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Glycosylation changes in different developmental stages of *Trichinella*

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Abstract: The *in situ* identification of carbohydrate structures in *Trichinella spiralis* intestinal larvae, adults and L1 muscular larvae was carried out by lectin histochemistry, with emphasis on the O-linked glycans. The absence of reactivity with two lectins-TML and MAL indicated that *Trichinella spiralis* does not synthesize sialic acid. Reactivity with HPA, VVL-B4, PNA and UEA-I staining suggested that *T. spiralis* synthesizes and expresses on its cuticle O-linked glycans analogous to Tn-antigen (GalNAc-α-Ser/Thr), T-antigen (Gal-β1,3–GalNAc-α-Ser/Thr) and also structures analogous to A-blood group antigens (GalNAc-α1,3-Gal-β1,3(4)-(Fuc-α1,2-)-R). Expression of the saccharidic moieties is stage-specific. Blood group-A and T-antigen structures were identified on the cuticle of the intestinal and muscular larvae. The Tn-antigen structure was missing in the intestinal larvae. Appropriate ligands for WGA were not identified in the adult individuals. The obtained results may contribute to a better understanding of the glycobiology of this parasitic nematode in relation to occupation of its intracellular niche. The presence of saccharidic structures analogous to some of those expressed on the intestinal epithelial cells may serve as a protective shield on the surface of the parasite.

Key words: *Trichinella spiralis*; lectin histochemistry; T-antigen; Tn-antigen; O-glycans

Abbreviations: AAA – *Artocarpus altilis* agglutinin; ESP – excretory/secretory products; Fuc-fucose; Gal – galactose; GalNAc – N-acetylgalactosamine; GlcNAc – N-acetylglucosamine; HPA – *Helix pomatia* agglutinin; lacdiNAc – di-N-acetyllactosamine; MAL – *Maackia amurensis* lectin; Neu5Ac – neuraminic (sialic) acid; PBS – phosphate-buffered physiological salt solution; PNA – peanut agglutinin; SNA I – *Sambucus nigra* agglutinin; TML – *Tritrichomonas mobilensis* lectin; UEA – *Ulex europaeus* agglutinin; VVL-B4 – *Vicia villosa* lectin, B4 isotype; WGA – wheat-germ agglutinin.

Introduction

Trichinellosis results from an infection by a parasitic nematode belonging to the genus *Trichinella*. The species most frequently associated with human infection is *Trichinella spiralis* (Owen, 1835) the species that is normally found in domestic pigs (Bruschi & Murrel 2000). Virtually all mammals are susceptible to the infection that is acquired by the ingestion of animal muscle tissue containing infectious *T. spiralis* larvae (L1) (Bruschi & Murrel 2000). They initiate the infection by penetrating columnar epithelium of the host small intestine, where they molt to adulthood (stages L2 – L3 – L4 – adults), mate and reproduce. The females shed newborn larvae, which leave the intestine via the blood stream and lymph. The *T. spiralis* life cycle is completed when these immature L1 larvae invade the striated muscle cells of the host (Bruschi & Murrel 2000; Schantz & Dietz 2001). During the intestinal phase of infection, larvae and adult parasites establish an intramulticellular niche composed by serial rows of enterocytes (Despommier et al. 1978; Wright 1979). The tyvelose-bearing excretory/secretory products (ESP) of L1 larvae probably help the process of invasion (McVay et al. 1998). However, the molecules due to which *Trichinella* adhere to the enterocyte or the epithelial cell target are still unknown (Moncada et al. 2003).

The parasitic helminths are in a close contact with the host tissues and fluids. The fundamental mechanisms implicated in the regulation of the host-parasite relationship are accomplished within the contact zone that includes the integument and digestive system of the parasite and also the host tissues and fluids. All parasites have carbohydrates on their surfaces, as a part of their cytoskeleton or in their internal structures, and these molecules have been found responsible for many of the biological functions of organisms (Reuter et al.

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Table 1. Carbohydrate binding specificity of the lectins used in this study.

Lectin	Abbreviation	Carbohydrate binding specificity	References
Triticum vulgaris agglutinin	WGA	$(GlcNAc)_n$>Neu5Ac	Goldstein et al. (1975)
Arachis hypogea agglutinin	PNA	Galβ(1,3)GalNAc	Lotan et al. (1975)
Helix pomatia agglutinin	HPA	GalNAc-α-	Roth (1984)
Vicia villosa lectin-isoform B_4	VVL	GalNAc-α-	Tollefsen & Kornfeld (1983b)
Ulex europaeus agglutinin-I	UEA-I	Fucα(1,2)Gal	Sugii & Kabat (1982)
Maackia amurensis lectin-II	MAL	Neu5Acα(2,3)Galβ(1,4)GlcNAc	Knibbs et al. (1991)
Tritrichomonas mobilensis lectin	TML	Neu5Ac	Babál et al. (1994)

1988). Therefore, the role of the surface glycoconjugates in parasitism by protozoans and helminths is becoming a subject of rising interest (Dell et al. 1999; Harnett & Harnett 2001).

