

Mini-review

Update on paramyosin in parasitic worms

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Abstract

Paramyosin was first identified as a structural component of invertebrate muscle. Analysis of crude, native, adult schistosome worm preparations identified a highly immunogenic protein which was later identified as paramyosin. Early vaccination/challenge studies with native paramyosin produced encouraging levels of protective efficacy against schistosomes, which led to the question as to how a subtegumental (muscular) protein could provide a target for vaccine-mediated immunological attack. Immunolocalisation studies of schistosomes confirmed the presence of paramyosin within the post-acetabular glands of cercariae and on the tegumental surface of lung schistosomula. Here we present an update on the more recent research on paramyosin in parasitic worms that has focused primarily in two directions: (i) further testing of the vaccine potency of paramyosin against schistosomes and other parasitic worms; and (ii) characterisation of the protein at the molecular and biochemical levels.

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Keywords: Paramyosin; Schistosome; Vaccine target; Surface protein**1. Introduction**

Paramyosin, a 98–101 kDa protein, is present in thick myofilaments of invertebrate muscle, forming medullary rods surrounded by a cortical array of myosin rods. For most of its length, paramyosin forms an alpha-helical coiled coil with repeated sequences of hydrophobic and charged amino acids, from which the molecule derives its strong binding affinity for other proteins. Paramyosin has been identified and localised within a number of parasitic helminths of humans and domestic animals. A summary of the major research findings with paramyosin in parasitic worms is presented in Table 1, the most recent of which are elaborated on below. Early functional studies correlated high levels of paramyosin within molluscan muscle with the physiological condition “catch”; a mechanism that allows long periods of muscle tension, while expending only small amounts of energy [1]. It became apparent that paramyosin functions

more than just as a myofibril protein in parasitic worms when it was noted that immunization of mice with homogenates of adult *Schistosoma mansoni* produced a strong immunological response that included the production of antibodies against a 97 kDa component [2]; this was later identified as paramyosin [3]. Monoclonal antibodies generated against this protein (designated Sm-97) were used in subsequent indirect immunofluorescence studies, detecting reactivity in regions just below the parasite surface, corresponding to either the tegument or the muscle layers of male and female adult *S. mansoni*.

2. Vaccine studies

Gobert [4] reviewed the early vaccination studies with paramyosin against schistosome infections. These included the key observations by Pearce using native protein against *S. mansoni*, the stimulation of anti-paramyosin IgE antibodies in mice, the identification of the schistosomulum stage as one of the targets of the anti-paramyosin immunological response, and the remarkably impressive

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Table 1
Significant research milestones with paramyosin

Date	Major research findings [Ref.]
1986	Immunological response detected against a 97 kDa component in <i>S. mansoni</i> homogenates [2]
1986	The 97 kDa protein identified as paramyosin [3]
1988	Localisation of paramyosin to the tegument of adult <i>S. mansoni</i> using anti- <i>Limulus</i> (horse-shoe crab) paramyosin antibodies [44]
1988/1989	Induction of resistance to <i>S. mansoni</i> infection in mice by purified native and recombinant parasite paramyosin [5,45]
1992	Paramyosins from <i>T. solium</i> and <i>S. mansoni</i> shown to bind collagen and C1 of complement [25]
1994	Immunoelectron microscopy localisation of paramyosin in the post-acetabular glands as well as in the tegument and muscle layers of transformed schistosomula of <i>S. japonicum</i> [46]
1997	Immunolocalisation of paramyosin in adults, cercariae and schistosomula of <i>S. japonicum</i> [47]
1998	Native and recombinant paramyosin elicit partial protective immunity against <i>S. japonicum</i> in sheep [12]
1999	Partial immunity produced in rodents against <i>Brugia malayi</i> using plasmid DNA encoding paramyosin [24]
2000	Protective immunity induced against <i>S. japonicum</i> by a DNA vaccine encoding paramyosin [10]
2001	Schistosome paramyosin shown to contain an Fc binding region [28]. Protective immunity generated in the murine model of cysticercosis using whole and fragments of recombinant <i>Taenia solium</i> paramyosin [16]
2002	Paramyosin identified as a calgranulin C-binding protein in <i>B. malayi</i> [30]
2002	Recombinant paramyosin induces protective efficacy against <i>S. japonicum</i> in buffalo [14]
2003	SCIP-1 confirmed as a tegumental surface-exposed form of paramyosin in <i>S. mansoni</i> [33]

reduction of *S. japonicum* (Philippine strain) worm burdens (mean percent reduction of 72.6%, 3 replicate experiments) in mice immunized (intraperitoneal injection; no adjuvant) with biochemically purified paramyosin from adult worms [2,5,6]. A summary of more recent vaccine studies targeting paramyosin is presented in Table 2.

