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Unique antigenic gene expression at different developmental stages of *Trichinella pseudospiralis*

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ABSTRACT

Parasite-induced and parasite-regulated larval capsule formation and host immunosuppression are two major characteristics that are unique in *Trichinella* spp. infections, but the molecule(s) and mechanism(s) that mediate these processes remain largely unknown. *Trichinella pseudospiralis* and *Trichinella spiralis*, are obviously different with respect to these two characteristics. A comparative study of these two species, in particular their antigen expression profiles at different developmental stages (the main molecules involved in the cross-talk or interaction between each parasite and its host), may help us better understand the parasite molecules and mechanisms involved. Here, we constructed cDNA libraries from *T. pseudospiralis* adults (Ad), newborn larvae (NBL) and muscle larvae (ML) mRNA and screened them with pig anti-*T. pseudospiralis* serum collected 26, 32 and 60 days post-infection (p.i.). The most abundant antigens were found to vary among life-cycle stages. Pyroglutamy peptidase 1-like and 6-phosphogluconolactonase-like genes predominated in the Ad stage and a serine protease (SS2-1-like gene) predominated in NBL similar to that observed in *T. spiralis*. Muscle larvae expressed proteasome activator complex subunit 3-like and 21 kDa excretory/secretory protein-like genes. This study indicated that parasites of two species may utilise different molecules and mechanisms for larvae capsule formation and host immunosuppression during their infections. Proteins of antigenic genes identified in this study may be also good candidates for diagnosis, treatment or vaccination for *T. pseudospiralis* infection, and also for the differential diagnosis of two species' infections.

1. Introduction

Trichinella spp. parasites are intracellular nematodes capable of surviving in many vertebrate hosts, and complete their entire life cycles within a single host. *Trichinella* infections involve an intestinal phase and a muscle phase. Importantly, the host immune system is strongly suppressed during the initial 2 weeks of the intestinal phase; thereafter, this suppression is markedly decreased and adults begin to be expelled from the intestine, although suppression remains detectable until 56 days p.i. The parasite-induced host immunosuppression may explain why the clinical symptoms (inflammation) of human trichinellosis begin at approximately 2 weeks p.i. The long interval between infection and onset of symptoms is

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a major public health challenge because it is extremely difficult to trace a pathogen to a specific event after a long incubation period. Inasmuch as symptoms of human trichinellosis resemble symptoms of many other conditions, this further delays accurate diagnosis. Capsule formation is the hallmark of the muscle phase, wherein larval parasites establish intracellular residency. Taken together, host immunosuppression and larval capsule formation are uniquely important characteristics of *Trichinella* infections.

Trichinella spiralis is the most frequent cause of human trichinellosis, as this species establishes large and long-lived populations in swine. It forms a canonical capsule around each muscle larva. No such capsule is formed around the larvae of *Trichinella pseudospiralis*, the only species in the genus known to infect not only mammals but also birds of prey. The absence of a capsule hinders recognition of infections with *T. pseudospiralis*. These species also induce different immune and inflammatory reactions in their hosts. In both primates and rodents, *T. pseudospiralis* is less pathogenic than is *T. spiralis*, generating considerably less inflammation because of its strong host immunosuppressive ability during the infection.

The majority of trichinellosis studies have focused on *T. spiralis*, and several *T. spiralis* proteins have been identified (Zarlenga et al., 2002). Furthermore, a global search of antigenic genes expressed at different developmental stages of *T. spiralis* was previously conducted by our group (Wu et al., 2009). That study indicated that *T. spiralis* expressed completely different highly abundant antigenic genes at different developmental stages, including a serine protease exclusively expressed in the Ad stage, a serine protease expressed in the NBL stage, and a serine protease inhibitor in the ML stage. However, few studies have investigated *T. pseudospiralis*. Moreover, it has not been possible to distinguish these two types of infections using serological methods due to a lack of species-specific antigens. Because these two species have such distinct characteristics, a global comparison of the antigenic genes expressed at different developmental stages of these two species offers an especially informative approach towards understanding the molecule(s) and mechanism(s) involved in host immunosuppression and larval capsule formation. This study may also reveal candidates for differential diagnosis, treatment and/or vaccination of the disease.