The aim of the present work was to probe and analyze *in situ* some of the terminally-positioned sugars, which are implicated in the oligosaccharide structures on the outer surface and also inside of the body of *T. spiralis* intestinal and muscle larvae and adult individuals by means of lectin histochemistry, with the emphasis on the O-linked glycans.

Material and methods

Sample preparations

Trichinella spiralis (ISS004) larvae were released by artificial digestion following the standard protocol according to Kapel & Gamble (2000) and kept in saline solution until inoculation of experimental mice.

Experimental inbred BALB/C, 6–8 weeks old male mice were inoculated with 500 infective *T. spiralis* larvae *per os* using a syringe fitted to a feeding needle. Mice were housed in plastic cages containing wood shavings and wooden sticks according to EU animal care standards and were given water and standard laboratory animal pellets *ad libitum*. The animals were divided into three groups. The intestinal stage was investigated 15 hours post inoculation (h.p.i.) and 6 days post inoculation (d.p.i.) – groups one and two, respectively; the muscular stage was investigated 40 d.p.i. – group three. Following the design of the experiment, euthanasia was performed by overdose of ether inhalation.

Small intestine excisions and muscle samples taken from hind legs and masseters were fixed in 4% formaldehyde in phosphate-buffered saline (PBS), pH 7.4 for 24 hours and longitudinal samples and cross section samples were processed in paraffin. After fixation, tissue samples were trimmed and embedded in paraffin and 5 μm thick sections were cut. In order to detect the nematodes series of sections per sample were stained with haematoxylin and eosin (H&E) and investigated in light microscopy.

Lectin histochemistry

The lectins used in this work and their specificities are shown in Table 1. Briefly, the work was performed using lectins that bind L-fucose (Fuc), sialic acid (Neu5Ac), N-acetyl-D-galactosamine (GalNAc), D-galactose (Gal) and N-acetyl-D-glucosamine (GluNAc).

Parallel sections of each specimen were histochemically stained with biotinylated lectins: TML (Calbiochem-Novabiochem, San Diego CA, USA), PNA, MAL-II and VVL-B_4 (Vector, Burlingame CA, USA), UEA-I, HPA, and WGA (Sigma-Aldrich). Their optimal concentrations were firstly tested on human colorectal carcinoma (from the archives of Department of Pathology, Faculty of Medicine, Comenius University in Bratislava) and then on mice small intestine of healthy control animals. Lectin histochemistry was performed as previously described (Babál & Gardner 1996). Briefly, deparaffinized sections were rehydrated in phosphate-buffered physiological salt solution (PBS) pH 7.4. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in PBS (pH 7.4) and endogenous biotin was blocked with Ultra V Block (Lab Vision Corporation) 10 min incubation. The sections were then incubated 60 min with the following biotin-conjugated lectins: WGA (2.5 μg ml^{-1}), UEA-I (50 μg ml^{-1}), PNA (50 μg ml^{-1}), HPA (20 μg ml^{-1}), VVL-B4 (3.3 μg ml^{-1}), MAL-II (2.5 μg ml^{-1}) and TML (6.6 μg ml^{-1}) diluted in PBS. In order to avoid possible nonspecific binding activity, some of the experiments were performed with lectins that had been preincubated with 0.1 M solution of the target monosaccharide for 30 min at room temperature: N-acetylneuraminic acid (Sigma-Aldrich) for TML, MAL and WGA, α-D-galactose-1-phosphate, dipotassium salt and D-galactose (Sigma-Aldrich) for HPA, VVL-B4 and PNA, N-acetyl-D-galactosamine (Sigma-Aldrich) for HPA and VVL-B4 and L-Fuc (Sigma-Aldrich) for UEA-I. Then, all of the sections were incubated 20 min with streptavidin-horse-radish peroxidase complex (Sigma-Aldrich). Peroxidase activity was developed with 3,3'-diaminobenzidine (Sigma-Aldrich) and the samples were counterstained with haematoxylin and mounted in acrylic resin. The slides were washed three times in PBS for 5 min between all steps.

The staining intensity of the nematode integument and inside the body was evaluated semi-quantitatively by two independent observers into four arbitrary categories: no labeling, weak, moderate and strong.

Results

Thirteen h.p.i. the second molt of *T. spiralis* was documented in the small intestine so that our 15 hours old larvae were expected to be in their L3-L4 stage. The adult stage occurs at least 26 h.p.i. (Kozek 1971).

