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## **Trichinella antigens: a review**

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## Review article

# *Trichinella* antigens: a review

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**Abstract** – This paper presents a review of the *Trichinella* antigens within the context of species variation. As with other parasites, *Trichinella* antigens can be classified according to their localisation as surface, excretory/secretory (ES) and somatic components. Surface antigens are mainly constituents of the outer cuticle although secretions from inner parts of the body wall as well as from the oesophagus can temporarily accumulate in the surface. ES antigens come mainly from the excretory granules of the stichosome, whereas somatic constitutive antigens come from the internal parts of the worms. ES products are considered very important from an immunological point of view as they are easily targeted by the immune system, whereas parasite death is required for exposure of somatic products. Some of the antigenic components have been characterised chemically. Phosphorylcholine is an important hapten that modulates the immune responses in *Trichinella* infections. Glycoproteins are the major components of surface and excretory/secretory products. A 43-kDa glycoprotein has been regarded as a good candidate for diagnosis and vaccination purposes. Recently some glycans have received special attention either as relevant epitopes or as parasite evasion strategies. © Inra/Elsevier, Paris.

### *Trichinella* / antigen / phosphorylcholine / glycoprotein / carbohydrate

**Résumé** – **Antigènes de *Trichinella* : une revue.** Cette revue concerne les antigènes de *Trichinella* dans le contexte des variations d'espèces. Comme dans le cas d'autres parasites, les antigènes de *Trichinella* peuvent être classés en fonction de leur localisation comme composants somatiques, de surface et d'excrétion-sécrétion (ES). Les antigènes de surface sont principalement des constituants de la cuticule externe, bien que les sécrétions des parties internes de la paroi corporelle ainsi que celles de l'œsophage s'accumulent parfois temporairement à la surface. Les antigènes d'excrétion-sécrétion proviennent principalement des granules excrétoires du stichosome tandis que les antigènes somatiques constitutifs appartiennent aux parties internes des vers. On considère que les produits d'excrétion-sécrétion sont très importants du point de vue immunologique car ils sont facilement accessibles par le système immunitaire tandis que la mort du parasite est nécessaire pour que les produits somatiques soient exposés. Quelques-uns des composants antigéniques ont été caractérisés.

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la phosphorylcholine est un haptène important qui module les réponses immunitaires dans les infections par *Trichinella*. Les glycoprotéines sont les composants majeurs des antigènes de surface et des produits d'excrétion-sécrétion. La glycoprotéine de 43 kDa a été considérée comme un bon candidat pour le diagnostic et pour les projets de vaccination. Récemment, quelques glycanes ont été l'objet d'une attention spéciale soit dans le cadre de la stratégie d'évasion des parasites soit parce qu'ils ont été considérés comme des épitopes appropriés. © Inra/Elsevier, Paris.

### *Trichinella* / antigène / phosphorylcholine / glycoprotéine / carbohydate

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## 1. INTRODUCTION

*Trichinella* worms are adenophorean nematodes whose endogenous life cycles successively involve the intestines, blood and muscles. This particular life cycle, including intra- and extra-cellular stages, promotes active host-parasite interactions thus facilitating antigen recognition by the host immune system. The immune response against *Trichinella* has been widely studied in both natural and experimental infections and the major interest has focused on relevant epitopes capable of eliciting responses that are protective against reinfections or of interest for diagnostic purposes. Some of them are stage specific. Furthermore, it is likely that the parasite antigens play an important role in parasite protection against the host environment along the successive steps of its life cycle, in addition to immunostimulatory effects in the hosts. As in other helminth species, *Trichinella* antigens can be divided into three components:

the surface, the excretory/secretory (ES) and residual somatic components.

It is assumed that surface antigens are the most important targets for the immune system because they provide a host-parasite interface but it has also been shown that internal antigens can be expressed in the outer cuticle thus mimicking those of surface origin. Secreted antigens can also favour parasite survival by blocking the immune system of the host [42].

