THE USE OF A SYNTHETIC ANTIGEN FOR THE SEROLOGICAL DIAGNOSIS OF HUMAN TRICHINELLOSIS

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Summary :

Hosts infected with *Trichinella* produce antibodies specific for an epitope common to the TSL-1 family antigens. This epitope contained uncommon terminal 3, 6-dideoxy-D-arabinohexose (so called tyvelose) residues. The disaccharide moiety was synthesized and an immunodiagnostic assay was developed, which was specific and sensitive in swine trichinellosis. We aimed to verify the specificity and sensitivity of this immunodiagnostic test in human trichinellosis. 15 sera from normal subjects, 12 from patients with other parasitic diseases and 50 from trichinellosis patients were tested. Indirect enzyme linked immunosorbent assay (ELISA) for specific IgG and an amplified ELISA for specific IgE were performed using β -tyvelose-GalNAc-bovine serum albumin (BSA) disaccharide conjugate or *T. spiralis* muscle larvae excretory/secretory (E/S) products, as antigens. Neither control sera nor other parasitic infection sera resulted positive both for IgG and IgE when synthetic or E/S antigens were used. In trichinellosis patient sera, specific IgG were present in 100 % of cases, irrespective of the antigen used, but whereas specific IgE were detected in 78 % using E/S antigens, a 100% positivity rate was obtained, using the β -tyvelose-BSA conjugate.

KEY WORDS : β -tyvelose, human trichinellosis, IgG, IgE, diagnosis.

Trichinellosis is a relevant zoonotic disease, with a worldwide distribution, resulting from ingestion of raw or undercooked meat containing infecting larval stages of nematodes of *Trichinella* species (Bruschi & Murrell, 1999).

One of the biggest problems of the serological diagnosis of human trichinellosis is the lack of availability of an antigen, to be used in immunoenzymatic tests, which does not cross react with other infectious agents and in particular with other nematodes, especially in those geographical regions where infections caused by the latter are endemic (Murrell & Bruschi, 1994). It has been shown that hosts infected with *Trichinella* (regardless of the species) produce antibo-

dies specific for the carbohydrate epitope which is common to the TSL-1 (*T. spiralis* antigens-group 1) family antigens (Appleton *et al.*, 1991), produced by L1 stichosome (Silberstein & Despommier, 1985), a specialized secretory organ containing cells called stichocytes. This epitope was characterized and contained uncommon terminal 3, 6-dideoxy-D-arabinohexose (so called tyvelose) residues (Wisniewski *et al.*, 1993). Tyvelose residues were also described in some Gram-negative bacterial lipopolysaccharides and in *Ascaris* eggs, but the configuration of tyvelose in β is peculiar to *Trichinella*. The glycans present in the TSL-1 family antigens are represented by tri- and tetra-antennary structures containing the tyvelose and are likely to be highly immunogenic (Reason *et al.*, 1994). Furthermore, the disaccharide moiety was synthesized and an immunodiagnostic assay was developed for the diagnosis of swine trichinellosis, resulting specific and sensitive (Wisniewski *et al.*, 1997). The aim of our study was to verify the specificity and sensitivity of this immunodiagnostic test in human trichinellosis.

MATERIALS AND METHODS

SERA

Fifteen sera from trichinellosis free subjects (normal subjects), 12 from other parasitic disease (caused by both protozoa and helminths) patients with a clinical and serological diagnosis, and 50 from trichinellosis patients, resulted positive in indirect immunofluorescence, were used. The trichinellosis patients were infected during an outbreak which occurred in Central Italy in 1988, caused by *T. britovi* (Frongillo *et al.*, 1992).

ANTIGENS

Synthetic antigen

β -tyvelose-GalNAc-bovine serum albumin (BSA) disaccharide conjugate, free of α -tyvelose was kindly provided by Heska Corporation, Fort Collins, Colorado.

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	IgG anti-E/S	IgG anti-sugar-conjugated albumin	IgE anti-E/S	IgE anti-sugar-conjugated albumin
Controls (n = 15)	0.054 ± 0.02° (0)*	0.061 ± 0.01 (0)	0.055 ± 0.01 (0)	0.049 ± 0.01 (0)
Other parasitic diseases (n = 12)	0.064 ± 0.02 (0)	0.058 ± 0.03 (0)	0.060 ± 0.01 (0)	0.045 ± 0.02 (0)

° mean optical density ± s.d. obtained at 1:20 dilution of the sera.

* positivity rates are shown in parentheses.