The staining was evaluated mainly toward the cuticle of the muscular larvae (L1 larvae) and also about the integument on the intestinal larvae and adults as well as the cover of embryos, where they were found, during the intestinal infestation. Concerning the intestinal larvae and the adults, the term "integument" seemed to be more appropriate because in many cases it was quite difficult to demarcate the cuticle from the underlying hypodermis. Apart from the external body surface, we noted also the staining on the luminal surfaces of the alimentary canal, the excretory canaliculi and the hypodermal glands of the intestinal forms, and the scattered

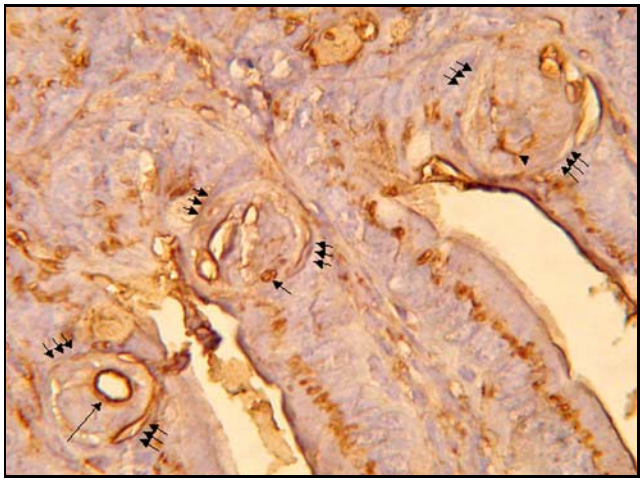

Fig. 1. Lectin histochemical staining for WGA of 15 hours old *Trichinella spiralis* in the mouse intestine; the triple arrows indicate one cross-sectioned female specimen. WGA stained the alimentary channel (long arrow), the hypodermal glands (short arrow) and the ovary (arrow block). WGA, Avidin-biotine-peroxidase complex (ABC-Px), diaminobenzidine (DAB). Magnification ×630.

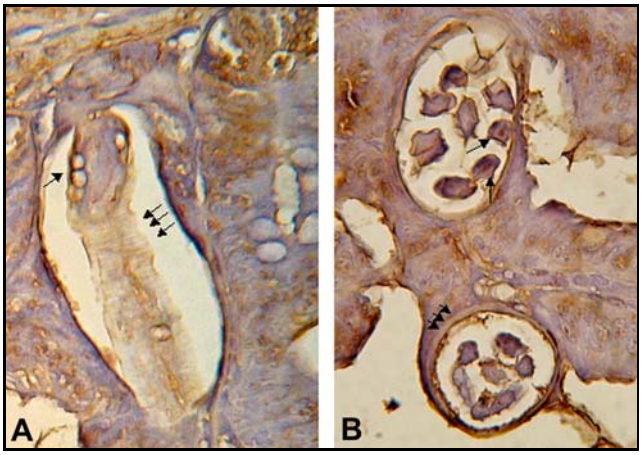

Fig. 2. Lectin histochemical staining for UEA-I of 15 hours old (A) and adult female *Trichinella spiralis* (B) in the mouse intestine. A – UEA-I stained the cuticle (triple arrow), the hypodermal glands looked empty (arrow); B – the cuticle of the adult specimen is negative for UEA-I (triple arrow), the cross-sectioned embryos showed some positive reaction, probably due to the stichosome (arrows). UEA-I, ABC-Px, DAB. Magnification ×630.

positive staining within the stichocytes in both forms, intestinal and muscular.

Sialic acid (Neu5Ac)

Evaluation of nematodes from all three age groups showed negative staining results with TML and MAL (data not shown). WGA reactivity was limited at the adult stage. This lectin stained intensely the lumen of the alimentary tract and the excretory canaliculi of the intestinal 15 hours old larvae and the L1 muscular larvae mainly, the stichocytes showed weak positivity. The body cover of the embryos was labeled moderately. Within the ovaries of the intestinal larvae and adult forms, the oocytes had a cover that reacted with WGA (Fig. 1). The staining intensity did not decrease significantly after WGA pre-incubation with Neu5Ac.

Fucose (Fuc)

UEA-I showed moderate labeling on the cuticle of 15-hours-old specimens (Fig. 2A). Compared to the intestinal larvae, the adult forms have lost the surface binding but moderate UEA-I staining was presented in the hypodermis. The embryos cover was also negative, however, in most of the cross-sectioned embryonal bodies we found moderate UEA-I staining that was scattered inside of the body, probably within the stichosome (Fig. 2B).

Concerning L1-larvae, there was weak thin outline on the cuticle. The stichocytes and also the luminal surface of the excretory canaliculi were strongly positive with UEA-I.