Chemical characterisation of *Trichinella* antigens has been attempted extensively. Among the surface and ES products, glycoproteins are the major components. Characterisation of their fine structure, localisation and their potential function have been the subject of many studies over the last few years. Phosphorylcholine (PC) is a very conserved and widely distributed molecule in nature and its presence in the somatic components of *Trichinella* is also well documented.

## 2. ANTIGENS ACCORDING TO THEIR ANATOMIC LOCALISATION

### 2.1. Surface antigens

Evidence that the host synthesises antibodies against different cuticular components has primarily been shown by using whole parasites and techniques such as indirect immunofluorescence [16, 90]. Electron microscope and ferritin-labelled conjugates [22] as well as scanning microscopy have shown precipitated antibodies on the larval and adult surface.  $^{125}\text{I}$ -labelled surface proteins have been solubilised in 2% SDS, then immunoprecipitated with rat immune serum and resolved in SDS-PAGE followed by autoradiography. The new-born larvae (NBL) exhibited four other major antigens of 20, 30, 58 and 64 kDa, whereas the muscle larvae (L1) exhibited four major antigens of 47, 55, 90 and 105 kDa, of which the 55- and 105-kDa antigens have lentil lectin binding capacities. Another group of antigens was identified in the cuticle of the L2-L4 stages with molecular weights of 20, 33, 40 and 56 kDa. Amongst these antigens the 56 kDa was different from the 55 kDa of the L1. Furthermore, among the antigens present in the L4 stage, only those of low molecular weight were shared with the adult stage [69, 70]. The authors also observed labelled surface antigens secreted into the culture medium. Changes in the molecular surface during culture time were observed by Makenzie et al. [53]. These changes were later assessed by Jungery et al. [41] where only one single 64-kDa component was identified on the surface of NBL following 2.5 or 6.5 h of culture. After 17.7 h or 1 night of culture, additional bands of 58, 34 and 32 kDa were also present. By using the same technique, Parkhouse and Ortega-Pierres [66] obtained similar results regarding the L1 stage, with slight differences for the NBL and the adult stages (64, 58, 34 and 30 kDa and 40, 33 and 20 kDa, respectively). In the adult, a 60-kDa band resulted

from the interaction of the 40- and 20-kDa bands, whereas the presence of the 58-kDa homologue dimer is a characteristic of the NBL stage [41].

Monoclonal antibodies have been very useful for surface antigen identification. Ortega-Pierres et al. [62] prepared seven monoclonal antibodies named NIM-M1 to NIM-M7. Monoclonal antibodies NIM-M5 and NIM-M6 reacted with the 64-kDa band solubilised from the NBL, although only the NIM-M5 was able to react with the surface of the live NBL. Additional bands of 58, 34 and 30 kDa appeared during culture, whereas the affinity of the NIM-M5 for the NBL surface decreased. By *in vivo* labelling of NBL, L1 and adults in culture, the authors observed shedding of the surface components that were replaced by new ones, concluding that the removal of surface components could be the mechanism by which the immune system recognises epitopes usually hidden in the intact worm. The monoclonal antibody NIM-M1 recognised four surface antigens on the L1 indicating that they share common epitopes. Monoclonal NIM-7 mainly reacted with the male copulatory bursa and slightly with some other areas of the male and female surfaces, thus suggesting a regional specialisation of surface antigens [63]. It was concluded by peptide analysis that all surface proteins may come from the same gene, which are built up on the surface as monomeric or dimeric either unglycosylated or glycosylated forms. Large amounts of partially purified antigens were obtained by treatment of L1 with cationic detergents and appeared to be highly protective as assessed by immunisation assays [35].

