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The Muscle Phase of Trichinellosis in Mice is Associated with Increased ST6GalNAc-1 Sialyltransferase Activity in Skeletal Muscle Fibers

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We previously showed that the de-differentiation of the occupied portion of muscle fibers toward Nurse cell after invasion by *Trichinella spiralis* is associated with increased intracellular accumulation of α-2,6-sialylated glycoproteins and novel gene activation of ST6GalNAc1. With this work we demonstrate ST6GalNAc1 expression in mouse skeletal muscles invaded by *T. spiralis*. Muscle samples were collected at certain time points after invasion. Immunochistochemistry was performed using rabbit polyclonal antibody against ST6GalNAc1 sialyltransferase. We found short up-regulation of the enzyme ST6GalNac1 that faded within the transformation of the occupied area into a Nurse cell. The enzyme ST6GalNAc1 is not synthesized in healthy mouse muscle tissue and is rarely expressed in normal tissues. It is responsible for the formation of the cancer-associated sialyl-Tn antigen in variety of carcinomas, blocking regular carbohydrate chain elongation. The functional role of this enzyme for the Nurse cell formation of T. spiralis in muscles has to be elucidated.

Key words: sialic acids, skeletal muscle *Trichinella*

Introduction

Sialic acids are called over than 40 modifications of the Neuraminic acid that occupy terminal position on the oligosaccharide chains of glycoproteins and glycolipids, and are thus involved in almost all types of recognition phenomena and adhesion mechanisms [10].

In skeletal muscles the sialic acids are important for the functional maintenance of glycoproteins involved in fiber structure and neuromuscular junctions [3, 7], for the development and regeneration [1], muscle excitability [6] and exercise performance [5]. However, even if sialylation in skeletal muscles is not as abundant as in other tissues, the muscles are very sensitive to losses of sialic acid due to mutations, which result in severe and progressive loss of motility [2, 11].

Among all known myopathies, the establishment of a Nurse cell-parasite complex resulting from infestation by the parasitic nematode *Trichinella* is a unique event.

This structure derives from a portion of the striated skeletal muscle fiber and develops within 15 to 20 days after a larva of *Trichinella* invades the fiber. After penetrating the skeletal muscle fiber, the larva induces morphological, functional and enzymatic changes. The occupied portion of the muscle fiber transforms into a structure called Nurse cell, capable of supporting the parasite for years [4]. During this process of de-differentiation, at least 53 genes associated with apoptosis, satellite cell activation and proliferation, cell differentiation, cell proliferation and cycle regulation, myogenesis and muscle development change in expression [13]. The affected areas lose their contractile properties but the membranes of the newly developing Nurse cells remain adherent within the construction of the contractile fiber. Considering the role of the sialic acids in cell adhesion we assumed that this process of transformation is associated with changes in sialylation of the occupied skeletal muscle fiber.

Our previous studies showed that the occupation of skeletal muscle fibers by *Trichinella spiralis* is associated with intracellular accumulation of α-2,3- and α-2,6-sialylated glycoconjugates, as well as elevated levels of free sialic acid, sialylated glycoproteins and total sialyltransfrase activity [8]. Further, up-regulation of sialyltransferase ST6GalNAc2 and a novel gene activation of sialyltransferase ST6GalNAc1 were found, too [9].

With this short report, by means of immunohistochemistry, we demonstrate a transient protein expression of the enzyme ST6GalNAc1 (ST6 [alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3]-N-acetylgalactosaminide alpha-2,6-sialyltransfrase 1, Mouse Genome Informatics Database) in mouse skeletal muscles, invaded by infectious larvae of the parasitic nematode *T. spiralis*.

Material and Methods

Ethical procedures

All animal experiments were performed in compliance with the Institutional Guidelines for Animal Experiments of the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, following the EU and locally established norms and procedures.

Parasites, invasion, sample collection and tissue preparation for basic pathomorphology

Infective *T. spiralis* larvae were isolated from previously invaded mice, between days 30 and 40 day post infection (d.p.i.) according to a routine protocol. ICR mice, 6-8 weeks old, were inoculated with 500 infective *T. spiralis* larvae *per os*. The animals (five per group) were sacrificed at day 0, 10, 14, 18, 24 and 35 post invasion (d.p.i.). Tissue specimens were excised from the femoral, pectoral and gluteal muscles and fixed with 10% neutral buffered formaldehyde. After processing, the specimens were embedded in paraffin. Tissue sections, 5 μm thick, from all experimental groups were submitted for staining with hematoxylin and eosin (H&E) for basic morphological evaluation and immunohistochemistry.