Galactose (Gal)

In most of the intestinal larvae the cuticle was well demarcated from the hypodermis, both stained moderately to strongly with PNA. Strong labeling with this lectin also showed the hypodermal glands (Fig. 3A).

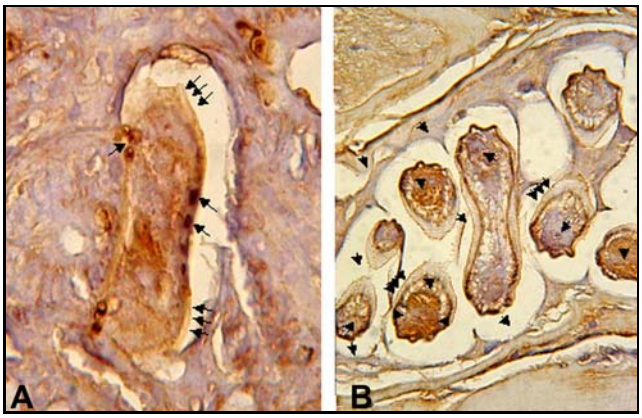

Fig. 3. Lectin histochemical staning for PNA of 15 hours old *Trichinella spiralis* in the mouse intestine (A) and L1 *Trichinella spiralis* muscular larva (B). A – PNA stained the cuticle (triple arrows) and the hypodermal glands (single arrows); B – PNA stained the cuticle (triple arrows), stichosome (arrow blocks) and excretory canaliculi (single arrows). PNA, ABC-Px, DAB. Magnification ×630.

Pre-incubation of PNA with galactose removed the staining of all of the indicated areas completely. The same procedure with α-D-galactose-1-phosphate significantly reduced the staining intensity.

The integument of the adult worms was stained intensely, as well as the embryos cover and the eggshell. Some specimens showed moderate PNA labeling scattered in the stichosome (data not shown). The stichosome of L1 larvae was strongly positive for PNA and the lumen of the excretory canaliculi, too. On the cuticle there was a contour of strong PNA positive staining (Fig. 3B). Pre-incubation of PNA with galactose completely removed the staining of all of the indicated areas, while the same procedure with α-D-galactose-1-phosphate did not affect the reaction intensity.

N-acetylgalactosamine (GalNAc)
In order to demonstrate the presence of this sugar we used two lectins: HPA and VVL-B4, both of which showed similar pattern of perinuclear granular positivity corresponding to the Golgi apparatus in the mouse intestine epithelial cells. We used this labeling as a reference point, which completely disappeared after GalNAc pre-incubation with the lectins. Pre-incubation of HPA with α-D-galactose-1-phosphate or galactose reduced moderately the perinuclear staining of epithelial cells. As for VVL-B4, after addition of galactose, only a weak staining was presented within the area of Golgi but treating with α-D-galactose-1-phosphate or GalNAc completely inhibited it.

Although in most intestinal specimens (larvae and adults) it was difficult to distinguish the cuticle and the underlying hypodermis, we found that HPA stained strongly the integument, the hypodermal glands and some of the luminal surfaces, probably alimentary, of the intestinal larvae. In some specimens there was a thin, moderately HPA-positive layer on the cuticle. The outer cover of the embryos was also strongly positive. HPA strongly reacted with the cuticle and the stichosome of the L1-larvae. After pre-incubation of HPA with galactose or α-D-galactose-1-phosphate, the thin line on the outer cuticle surface and the staining inside of the body and the glands were not affected (Fig. 4A). HPA treating with GalNAc resulted in complete removal of the labeling. Pre-incubation of HPA with galactose removed the integument staining of the adults but treatment of this lectin with α-D-galactose-1-phosphate and GalNAc did not affect it substantially.

VVL-B4 stained weakly the cuticle, hypodermis and the hypodermal glands of the intestinal larvae. There was a strong scattered positivity within some of the specimens. Pre-incubation of VVL-B4 with GalNAc virtually did not change the intensity of the positive staining of the indicated areas with the exception for the scattered positivity that was reduced to weak staining. VVL-B4, pre-incubation with α-D-galactose-1-phosphate or galactose resulted in almost complete abolishment of the staining of the intestinal larvae.

The cuticle and the hypodermal glands of the adult worms, as well as the cover of the embryos, were stained moderately to strongly. On L1-larvae, the VVL-B4 positive staining was presented as a thin vivid line on the cuticle surface and weak scattered veil within the stichosome, but the capsule padding was negative (Fig. 4B). When observed, the hypodermal glands were strongly stained. Preincubation of VVL-B4 with GalNAc, α-D-galactose-1-phosphate or galactose resulted in complete removal of the staining.