2. Materials and methods

2.1. Parasites

T. pseudospiralis (ISS13) ML were isolated from infected mice at 35 days p.i. by artificial digestion with pepsin-HCl. Ad were isolated from infected mouse intestines at 3 days p.i., and NBL were isolated from female adults (Liu et al., 2007).

2.2. Construction and immunoscreening of cDNA libraries

cDNA libraries from Ad, NBL and ML of *T. pseudospiralis* were constructed as described (Liu et al., 2007). An SPF pig was experimentally infected *per os* with 20,000 ML of *T. pseudospiralis*. Serum was collected prior to infection as a negative control, and at 26, 32 and 60 days p.i. A total of 60,000 plaque forming units from each library were screened with the pig anti-*T. pseudospiralis* sera.

2.3. Sequence analysis

Selected clones were sequenced from both the 5′ and 3′ ends using the dideoxy chain-termination method on an automated DNA sequencer. The DNA sequences were analysed with DNASIS software and compared with the GenBank nucleotide and protein databases using the NCBI-BLAST network server (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Results and discussion

Previous works have indicated that different antigens are expressed at different developmental stages of *T. spiralis* (Wu et al., 2009); a similar pattern was observed in *T. pseudospiralis* in this study. The different parasitological and pathological characteristics of *T. pseudospiralis* and *T. spiralis* infections could be attributed to differences in the excretory–secretory (ES) products released by each species; even the genes shared by both species have significant diversity in their cDNA and amino acid sequences, and in the molecular masses and antigenicity of their proteins (Kuratli et al., 2001). In this study, with the exception of a common serine protease gene expressed in the NBL stage, all other highly abundant antigenic genes were different from those found in *T. spiralis* (Tables 1 and 2). This observation suggests that the mechanisms and molecules involved in infections with these two species, especially in host immunosuppression and larval capsule formation are appreciably different.

Pyroglutamyl-peptidase 1-like and 6-phosphogluconolactonase-like genes were identified as the major antigenic genes from the *T. pseudospiralis* Ad stage. Pyroglutamyl-peptidase 1 belongs to the C15 family of cysteine peptidases. Parasite-expressed peptidases are emerging as novel virulence factors and therapeutic targets in parasitic infections. A homologue of pyroglutamyl-peptidase 1 in *Trypanosoma brucei* was found to be insensitive to host plasma cysteine peptidase inhibitors and involved in the abnormal degradation of thyrotropin-releasing hormone and gonadotropin-releasing hormone. It is also thought that the N-terminal block of other neuropeptides with pyroglutamyl moieties may contribute to some of the endocrine lesions observed in African trypanosomiasis (Morty et al., 2006). The second enzyme in the pentose phosphate pathway is 6-phosphogluconolactonase which plays a key role in glycolysis. Interestingly, patients infected with *Trichinella* exhibit a temporary decrease in blood glucose levels from 8 to 28 days p.i. Moreover, hormone changes accompany immunosuppression, and decreased blood glucose can also cause an increase in insulin levels (Rantala et al., 2012). Thus, it may be hypothesised that the strong immunosuppression observed in the intestinal phase of

Table 1
Positive clones and antigenic genes from cDNA libraries of *T. pseudospiralis* Ad, NBL and ML.

