POSSIBLE PRESENCE OF COMMON TYVELOSE-CONTAINING GLYCANS IN *TRICHINELLA* L1 LARVAE AND EMBRYONATED EGGS OF SEVERAL NEMATODES

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Summary :

A monoclonal antibody (mAb US4) recognising an epitope containing tyvelose within the *T. spiralis* L-1 muscle larvae (TSL-1) antigens was tested in western-blot against various antigenic preparations from different stages of the following nematodes: *T. spiralis* (L1,adult), *T. muris* (egg, L1, L3, adult), *Ascaris suum* (egg, adult), *Toxocara canis* (egg, adult), *Anisakis simplex* (L3) and *Haemochus contortus* (egg). Positive reaction was present in antigen preparations from L1 larvae of *T. spiralis and T. muris* and from embryonated eggs of *T. muris, A. suum, T. canis* and *H. contortus*.

KEY WORDS : TSL-1 antigens, tyvelose, glycans, *Trichinella*, nematodes.

TSL1 antigens are a group of glycoproteins that express an immunodominant carbohydrate epitope (Ortega-Pierres *et al.*, 1996). They primarily locate at the cuticle surface and in the alpha- and beta-stichocyte granules of the muscle larvae of *Trichinella spiralis* (Ellis *et al.*, 1994) but are absent in adult worms. Fine structural analysis of this carbohydrate led to identification of a 3,6-dideoxy-D-arabino-hexose (Tyvelose) (Wisnewski *et al.*, 1993), an unusual sugar previously found in gram-negative bacteria and in the ascaroside alcohols of the *Ascaris* eggs (Jezyk & Fairbairn, 1967) although in a different isomeric form. More recently it has been shown that tyvelose is the terminal residue of a tetrasaccharide epitope(Ellis *et al.*, 1997).

In previous studies a monoclonal antibody (mAb) named US4 was characterised as recognising an epitope containing tyvelose within the TSL1 antigens (Romaris et al., unpublished results). In the present work we investigate the presence of this tyvelose-containing glycans in other helminths.

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MATERIAL AND METHODS

PARASITES

The following parasites and stages were used: L1 larvae and adult worms from *T. spiralis* (isolate GM-1 MFEL/SP/63/ISS48), embryonated (EE) and non-embryonated eggs (NEE); L1, L3 and adult worms from *Trichuris muris* (Edimburgh isolate); eggs at different embryonic stages (EE 1-3 months) and adult worms from *Ascaris suum*; EE and adult worms from *Toxocara canis*; EE from *Haemonchus contortus* and L3 larvae from *Anisakis simplex*.

ANTIGENS

Crude saline extracts (CSE) from larvae and adult worms were prepared by sonication. Crude extracts from eggs (ECSE) were prepared by freeze-thaw in liquid N2 followed by manual homogenisation and sonication.

Excretory/secretory (ES) products from L1 larvae of *T. spiralis* and adult worms from *T. muris* were obtained by incubating worms in MEM + Earle's salts added with L-glutamine, Hepes and antibiotics, for 24 hours.

MONOCLONAL ANTIBODY

The monoclonal antibody (mAb) US4 was obtained by Dr. Ubeira (Dept. Microbiology and Parasitology, University of Santiago de Compostela, Spain) in immunodefficient mice expressing the Xid-gene, following infection with L1 larvae and further immunization with the cytosolic fraction of *T. spiralis* L1 larvae (ISS48 isolate). It is of the IgG1K isotype (Romaris *et al.*, unpublished results).

ELECTROPHORESIS AND WESTERN-BLOTTING

SDS PAGE was carried out following conventional methods using the Mini-Protean system (Bio-Rad). Samples (aprox. 5-10 µg) were run in 5-20 % gradient gels under reducing conditions. Separated protein bands were visualised by silver staining.

Western-blotting was carried out according to standard protocols. The mAb US4 was used at 1:10000. Immunoreactive bands were detected using horseradish peroxidase-labeled anti-mouse IgG + IgM (Caltag) at 1:3000 dilution and 4-chloro-1-naphthol as substrate.

RESULTS

The recognition profiles in western-blot of mAb US4 with different antigen preparations is summarised in Figure 1. Among larval stages positive reaction was only present in CSE and ES from L1 larvae of *T. spiralis* and *T. muris* with a triplet of bands between 49 and 53 kDa plus an additional one at 66 kDa in *T. spiralis* and one band of about 60 kDa in *T. muris*. Positive reactions were also observed in CSE from embryonated eggs of all species with two bands at about 55 and 60 kDa in *T. muris* , two bands at about 60 and 65 in *T. canis* and *H. contortus* and one band of 55 kDa in *A. suum*. No recognition was apparent in CSE from non-embryonated eggs of all species.