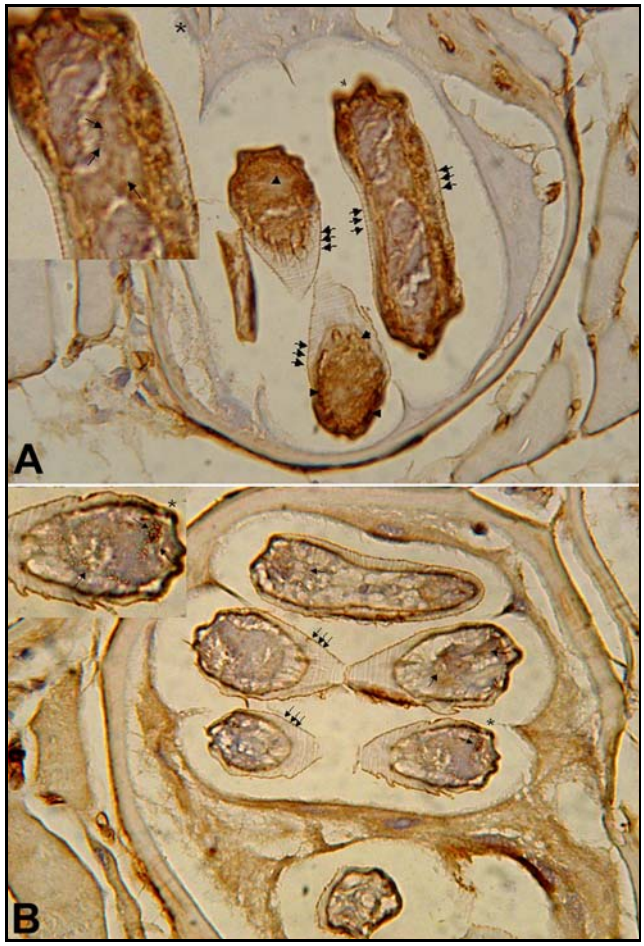

Fig. 4. Lectin histochemical staining for HPA (A) and VVL-B4 (B) of L1 *Trichinella spiralis* muscular larva. A – HPA stained intensely the cuticle and the underlying hypodermis (triple arrows) and the stichosome (arrow block). The enlarged area (star) shows the excretory canaliculi (single arrows) positive for HPA; B – the cuticle (triple arrows) and the excretory canaliculi (single arrows) in the enlarged area (star) were positive for VVL-B4. HPA/VVL-B4, ABC-Px, DAB. Magnification ×630.

Discussion

Lectins are proteins or glycoproteins that specifically recognize and label sugar moieties or certain glycosidic linkages. They have been confirmed as an excellent tool for characterization of carbohydrates that form oligosaccharide component of glycoconjugates, because of their ability to discriminate a variety of complex glycan structures that are found on the cellular surface, in the extracellular matrix and attached to soluble glycoproteins (Spicer & Schulte1992).

Like many other nematodes, *Trichinella* synthesizes a great variety of glycans exposed on its cuticle. Those, whose structure had been elucidated are N-linked and the preferred antenna building block is GalNAc-β-1,4-GlcNAc or "lacdiNac" antennae (Reason et al. 1994; Morelle et al. 2000). The existence of O-linked structures is also expected since the enzymatically cleaved N-glycans were found to react only with a part of tested protective antibodies (Ellis et al. 1994). The present work used a set of lectins in combination with several target monosaccharides for characterization of the carbohydrates that form the oligosaccharide chains of *Trichinella* glycans, mainly on its cuticle.

All *Trichinella spiralis* developmental stages investigated in this study were found to be negative for both sialic acid specific lectins TML and MAL. However,

WGA is also known to possess affinity towards sialic acid residues, although this is much weaker than the affinity to GlcNAc (Gallagher et al. 1985). The partial decrease of staining, with WGA after Neu5Ac pretreatment may result from the sterical conformation changes and does not indicate the existence of sialic acid in *Trichinella*. This is supported also by the absence of TML reactivity in the parasite. Actually, no data on sialic acids presence in parasitic nematodes were found in the available literature. The absence of sialic acids has been reported in the nematodes *Panagrellus redivivus* (L., 1767) and *Caenorhabditis elegans* (Maupas, 1900) (Bacic et al. 1990; Reuter et al. 1991). The analysis of N-linked glycans of adult *T. spiralis* also failed to demonstrate the presence of acidic saccharides (Morelle et al. 2000). The lack of reactivity with TML and MAL demonstrated in this work is supported by the observation described by Gruden-Movsesijan et al. (2002) on blotted ESP from muscular larvae with the absence of SNA I (*Sambucus nigra* agglutinin) staining. This allows us to conclude that *T. spiralis* does not synthesize sialic acids.

