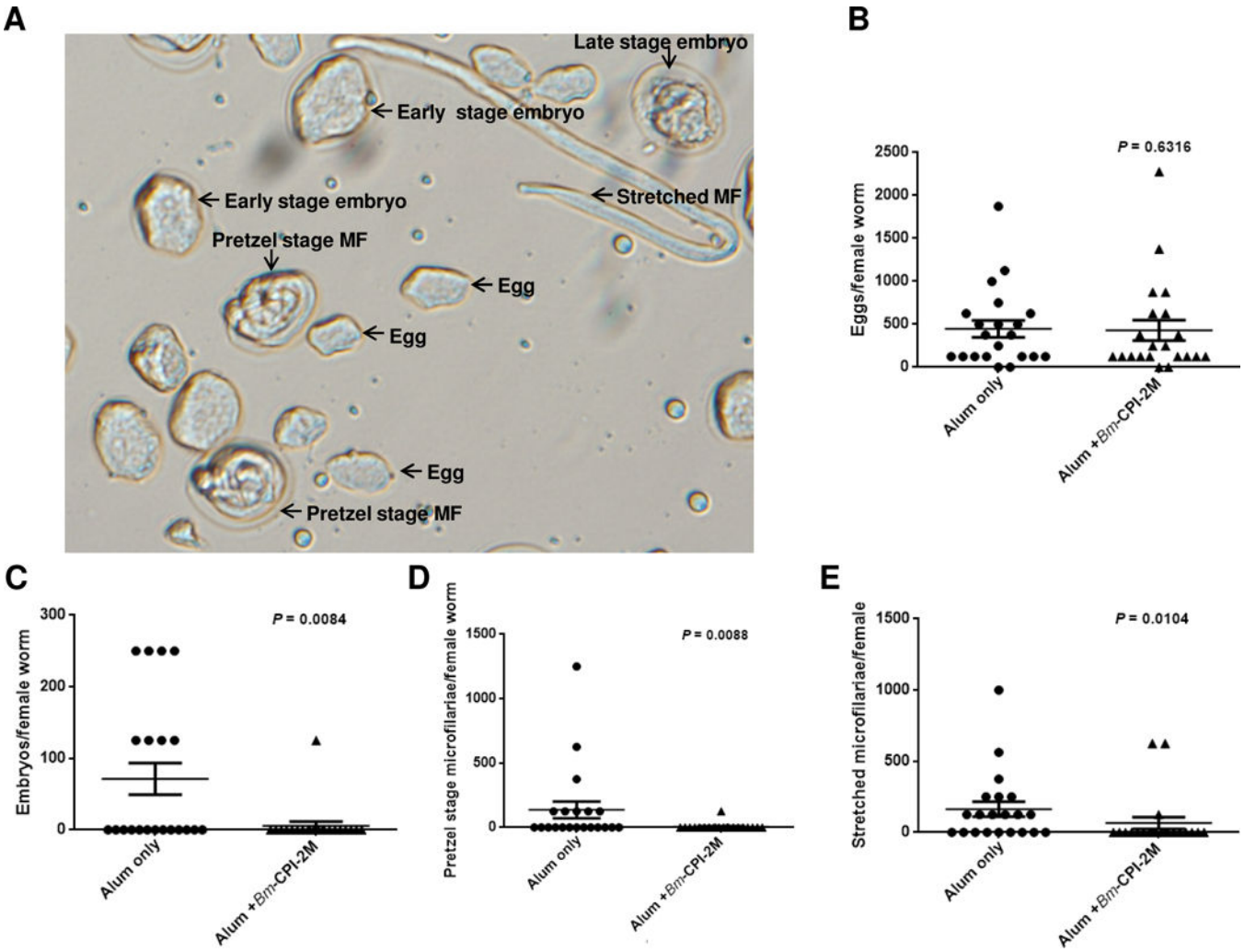


Fig. 2.

Embryogram data of female *Brugia malayi* worms harvested from alum only and *Bm*-CPI-2M vaccinated gerbils. (A) Embryogram picture of female *B. malayi* showing different developmental stages including eggs, early embryos, late embryos, pretzel stage microfilariae (MF) and stretched MF (40× magnification). (B) Eggs/female worm. (C) Embryos/female worm. D) Pretzel stage MF/female worm. (E) Stretched MF/female worm. The lines represent the mean with S.Ds.

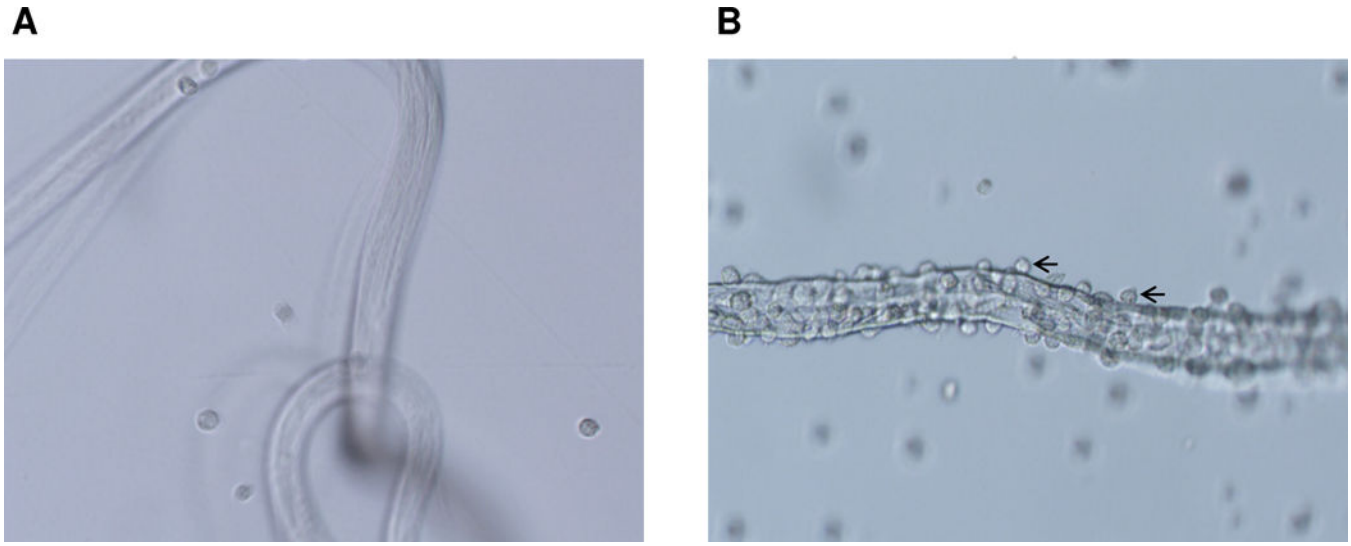


Fig. 3.

Images of *Brugia malayi* L3s in in vitro killing assays. (A) L3s cultured in the presence of peritoneal exudate cells (PECs) plus 1:20 dilution of gerbil sera from the alum control group. (B) L3s cultured in the presence of PECs plus 1:20 dilution of gerbil sera from the *Bm*-CPI-2M immunized gerbils. Arrows indicate adherence of cells to dead L3s (40× magnification).