DISCUSSION

The results from this preliminary study seem to show that the epitope recognised by mAb US4 is restricted to the early stages of nematode life cycles as only L1 infective larvae or embryonated eggs containing infective stages show positive reaction. Further immunocytochemical studies are required for a precise localisation of this epitope, either in the infective larvae or in the layers of the egg shell containing these infective larvae. In any case, this restriction to infective stages could indicate a role in penetration and establishment of these nematodes within the intestine of their hosts. Regarding chemical nature of the epitope targeted by mAb US4 it was suggested to be a tetrasaccharide containing tyvelose as this mAb was specifically inhibited by the synthetic tetrasaccharide β-D-Tyv-(1,3)-β-D-GalNAc-(1,4)-[α-L-Fuc-(1,3)]-β-D-GlcNAc- (Romaris *et al.*, unpublished results).

Non-specific binding of mAbs US4 to carbohydrates is unlikely as recognition is restricted to embryonated eggs and L1 stages despite that highly carbohydrate content is shown by the other stages. Furthermore, no

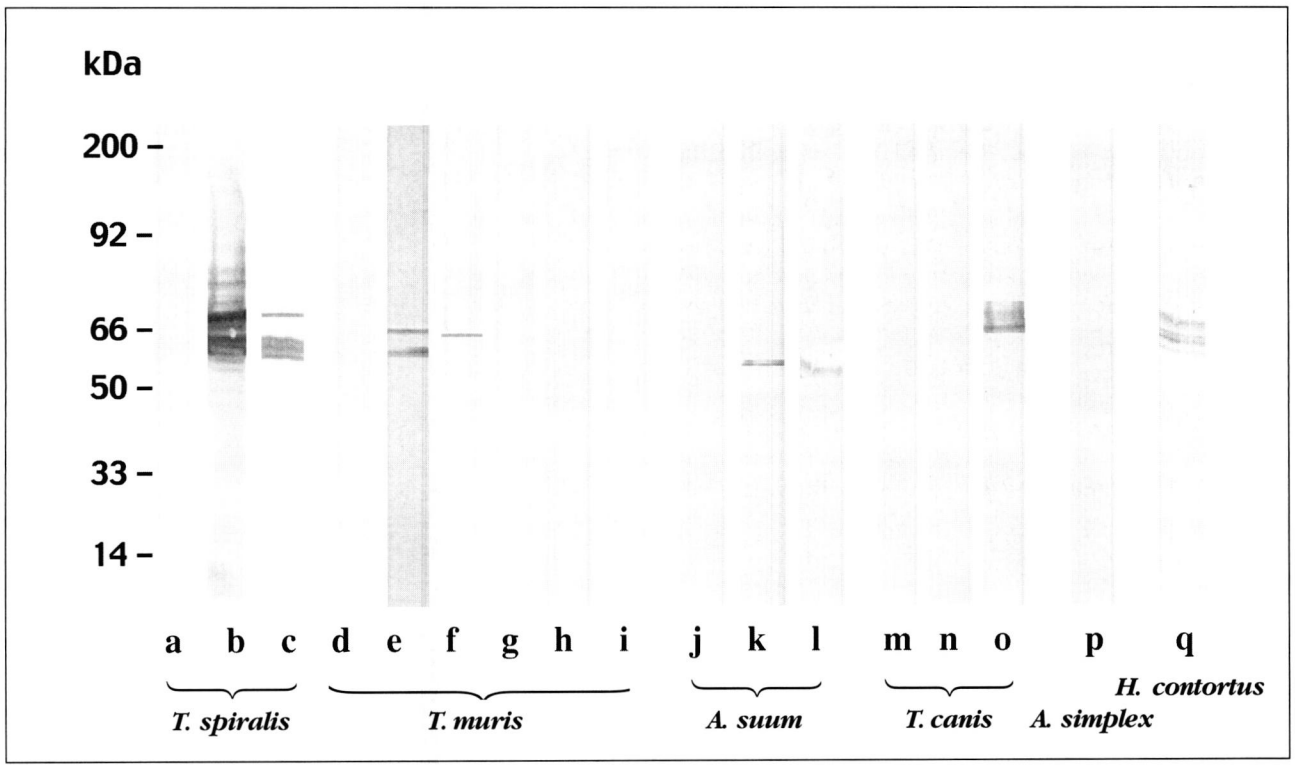

Fig. 1. – Recognition profiles of mAb US4 in western-blot with various antigen preparations from several stages of *Trichinella spiralis* and other nematodes. **a**. CSE from adults, **b**. CSE from L-1 larvae, **c**. ES from L-1 larvae, **d**. CSE from non-embryonated eggs, **e**. CSE from embryonated eggs, **f**. CSE from L-1 larvae, **g**. CSE from L-3 larvae, **h**. CSE from adults, **i**. ES from adults, **j**. CSE from adults, **k**. CSE from one month embryonating eggs, **l**. CSE from three month embryonating eggs, **m**. CSE from adults, **n**. CSE from non-embryonated eggs, **o**. CSE from embryonated eggs, **p**. CSE from L-3 larvae, **q**. CSE from embryonated eggs.
CSE = crude saline extract, ES = excretory-secretory products.

positive reaction was observed with other mAbs (named US5, US9) of the same subclass (data not shown).

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