Experiments on mice vaccinated and then challenged with native paramyosin (plus adjuvant) against Chinese strain *S. japonicum* were unable to replicate the earlier study with the Philippine form; moderate or no protective efficacy was induced by cutaneous or intraperitoneal injection, respectively. Partial sequencing of cDNAs encoding paramyosin from these two geographical strains of *S. japonicum* [8] showed some point variations in nucleotide sequence resulting in corresponding amino acid differences between the Chinese and Philippine forms, which could partially explain the differing vaccination results. These differences were further investigated when the complete cDNA sequences for the Chinese and Philippine strains of *S. japonicum* were obtained [9,10]. This allowed a more rigorous comparison of sequence differences in paramyosin between discrete strains of *S. japonicum* and between *S. japonicum* and *S. mansoni*. Sequence homologies between

geographical strains (Chinese, Philippine and Japanese *S. japonicum*) and between species (*S. mansoni* and *S. japonicum*) were compared both at the nucleotide and amino acid levels [10]. Unsurprisingly, higher levels of sequence homology occur within the *S. japonicum* strains compared with those of *S. mansoni* and *S. japonicum*.

A DNA vaccine comprising full length Chinese *S. japonicum* paramyosin (Sjc97) was trialed [10]. Intramuscular injection into mice resulted in the stimulation of IgG2a and IgG2b antibody isotypes and the antibodies produced by vaccination were effective in mediating macrophages to kill schistosomula in vitro. These antibodies also reacted with native paramyosin in Western blots. The cellular response to the DNA vaccine included increased IL-2, IFN- γ stimulation with decreased levels of IL-4 and IL-5. These responses resulted in a reduction of associated pathology due to declines in the overall worm burden, egg density in livers and numbers of eggs in the spleen.

Unlike the African forms, schistosomiasis japonica is a zoonosis and it is important to consider the results of vaccine studies with paramyosin on other mammalian hosts (such as water buffalo, important working animals in China) that live in close proximity to humans and which act as major reservoirs of human transmission [11]. Moderate levels of protection against *S. japonicum* challenge of sheep were presented in a report by Taylor et al. [12] where a recombinant fragment of the 3' end of paramyosin and the native protein (both with Freund's adjuvant) were utilized. Native paramyosin provided greater reduction in worm burdens than the recombinant form of the protein. Chen et al. [13] investigated the effectiveness of paramyosin against *S. japonicum* challenge in pigs where recombinant protein adjuvanted with alum or TiterMax again resulted in only a moderate reduction in worm numbers. In addition, modest levels of reduction in granuloma density and fecal egg counts were observed presumably resulting in no change to the associated long term pathology. McManus et al. [14] used recombinant paramyosin in vaccination trials in mice and also in Chinese water buffalo, with subsequent challenge by *S. japonicum*. Clear increases in specific IgG antibodies were detected with reduction of worm burdens and/or eggs in mice and in two of three buffalo vaccine trials undertaken. These reports provide some encouragement for future use of a paramyosin- (protein or DNA) based vaccine to protect domestic livestock animals against schistosomiasis japonica. Such a vaccine would provide better health and performance levels for the farm animals, and reduce the levels of transmission of *S. japonicum* to humans. The importance of providing protection to livestock has also been recently mentioned in a review article of Kojima [15].

The reports of paramyosin-based vaccination against schistosomes led to the study and characterisation of the protein in other organisms. Several studies have demonstrated the potential value of paramyosin as a vaccine target that warrants its further characterisation both in *Taenia solium* and *T. saginata*. This is despite the fact that whereas