Although in this study we used WGA in context of its ability to recognize also sialic acid residues, the monosaccharide that WGA binds with high affinity is GlcNAc. This lectin was shown to be strongly inhibited by short sequences of GlcNAc units, however, GlcNAc itself is a poor inhibitor (Goldstein et al. 1975). WGA also possesses high affinity to polylactosamine glycans, where it has been clearly shown that it interacts with the internal GlcNAc residues (Gallaghar et al. 1985). On mammalian cell surfaces, GlcNAc residues have been found in continuous sequences only in N-linked oligosaccharides, where they form the core that is attached to the asparagine. The binding of such components is weak in the absence of the bisecting GlcNAc residue that is present in the hybrid type N-bound oligosaccharides (Yamamoto et al. 1981). GlcNAc is highly represented in *T. spiralis* N-glycans, where it is either in terminal position or it is implicated in the so called "lacdiNAC" (GalNAc-β1,4-GlcNAc) antennae. This structure is restricted in mammalian glycoproteins but in many helminths, including *Trichinella*, it is the preferred building block (Morelle et al. 2000; Appleton et al. 2001). We observed limited reactivity of the adult worms with WGA. Indeed, bisecting GlcNAc is unlikely to exist in N-linked oligosaccharides, nor the polylactosamine sequences (Morelle et al. 2000). Our results showed that the larval stages (intestinal and muscular) and the embryos express the appropriate ligand for this lectin due to their reaction with WGA. L1 muscular larvae synthesize several ES glycoproteins that bind WGA but it is not likely they to possess "lacdiNAc" structure (Gruden-Movsesijan et al. 2002).

The lectins used in our study that are assumed to recognize structures common for the O-linked glycans were HPA, VVL-B4, PNA and UEA-I.

In the nematodes, the changed pattern of labeling with VVL-B4, HPA and PNA after pre-incubation with the galactosides indicated that the intestinal larvae express α-D-galactose-1-phosphate and also Gal-β1,3-GalNAc structure (T-antigen) on their cuticle. GalNAc inhibited the reaction with HPA but did not affect the weak superficial staining with VVL-B4. On the other hand, the UEA-I labeling demonstrated that the cuticle of 15-hours-old larvae possesses α1,2-fucosyl residues. Unlike most other lectins, HPA has a sugar combining site, the size of which may not be larger than that of one α-linked GalNAc residue (Hammarström & Kabat 1969). Furthermore, this lectin interacts not only with the single such sugar residue linked to protein, but also with GalNAc residues located at the non-reducing terminus of O-glycosidically linked oligosaccharides. The internal GalNAc residues are not reactive with the *Helix pomatia* (L., 1758) agglutinin (Roth, 1984). This part of our results may lead to the assumption that the terminal GalNAc residue expressed on the cuticle of the intestinal *T. spiralis* larvae, which reacts with HPA only, belongs to structures analogous to A-blood group and not to Tn-antigens. The VVL-B4 does not bind GalNAc residues of the glycans probably due to the steric hindrance of the fucosyl residue. Indeed, it has been reported that B4-isoform of VVL failed to agglutinate A-blood group erythrocytes but was clearly shown to bind GalNAc-α-Ser/Thr (Tn-structure) (Tollefsen & Kornfeld 1983a, b; Puri et al. 1992).

UEA-I labeling was missing on the cuticle of the adult forms. The lack of fucose at the outer position in the structure of N-bound glycans of adult *T. spiralis* crude extract was reported several years ago (Morelle et al. 2000) and the authors assumed that most of the complex structures were core fucosylated. UEA-I is known to recognize α1,2-bound fucose, a sugar residue situated closely to the outer terminus of the oligosaccharide (Watkins 1980; Sugii & Kabat 1982). Since there was UEA-I labeling of the hypodermis of the adult forms it is tempting to speculate that they synthesize O-bound glycans containing α1,2-Fuc but do not express oligosaccharides similar to A-blood group on their cuticles. Our results showed that *Trichinella* probably exposes on its cuticle both Tn- and T-antigens that were demonstrated by the interaction with VVL-B4 and PNA.

The cover of *T. spiralis* embryos was shown to express carbohydrates similar to T-antigen due to the interaction with PNA and also structures analogous to Tn-antigen because of the binding of HPA and VVL-B4. The lack of UEA-I staining on the cuticle showed that during the embryonic stage A-blood group antigens are not displayed on the surface. However, such structures are synthesized in the stichosome. Unlike to the adult individuals, HPA pre-incubation with galactose did not affect the staining on the embryos.