Representative clone	Library (clones)	Sera days	Accession no.	Similar sequence	
				Protein	*E* value
Tp-AD3-60D-9.17-5	Ad (23)	60	JN173261	Pyroglutamyl-peptidase 1 [*T. spiralis*]	6e−17
	NBL (9)	60			6e−17
Tp-AD3-32D-1	Ad (3)	26	EF051033	6-Phosphogluconolactonase [*B. mori*]	0.37
	Ad (16)	32			0.37
	Ad (3)	60			0.37
	ML (2)	60			0.37
Tp-ML-60D-RW29	ML (11)	60	EF043393	PA28 complex subunit 3 [*T. spiralis*]	7e−115
Tp-NBL-26D-5	NBL (6)	26	JN166799	NBL SS2-1 [*T. spiralis*]	0.0
	NBL (4)	60			0.0
Tp-ML-60D-RW15	ML (8)	60	–	21 kDa protein [*T. pseudospiralis*]	1e−91
Tp-ML-60D-RW26	ML (3)	60	EF051032	28 kDa protein [*T. pseudospiralis*]	3e−123
Tp-AD3-26D-7	Ad (1)	26	JN166800	Antigen gene [*T. spiralis*]	2e−141
Tp-NBL-26D-10	NBL (1)	26	JN173264	Myosin head [*T. spiralis*]	5e−154
Tp-AD3-32D-28	Ad (1)	32	JN173260	Splicing factor 3B [*T. spiralis*]	2e−68
Tp-NBL-60D-W3	NBL (1)	60	JN173267	Tropomyosin [*T. spiralis*]	2e−77
Tp-ML-60D-RW19	ML (1)	60	EF051034	Uracil-DNA glycosylase [*T. spiralis*]	5e−146
Tp-ML-60D-RW23	ML (1)	60	EF051035	HEAT repeat domain [*T. spiralis*]	0.0

T. pseudospiralis infection is linked to hormonal changes induced by molecules from the parasite adult.

The same serine protease, SS-1, was identified as the most abundant antigenic protein in the NBL of *T. spiralis* and *T. pseudospiralis*. Serine protease activity has been reported in *T. spiralis* at different developmental stages (Todorova and Stoyanov, 2000). Parasite proteases have important functions in host-parasite interactions, such as enabling host tissue penetration, digesting host tissue for parasite nutrition and facilitating the evasion of the host immune response (Tort et al., 1999). Rees-Roberts et al. (2010) reported that a serine protease secreted by *Brugia malayi* could cleave complement C5a to inhibit granulocyte chemotaxis, resulting in the suppression of inflammation and facilitating the invasion of the parasite.

Proteasome activator PA28 complex subunit 3 was identified as the most abundant antigenic gene in the ML stage of *T. pseudospiralis*. PA28 is a cytokine-inducible proteasome regulator. The proteasome system plays a fundamental role in immune regulation through a variety of mechanisms, including the processing of intracellular antigens into peptides that bind to MHC class I molecules for presentation to CD8+ T cells, lymphocyte survival and the regulation of cytokine production and inflammation (Sijts and Kloetzel, 2011). The proteasome may also play a role beyond antigen processing and immune recognition in cellular adaptation to oxidative stress (Pickering et al., 2010). Both PA28 and the immuno-subunits of the proteasome can dramatically accelerate the generation of a subset of MHC class I-presented antigenic peptides, and the role of PA28 in the production of MHC class I ligands is also much more significant than expected (de Graaf et al., 2011). The relationship between PA28 and CD8+ T cells is indirect; PA28 of *T. pseudospiralis* most likely contributes to the moderate host immunosuppression in the muscle phase because the decreased CD4+/CD8+ ratio is a typical signal of host immunosuppression.

The results of this study also suggest that stronger host immunosuppression in the intestinal phase than it in the muscle phase may occur because immunosuppression is regulated by different molecules using different mechanisms in each stage. Thus, in *T. pseudospiralis* infection, strong immunosuppression may be induced by pyroglutamyl-peptidase 1,6-phosphogluconolactonase and a serine protease from Ad and NBL stages parasites in the intestinal phase, and moderate immunosuppression is induced by PA28 from ML in the muscle phase. The obvious decrease in suppression beginning at 2 weeks p.i. in intestinal phase may ensue from the reduced quantities of molecules from Ad and NBL following the expulsion of the Ad worms from the intestine. The same situation may also occur during *T. spiralis* infection in which the altered host

Table 2
Highly abundant antigenic genes expressed in *T. pseudospiralis* and *T. spiralis*.