Table 2
Vaccine studies targeting paramyosin

Parasite	Host	Vaccine formulation ^a	Protection–reduction in worm burden (%)	Protection–reduction in egg numbers (%)	Comments [Ref.]
<i>Schistosoma japonicum</i>	Mice	Full length RP	32	66 in liver.	Reduction in worm burdens and/or eggs in mice and in two of three buffalo vaccine trials undertaken [14]
	Water buffalo		34	49 in liver.	
	Sheep	NP	48	43 in fecal samples.	Recombinant protein fragment consisted of third of protein corresponding to the 3' end of the nucleotide sequence [12]
		RP fragment	17	68 in liver.	
	Mice	NP	21	16 in fecal samples.	No protective immunity generated by intraperitoneal injection of vaccine [7]
				53 in liver.	
<i>Schistosoma mansoni</i>	Pig	Full length RP	32–35	Not reported.	No reduction in pathology observed [13]
	C57BL/6 and BALB/c mice	DNA encoding full length protein	35–41	Egg numbers highly variable in both treated and controls.	No protection observed in BALB/c mice [10]
		NP	24–53	44–73 in liver.	
	Mice	NP and RP	26–33	57–83 in spleen.	Protection levels similar to vaccination with freeze thawed schistosomula [41]
				Not reported.	
<i>Taenia solium</i>	Mice	Full length RP	52	Not applicable.	Recombinant protein corresponded to 50% of the complete cDNA [5]
		Amino-terminal fragment	47–87		
		Central fragment	–25–36		
		Carboxyl-terminal fragment	34–52		
<i>Brugia malayi</i>	Rodents (jirds)	DNA encoding 70% protein	none	Not reported.	Cellular responses attributed to amino terminal region while humoral responses attributed to carboxy terminal region [16]
					Strong antibody responses produced [23,48]

^a RP, recombinant protein; NP, native protein.

immunolocalisation studies have identified surface expression of paramyosin in schistosomes, similar experiments in *Taenia* have not yet been undertaken. The potency of a paramyosin vaccine against cysticercosis was evaluated by vaccinating mice with full length recombinant protein (863 amino acids) derived from *T. solium* and challenging them with metacestodes of *T. crassiceps* [16]; the vaccinated groups showed a significant reduction in worm burdens after the challenge infection. cDNA fragments corresponding to the amino (amino acids 1–268), central (amino acids 269–551) and carboxyl (amino acids 551–863) regions of the protein were cloned, expressed, purified and used to vaccinate mice in order to determine which region of paramyosin was responsible for conferring protection [16]. The levels of protection obtained with all three fragments were less consistent compared with the entire recombinant protein and the amino terminal region produced the highest and most significant levels of protection. In direct contrast, antibodies in sera from mice vaccinated with full length recombinant paramyosin reacted more strongly against the carboxyl and central regions of the protein. T helper cell type 1 (Th1) immune responses, as indicated by elevated levels of IFN- γ and IL-2, but not IL-4 (indicative of a Th2 response), was generated predominantly by the amino

terminal region, contrasting with the antibody responses obtained. Epitope mapping showed that amino acids 176–192 contained the immunodominant murine CD4+T cell epitope of *T. solium* paramyosin [17].

Gazarian et al. [18] screened a 12-mer random peptide phage display library with antibodies raised against a synthetic peptide corresponding to the amino-terminal 20 aa sequence of *T. solium* paramyosin; positive clones shared a consensus motif with structural similarity to the Arg10–Thr16 region within the sequence. More recently, some initial protection studies using genetic immunization showed that the amino terminal fragment of *T. solium* paramyosin cloned into a plasmid expression vector with a cytomegalovirus promoter, together with IL-12-expressing plasmids induced 79% protection, suggesting that this immunization strategy might result in development of a cost-effective vaccine against cysticercosis [19].

In human studies, Vazquez-Talavera et al. [20] used Western blotting to demonstrate that sera from neurocysticercotic patients were strongly reactive against the carboxyl end region, with poor recognition of the central and amino regions. In contrast, the cellular immune response of patients did not show preferential recognition of any region of paramyosin. In similar fashion, the humoral

immune response against *T. saginata* paramyosin is preferentially directed against the carboxyl terminal [21]. Furthermore, recombinant paramyosin from *T. saginata* was recognised by sera from 31.6% of *T. solium* seropositive cysticercosis patients and 100% of acute cysticercosis patients [21].

Vargas-Parada and Laclette [22] described the structure of the gene coding for the paramyosin of *T. solium*. Comparison with the paramyosin genes of *Drosophila melanogaster* and *Caenorhabditis elegans* showed a lack of conservation of exon/intron organization in contrast to other muscle genes. No evidence of alternative splicing sites was found, excluding the possibility that *T. solium* expresses a mini-paramyosin like *D. melanogaster*.