Concerning the *T. spiralis* L1 muscular larvae, the results showed that during this stage carbohydrates similar to A-blood group antigens, Tn- and T-antigens illustrated by UEA-I, HPA, VVL-B4 and PNA labeling, respectively, are present on the cuticle. These data for the first time provide histochemical proof of O-bound glycans existence in *T. spiralis*. The presence of termi-

nal α-Gal residues and also T-antigen structure have been indicated by probing of blotted *T. spiralis* muscle larvae excretory/secretory products (ESP) with *Artocarpus altilis* ((Parkinson) Fosberg, 1941) agglutinin (AAA) (Gruden-Movsesijan et al. 2002).

This study was designed to describe some sugar moieties of the glycans in different stages of *T. spiralis* life cycle. The obtained results may contribute to a better understanding of the glycobiology of this parasitic nematode in relation to occupation of its intracellular niche. In summary, *T. spiralis* does not synthesize sialic acid, which was illustrated by the absence of reactivity with two lectins, TML and MAL. The intestinal larvae expose α-D-galactose-1-phosphate on their surface since their HPA and PNA labeling was inhibited by this target sugar. *T. spiralis* synthesizes and expresses on its cuticle O-linked glycans analogous to Tn-antigen (GalNAc-α-Ser/Thr), T-antigen (Gal-β1,3-GalNAc-α-Ser/Thr) and also structures similar to A-blood group antigens (GalNAc-α1,3-Gal-β1,3(4)-(Fuc-α1,2-)-R). A-blood group antigens are stage-specific since they were presented on the cuticle of intestinal and muscular larvae, but they were missing on the surface of the adults and embryos. Tn-antigen-identical structure is also stage-specific since it was absent in the intestinal larvae. Apart from the adult individuals, the other three stages synthesize appropriate ligands for WGA. The L1 muscular larvae showed the richest variety of carbohydrates, including the O-linked glycans, whose more precise identification and their significance for sustaining of the intracellular environment would be a subject of our future work.

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References

Appleton J., Dell A., Nitz M. & Bundle D. 2001. Novel N-glycans of the parasitic nematode *Trichinella spiralis*. Trends Glycosci. Glycotech. **13:** 481–492.

Babál P. & Gardner W.A. 1996. Histochemical localization of sialylated glycoconjugates with *Tritrichomonas mobiensis* lectin (TML). Histol. Histopathol. **11:** 621–631.

Babál P., Pindak F.F, Wells D.J. & Gardner W.A. 1994. Purification and characterization of a sialic acid-specific lectin from *Tritrichomonas mobilensis*. Biochem. J. **299:** 341–346.

Bacic A., Kahane I. & Zuckerman B.M. 1990. *Panagrellus redivivus* and *Caenorhabditis elegans*: evidence for the absence of sialic acids. Exp. Parasitol. **71:** 483–488.

Bruschi F. & Murrel K.D. 2000. Trichinellosis, pp. 917–925. In: Guerrant R.L., Walker D.H. & Weller P.F. (eds), Tropical Infectious Diseases: Principles, Pathogens and Practice, **2** (85), Churchill-Livingstone, Philadelphia PA.

Dell A., Haslam S.M., Morris H.R. & Khoo K.H. 1999. Immunogenic glycoconjugates implicated is parasitic nematode diseases. Biochim. Biophys. A. **1455:** 353–362.

Despommier D.D., Sukhdeo M. & Meerovitch E. 1978. *Trichinella spiralis*: site selection by the larva during the enteral phase of infection in mice. Exp. Parasitol. **44:** 209–215.

Ellis L.A., Reason A.J., Morris H.R., Dell A., Iglesias R., Ubeira F.M. & Appleton J. 1994. Glycans as targets for monoclonal antibodies that protect rats against *Trichinella spiralis*. Glycobiology **4:** 585–592. DOI 10.1093/glycob/4.5.585

Gallagher J.T., Morris A. & Dexter T.M. 1985. Identification of two binding sites for wheat-germ agglutinin on polylactosamine-type oligosaccharides. Biochem. J. **231:** 115–122.

Goldstein I.J., Hammarström S. & Sunblad G. 1975. Precipitation and carbohydrate-binding specificity studies on wheat germ agglutinin. Biochim. Biophys. Acta **405:** 53–61.

Gruden-Movsesijan A., Ilic N. & Sofronic-Miloaljevic L. 2002. Lectin-blot analyses of *Trichinella spiralis* muscle larvae excretory-secretory components. Parasitol. Res. **88:** 1004–1007.

Hammarström S. & Kabat E.A. 1969. Purification and characterization of a blood group A reactive hemagglutinin from the snail *Helix pomaia* and a study of its combining site. Biochemistry **8:** 2696–2705.