Species	Representative clones	Protein homology	Clones distribution		
			AD	NBL	ML
T. pseudospiralis	Tp-ML-60D-RW45	6-Phosphogluconolactonase [*B. mori*]	22	0	2
	Tp-AD3-60D-9.17-5	Pyroglutamyl-peptidase 1 [*T. spiralis*]	23	9	0
	Tp-NBL-26D-5	NBL SS2-1 [*T. spiralis*]	0	10	0
	Tp-ML-60D-RW29	PA28 complex subunit 3 [*T. spiralis*]	0	0	11
	Tp-ML-60D-RW15	21 kDa protein [*T. pseudospiralis*]	0	0	8
	Tp-ML-60D-RW26	28 kDa protein [*T. pseudospiralis*]	0	0	3
T. spiralis	Ts Adsp-1-8	Serine protease family [*T. spiralis*]	16	0	0
	NBLWN18	NBL SS2-1 [*T. spiralis*]		70	0
	MLWM5	Serine proteinase inhibitor [*T. spiralis*]	0	0	10

immunosuppression in different phases is most likely regulated by the Ad and NBL stage-specific serine protease (strong suppression) in the intestinal phase and by the ML serine protease inhibitor (moderate suppression) in the muscle phase.

The alleviation and eventual resolution of the immunosuppression 2 weeks p.i. may be a unique strategy the parasites use to protect themselves. Strong host immunosuppression during the muscle phase (long term parasitism phase) may pose risks for parasites and hosts, alike, if immunosuppressed hosts are easily infected with other pathogens.

At present, immunological methods based on ES antigens of ML are not accepted for parasite inspection in slaughtered animals or for diagnosis of human trichinellosis, even though these methods are more sensitive (<0.01 larvae/g) than trichinelloscopic examination (3–5 larvae/g) and digestion methods (1–3 larvae/g). The main limitation to using ES antigens is the delay of 4–7 weeks for production of host antibodies against them (Kapel and Gamble, 2000), whereas *Trichinella* NBL can develop into infectious larvae at 17 days p.i. Antigens from Ad and NBL can be recognised by early infection serum; however, the difficulty in their preparation limits their application and in vitro reproducibility has not yet been satisfactorily achieved. Yet, the selection and expression of the antigenic genes from Ad and NBL may be the best way to resolve this problem. The antigenic genes identified in different developmental stages of *T. pseudospiralis* and *T. spiralis* cannot only explain why ES antigens from ML cannot be recognised by serum from early stages of infection, but may also provide candidate molecules for the early diagnosis of two species' infections.

The study results also suggest that the immunological methods used here are not suitable for the isolation of molecules involved in larval capsule formation. We did not find more antigenic genes that were differentially expressed and potentially involved in capsule formation between the two species at the NBL stage, when capsule formation begins, with the exception of a serine protease gene common to the two species and a unique pyroglutamyl-peptidase 1-like gene in *T. pseudospiralis*. It is possible that the molecules involved in capsule formation are expressed by the NBL inside the muscle cells but not exposed to the host immune system, resulting in a lack of antibody production and precluding the use of immunological methods to identify these molecules. It has also been proposed that the collagen capsule is derived predominantly from a host response to the reprogrammed muscle cell, rather from the parasite itself (Haehling et al., 1995). Differences in capsule formation between *T. spirals* and *T. pseudospiralis* may therefore reflect differences in antigenicity between parasites otherwise considered equivalent in their abilities to penetrate, reprogram and survive within the muscle cell.

4. Conclusion

The host immunosuppression and larval capsule formation induced by *T. spiralis* and *T. pseudospiralis* may involve fundamentally different mechanisms that are regulated by distinct sets of molecules. The antigenic genes identified in this study may also be good candidates for the diagnosis, treatment and vaccination for *T. pseudospiralis* infection, and as well as for the differential diagnosis of two species' infections.

Conflict of interest statement

The authors declare that they have no competing interests.

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