Table I. – Results obtained with controls.

Sera	IgG anti-E/S	IgG anti-sugar-conjugated albumin	IgE anti-E/S	IgE anti-sugar-conjugated albumin
Trichinellosis patients (n = 50)	0.440 ± 0.01°	0.452 ± 0.01	0.070 ± 0.016*	0.296 ± 0.02
1:20	–	–	–	3 (6)
1:40	–	–	17 (34)	16 (32)
1:80	8 (16)**	8 (16)	18 (36)	25 (50)
1:160	24 (48)	24 (48)	4 (8)	6 (12)
1:320	18 (36)	18 (36)	–	–
Total	(100)	(100)	(78)	(100)

° Mean optical density ± s.d. obtained at 1:20 dilution of the sera.

* At 1:20 dilution, using E/S antigens IgE were not detected.

** Positivity rates are shown in parentheses.

Table II. – Results obtained with patients.

E/S antigens

E/S antigens were collected after incubation of *T. spiralis* L1 larvae at 37°C in MEM-Eagle (Gibco, Paisley, Scotland). The supernatant, collected after centrifugation, was filtered through Millipore filters (0.22 µ), concentrated with 7.5 % PEG 6,000 to a final concentration of 700 µg/ml and stored at – 80°C until use.

INDIRECT ELISA

Indirect ELISA for the detection of specific IgG (Bruschi *et al.*, 1990) were performed. 125 ng of synthetic antigen, dissolved in Tris-EDTA-NaCl buffer, pH 7.3, or 100 µg/ml of E/S antigens were added to each well, after that plates were stored at + 4°C overnight.

After washing with phosphate buffer saline, pH 7.2 with 0.05 % Tween, human sera were added at different dilutions in phosphate buffer saline (PBS)-BSA. The presence of antibodies were revealed using a peroxidase-conjugated anti human IgG (Sigma St. Louis, Mo) and orthophenyldiamine (Sigma St. Louis, Mo) and hydrogen peroxide as substrate. The reaction was stopped after 15 min. with H₂SO₄ 0.5 M and reading was performed at 492 nm.

AMPLIFIED ELISA

An amplified ELISA for specific IgE previously described (Tassi *et al.*, 1990; Piergili-Fioretti *et al.*, 1992) was performed.

RESULTS

The results show that neither control sera nor other parasitic infection sera resulted positive in any case for both IgG and IgE. when synthetic or E/S antigens were used (Table I). In trichinellosis patient sera specific IgG were present in 100 % of cases, irrespective of the antigen used, but whereas specific IgE were detected in 78 % using E/S antigens, a 100 % positivity rate was obtained, using the β-tyvelose-GalNac BSA conjugate (Table II).

DISCUSSION

The parasitic nematode *Trichinella* produces a great amount of proteins able to induce a strong humoral response in infected hosts (Silberstein & Despommier, 1985). Among them the TSL-1 family antigens play a crucial role in host-parasite relations and despite the fact that they represent only 3 % of total protein present in muscle larvae, between 26 and 86 % of all *Trichinella*-specific antibodies present in an infected host serum reacts with the carbohydrate common to these proteins, β-tyvelose (Denkers *et al.* 1990, 1991). Furthermore, Denkers *et al.* (1991) have clearly shown the incapability of other helminthic antigens to inhibit the binding of a carbohydrate epitope-

specific monoclonal antibody with TSL-1 antigens, suggesting the peculiarity of this epitope present in *Trichinella*. The absence of positive results observed in sera from other parasite infected subjects is in line with this observation. All trichinellosis patient sera reacted with β -tyvelose-conjugated albumin as well as with E/S antigens. Considering that sera evaluated were from patients infected with *T. britovi*, our results confirm the existence of cross-reactions between the TSL-1 antigens from different *Trichinella* species (Gamble, 1994).

Our results show that trichinellosis patients produce not only IgG (even if at a lesser extent) but also IgE specific for the β -tyvelose epitope. This is the first observation in humans to our knowledge but the meaning in the light of host-parasite relations is far to be clarified. It is noteworthy that using only synthetic antigen we obtained, by an amplified ELISA, a 100 % sensitivity for specific IgE, which was not even approached using E/S antigen (78 %).

The availability of an antigen which is not responsible for false positive reactions represents the gold standard for a serological test, particularly in case of parasitic infection diagnosis where cross-reactions are very common. The specificity of the synthetic antigen used in our study is of importance as it can be used to confirm trichinellosis and rule out cross reactions.

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