Harnett W. & Harnett M.M. 2001. Modulation of the host immune system by phosphorilcholine-containing glycoproteins secreted by parasitic filarial nematodes. Biochim. Biophys. Acta **1539:** 7–15. DOI 10.1016/S0167-4889(01)00101-X

Kapel C.M.O. & Gamble H.R. 2000. Infectivity, persistence, and antibody response to domestic and sylvatic *Trichinella* spp. in experimentally infected pigs. Int. J. Parasitol. **30:** 215–221. DOI 10.1016/S0020-7519(99)00202-7

Knibbs R.N., Goldstein I.J., Ratclife R.M. & Shibuya N. 1991. Characterization of the carbohydrate binding specificity of the leukoagglutinin lectin from *Maackia amurensis*. Comparison with the other sialic acid-specific lectins. J. Biol. Chem. **266:** 83–88.

Kozek W.J. 1971. The molting pattern in *Trichinella spiralis*. A light microscope study. J. Parasitol. **57:** 1015–1028.

Lotan R., Skutelsky E., Danon D. & Saron N. 1975. The purification, composition and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). J. Biol. Chem. **250:** 18–23.

McVay C.S., Tsung A. & Appleton J.A. 1998. Participation of parasite surface glycoproteins in antibody-mediated protection of epithelial cells against *Trichinella spiralis*. Infect. Immun. **66:** 1941–1945.

Moncada D.M., Kammanadiminti S.J. & Chadee K. 2003. Mucin and Toll-like receptors in host defense against intestinal parasites. Trends Parasitol. **19:** 305–311. DOI 10.1016/S1471-4922(03)00122-3

Morelle W., Haslam S.M., Morris H.R. & Dell A. 2000. Characterization of the N-linked glycans of adult *Trichinella spiralis*. Mol. Biochem. Parasitol. **109:** 171–177. DOI 10.1016/S0166-6851(00)00241-3

Puri K.D., Gopalarkrishnan B. & Surolia A. 1992. Carbohydrate binding specificity of the Tn-antigen binding lectin from *Vicia villosa* seed (VVL-B4). FEBS Lett. **312:** 208–212.

Reason A.J., Ellis L.A., Appleton J.A., Wisnewski N., Grieve R.B., McNeil M., Wassom D.L., Morris H.R. & Dell A. 1994. Novel tyvelose-containing tri- and tetra-antennary N-glycans in the immunodominant antigens of the intracellular parasite *Trichinella spiralis*. Glycobiology **4:** 593–603. DOI 10.1093/glycob/4.5.593

Reuter G., Aumann J., Wyss U., Jansson H.B. & Schauer R. 1991. *Panagrellus redivivus*: failure to find evidence for the occurrence and biosynthesis of sialic acids. Exp. Parasitol. **73:** 389–395.

Reuter G., Kelm S. & Schauer R. 1988. Chemistry and biology of cell surface glycoconjugates. Acta Histochem. Suppl. **36:** 51–79. Roth J. 1984. Cytochemical localization of terminal N-acetyl-G-galactosamine residues in cellular compartments of intestinal goblet cells: implications for the topology of O-glycosylation. J. Cell. Biol. **98:** 399–406.

Schantz P.M. & Dietz V. 2001. Trichinellosis, pp. 521–533. In: Gillespie S. & Pearson R.D (eds), Principles and Practice of Clinical Parasitology, 19b, John Wiley & Sons Ltd., Sussex.

Spicer S.S. & Schulte B.A. 1992. Diversity of cell glycoconjugates shown histochemically: a perspective. J. Histochem. Cytochem. **40:** 1–38.

Sugii S. & Kabat E.A. 1982. Further immunochemical studies on the combining sites of *Lotus tetragolbulus* and *Ulex europaeus I* and *II* lectins. Carbohydr. Res. **99:** 99–101.

Tollefsen S.E. & Kornfeld R. 1983a. Isolation and characterization of lectins from *Vicia villosa*. J. Biol. Chem. **258:** 5165–5171.

Tollefsen S.E. & Kornfeld R. 1983b. The B4 lectin from *Vicia villosa* seeds interacts with N-Acetylgalactosamine residues α-linked to serine or threonine residues in cell surface glycoproteins. J. Biol. Chem. **258:** 5172–5176.

Watkins W.M. 1980. Biochemistry and genetics of the ABO, Lewis and P blood group systems. Adv. Hum. Genet. **10:** 1–136.

Wright K.A. 1979. *Trichinella spiralis*: an intracellular parasite in the intestinal phase. J. Parasitol. **65:** 441–445.

Yamamoto K., Tsuji T., Matsumoto I. & Osawa T. 1981. Structural requirement for the binding of oligosaccharides and glycopeptides to immobilized wheat germ agglutinin. Biochemistry **20:** 5894–5899.

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