Paramyosin has also been sequenced in the liver fluke *Opisthorchis felineus* which has varying levels of sequence similarity at the nucleotide/amino acid levels relative to other helminth paramyosins including those from *S. japonicum* (70%/83%), *S. mansoni* (68%/83%), *Echinococcus granulosus* (66%/71%) and *Taenia solium* (66%/72%) [23]. This information allowed the generation of a recombinant fragment from the central region of the protein which was used to generate mouse hyperimmune serum which successfully bound native paramyosin in *O. felineus* extracts.

Vaccine studies with *Brugia malayi* DNA encoding paramyosin failed to provide protection against infection [24]. Although, the plasmid DNA did generate strong cellular and humoral responses these did not result in a reduction in worm burdens. DNA vaccination induced both Th-1 and Th-2 responses as indicated by the stimulation of IgG1 and IgG2a antibody responses, but the DNA vaccine induced lower levels of antibody response compared with those produced by native or recombinant paramyosin [24].

In summary, helminth paramyosins have been shown to be immunogenic and show promise for use as vaccines. However, they can be difficult to express and purify in recombinant form and, generally, they produce relatively modest reductions in worm and/or egg burdens in vaccination/challenge experiments. It may be necessary to explore alternative vaccination strategies to improve their vaccine efficacy and/or utilize them in combination with additional target antigens [25,26].

3. Non-muscle functions of paramyosin

Possible functions of paramyosin in non-muscle regions have been suggested by a number of studies. Paramyosin had previously been shown to bind to collagen [27] and since C1q of the complement system has a collagen-like region, competitive binding assays performed confirmed that paramyosin did, indeed, bind C1q and inhibits complement C1 function. Laclette et al. [27] suggested that paramyosin may also inhibit the complement cascade of the immune system. They

examined paramyosin from *S. mansoni*, *T. solium* and the mussel *Mytilus edulis*. Paramyosin from all of these organisms inhibited complement C1 function in vitro. Further immunomodulatory functions for paramyosin have been presented by Loukas et al. [28] who showed the protein acts as a Fc receptor on the surface of mechanically transformed *S. mansoni*. These findings indicated that paramyosin may act as an immunological defense mechanism by absorbing antibodies, and may be one explanation for the surface expression of the protein. It is noteworthy also that, similar to the findings of Laclette et al. [27] and Loukas et al. [28], recombinant paramyosin from the cattle tick *Boophilus microplus* binds both IgG and collagen [29].

Another important immunoregulatory function of paramyosin was reported by Akpek et al. [30] who determined that paramyosin is a major binding component for calgranulin in *Brugia malayi* microfilariae. Released by neutrophils, calgranulin is a protein involved in host defense and its binding to paramyosin appears to contribute to keratitis caused by filariasis. This interaction was identified by incubating homogenates of adult worms with recombinant calgranulin and affinity purifying the antibodies generated against this calgranulin-binding protein complex. These antibodies were then used to screen a *B. malayi* microfilariae cDNA library and positive clones were all found to contain paramyosin sequences. Binding of paramyosin and calgranulin was also confirmed in vitro using radiolabelled calgranulin and SDS-PAGE analysis. To determine whether the complex of calgranulin and its binding protein could induce keratitis mimicking onchocercal human corneal disease, BALB/c mice preimmunized with the binding complex were challenged with intracorneal binding complex or live *Brugia* microfilariae. Test animals showed development of severe keratitis that mimicked, both clinically and histopathologically, the human onchocercal corneal disease, demonstrating the antigenic specificity of the paramyosin–calgranulin binding complex.

Paramyosin has also been cloned from *Anisakis simplex* by Perez-Perez et al. [31]. *A. simplex*, a nematode parasite of fish, can cause severe allergic reactions in humans. A cDNA library was constructed and screened firstly using hyperimmune serum from a rabbit injected with *A. simplex*. Positive clones were further screened using human sera containing highly elevated anti-*A. simplex* IgE antibodies. From the positive clones, two sequences coding for the full length paramyosin were isolated, expressed in *E. coli* and purified. The frequency of specific IgE binding to the recombinant and native forms of paramyosin using the sera of 26 *A. simplex*-sensitive individuals was 23% and 88%, respectively. This study identified paramyosin (termed Anis 2) as a new *A. simplex* allergen in humans although the recombinant protein did not maintain the complete allergenic properties of the native paramyosin, considering its lower IgE binding capacity of the recombinant protein. Nevertheless, both proteins had the same specific IgE

inhibition capacity inhibiting 11% of the specific IgE binding to a total parasite extract.