Immunohistochemistry

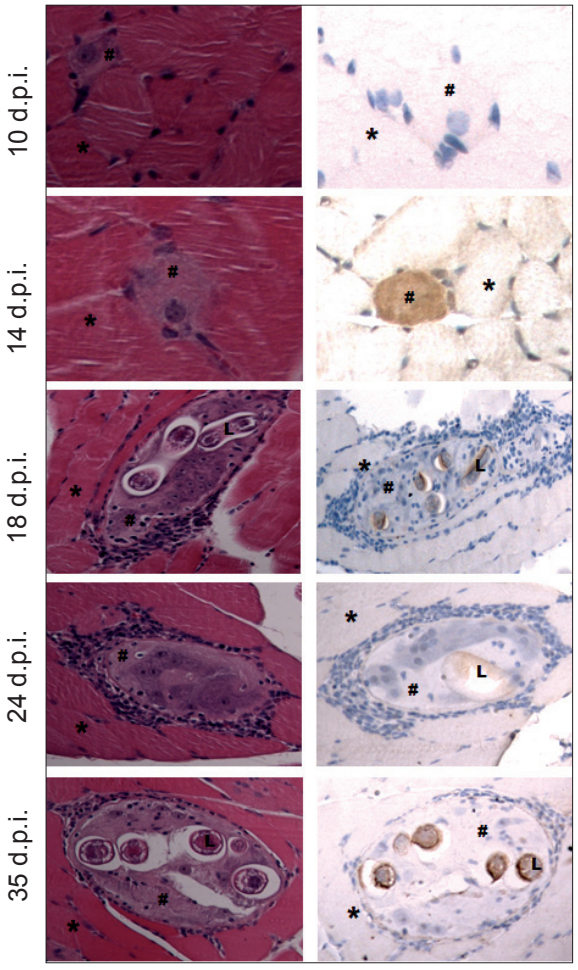
Selected tissue sections from all experimental groups were heated for 5 min in 10 mM Tris-1.25mM EDTA revitalizing buffer at pH 9.2. The endogenous peroxidase activity was blocked by 0.3% solution of H2O2, then 2.5% normal goat serum (Vector Laboratories Ltd, Burlingame, CA, USA) was used to prevent non-specific antigen activity. Polyclonal antibody against ST6GalNAc1 (Aviva Systems Biology Cor., San Diego, CA, USA) was applied overnight at 4°C in dilution 1:50. The sections were then treated with a secondary antibody (ImmPress HRP anti-rabbit IgG polymer detection kit, Vector Laboratories) for 30 min, a color reaction was developed with DAB Peroxidase

(HRP) Substrate Kit (Vector Laboratories) and the sections were counterstained with hematoxylin. The immunohistochemical staining was evaluated as negative (-) and positive (+).

Results and Discussion

The results are shown in **Figure 1**.

The occupied sites in skeletal muscle specimens from day 10 p.i. were distinguished by the enlargement and centralisation of the fiber nuclei. Following the time course of muscle invasion, the affected area of the sarcoplasm disintegrated progressively and by the day 35 p.i. the de-differentiation of the occupied fiber resulted in a Nurse cell completion. During this period the affected sarcoplasm changed from eosinophilic to basophilic and then back to light eosinophilic. The enlarged nuclei persisted also in the mature Nurse cell containing coiled larvae. Between days 18 and 24 p.i. the developing Nurse cells were surrounded by cells of inflammatory response. Protein expression of the sialyltransferase ST6GalNAc1 was observed within the invaded sarcoplasm only at day 14 p.i.



The enzyme ST6GalNAc1 catalyzes the transfer of a sialic acid in an alpha-2,6 linkage to O-linked N-acetylgalactosamine (GalNAc) residues and is responsible for the formation of the cancer-associated sialyl-Tn antigen (SiA-α-2,6-GalNAc-α-1-Ser/Thr) [12]. According to the Mouse Genome Informatics database and our molecular studies [9] expression of this enzyme is absent in healthy skeletal muscles. It is typical however for the alimentary system and its expression is considered as a prognostic marker for many cancer diseases characterized by increased levels of the sialyl-Tn antigen [12]. The biological function of the short expression of ST6GalNAc1in the developing Nurse cell of *T. spiralis* should be further elucidated.

Fig. 1. Immunohistochemistry of mouse skeletal muscles with Trichinella spiralis at days 10, 14, 18, 24 and 35 post invasion (d.p.i.) with polyclonal Ab against ST6GalNAc1. Star – non-occupied skeletal muscle fibre, hashtag – occupied sarcoplasm, L – larva. Obj. magn. 40×.

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