Following partial purification and analysis by mass spectrometry, a 94-kDa complement inhibitor protein, named SCIP-1, identified in *S. mansoni* [32] has now been confirmed as a surface-exposed form of paramyosin [33]. Immunofluorescence showed that anti-paramyosin antibodies labelled the surface of live schistosomula and adult worms. Like SCIP-1, purified native paramyosin reacted with a polyclonal rabbit anti-human CD59 antiserum. Also, complement components C8 and C9 bound to recombinant and native paramyosin. Paramyosin inhibited Zn^{2+} -induced C9 polymerization and poly-C9 deposition onto rabbit erythrocytes (E_R). In addition, paramyosin inhibited lysis of E_R and of sensitized sheep erythrocytes by human complement. Anti-paramyosin antibodies enhanced in vitro killing of schistosomula by normal and C4-depleted human complement. Taken together, these findings suggest that a surface expressed form of *S. mansoni* paramyosin inhibits activation of the terminal pathway of complement and thus has an important immunomodulatory role in schistosomiasis.

4. Immunolocalisation studies

Immunolocalisation of paramyosin has focused predominantly on schistosomes (as reviewed in Refs. [4,34], and has demonstrated the presence of the protein on the surface of lung schistosomula and adult parasites and in the post-acetabular glands of cercariae. More recent studies have continued to support the lung schistosomula as the target for early paramyosin-mediated vaccine damage. Hernandez et al. [35] demonstrated the binding of IgA from humans with a 97 kDa protein from adult schistosomes. This 97 kDa band was confirmed to be paramyosin by screening a *S. japonicum* cDNA library with polyclonal antibodies to paramyosin and IgA antibodies. The production of IgA from mucosal tissues in response to paramyosin, may contribute to the elimination of the parasite while within the lungs of the host. The presence of paramyosin on the surface of schistosomula was further suggested by molecular analysis of *S. mansoni* by Harrop et al. [36]. Paramyosin was detected in a cDNA library of lung schistosomula probed with sera generated from schistosomula-released antigens. Other localisation work includes a study on *Fasciola hepatica* [37], which similar to those on schistosomes, noted heavy labelling in the muscle region and, to a lesser degree, in the parasite tegument, again suggesting non-muscular functions for the protein.

5. Immunological correlates with human schistosome infection in endemic areas

Correlation of immunological responses to schistosome infection in humans from endemic areas has provided a

long-term view on the schistosomiasis and the factors that can be attributed to susceptibility or resistance to infection. In addition, such studies can provide insights into the selection of appropriate antigens as vaccine targets and also for vaccine formulation prior to clinical testing in humans. Studies on endemic *S. mansoni* in north eastern Brazil [38] and Egypt [39] and *S. japonicum* in China [40] and the Philippines [41,42] tested for immunological (antibody/cytokine) correlates of apparent resistance and apparent susceptibility in relation to specific antigens, including paramyosin, in individuals of cohorts followed epidemiologically for extended periods of time. In general, immunological markers for both resistance and susceptibility to reinfection were identified for paramyosin, highlighting the need to limit specific antigen stimulation to the desired immune responses so as not to lead to increased susceptibility to infection or exacerbating pathology. The mechanism induced in resistant subjects resulted from the ability of paramyosin to induce a strong cell-mediated immune response, typified by elevated levels of interferon gamma [38,39,41]. In terms of human vaccine development such a paramyosin-induced response stimulating resistance to infection could be boosted with the use of an appropriate adjuvant.

6. Projections

The progression of research on paramyosin is providing new insights into the molecular and immunological characteristics of this protein in a range of parasitic worms, most notably the schistosomes. The development of a paramyosin-based vaccine, effective against *S. japonicum*, has broadened, focusing in China on livestock, since deposition of eggs by infected animals, notably water buffalo, accounts for the bulk of transmission to humans [43]. Development of such a “transmission-blocking” vaccine is an attractive practical approach that may additionally serve as a prelude to human vaccination trials [43]. Research investigating the functions of paramyosin in non-muscular regions has indicated that it is a multi-faceted modulator of the host immune system. The ability of paramyosin to bind complement, immunoglobulin and secreted components of the cellular immune response makes it an integral part of the potent defense armamentarium employed by parasites. Future research will no doubt extend into additional studies of paramyosin that will further explore the role(s) this multifunctional protein plays in the parasitic biology of these organisms.

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