Immunocytochemical studies using the monoclonal antibody NIM-M1 have shown that surface and protective antigens originate in the stichosome of the larvae [56, 64] and more precisely in the  $\alpha$ -granules [92, 94]. According to these last authors, the  $\alpha$ -granules are the main source of surface antigens which would then be secreted via the oesophagus. Eleven groups of antigens rang-

ing from 32 to 220 kDa were identified by Boireau et al. [7] based on antigen recognition by a panel of monoclonal antibodies raised against larval extracts. Antigens from groups 1, 2, 3, 4 and 6 are localised in either the cuticle, oesophagus or longitudinal bands, whereas those of groups 5 and 7, apart from the cuticle and some parts of the oesophagus, are present in the hypodermis, haemolymph and genital primordium of the larva and adult stages. Some antigens from groups 1, 2 and 3 are not present in the adult stage, thus indicating a loss of epitopes during cuticle shedding after the first moult.

## 2.2. Excreted/Secreted (ES) antigens

The first evidence of the presence of antigens in the excreted/secreted products from various stages of *Trichinella* was found by Oliver Gonzalez [61] by observing precipitates around the mouth of L1 and around the mouth, anus and vulva of adult females when incubated with immune serum. Direct evidence of the protective capacity of the ES antigens was later demonstrated by Campbell [13] who observed the rapid elimination of adult females together with a reduction in the number of muscle larvae. The composition of ES antigens from the L1 larvae was first partially determined by Mills and Kent in 1965 [59] where, by ionic interchange chromatography, the glycoproteins were resolved into four bands, whereas by cellulose acetate electrophoresis, three bands were observed. Later, the complex composition of *Trichinella* ES antigens was assessed by SDS-PAGE with marked differences in the L1 and adult profiles as revealed by antigen recognition patterns of the rabbit immune serum [83, 103]. The stichosome is the main source of ES antigens as shown by tissue staining with enzyme- and fluorochrome-labelled antibodies [16, 20, 38]. A fraction highly rich in stichocyte granules named S3 was obtained by differential centrifugation of the L1 crude extract [20]. Four types of stichocyte-derived gran-

ules ( $\alpha 0/\alpha 1$ ,  $\alpha 2$ ,  $\beta$  and  $\gamma$  or  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) have been described in the L1 stage [81, 93]. The 50–55-kDa antigens are secreted by  $\alpha$ -stichocytes, whereas the 48-kDa antigen is present in  $\beta$ -stichocytes [84]. It is not clear whether the various antigen locations within the stichosome are due to different structures or whether they come from a common precursor which undergoes post-translational changes in different regions of the stichosome. It was also observed that NBL start to form the stichosome and secretory granules soon after invasion of the muscle cells [33].

At least 28 bands with molecular weights ranging from 11 to 200 kDa were seen in the S3 fraction by SDS-PAGE analysis [21] and 37 bands (22 of which contained carbohydrates) by isoelectrofocusing. Twenty of the S3 components were identified as antigens by immunoelectrophoresis and then purified by immunoaffinity chromatography as the pooled acid wash (PAW) fraction containing highly protective immunogens. Of the 11 groups of antigens classified by Boireau et al. [7], those of group 10 are restricted to the stichosome, whereas groups 8 and 9 share some components with the genital primordium or the haemolymph. Recently, a glutathione-S-transferase was isolated from crude extracts of L1 composed of two polypeptides of 28.5 and 28 kDa localised in  $\alpha$ - and  $\beta$ -stichocytes of L1 and in some granules of the stichocytes of 72-h adults [78].

Gamble [30] measured the protective capacity of the 45-, 49- and 53-kDa ES antigens after purification by affinity chromatography using the 7C2C5 monoclonal antibody. The glycoprotein nature of these antigens was determined. They were present in the stichocytes of the L1 larvae, whereas they were absent in the adult and NBL stages. Protection has been estimated by the accelerated expulsion of adult worms as well as by the reduction of female fecundity. Later, by chromatofocusing, Despommier et al. [23] showed that a 43-kDa protein from ES was present not only in the  $\alpha$ -sti-

chocytes but also in the cuticle, cytoplasm and nucleoplasm of the nurse cell from day 9 post-infection onwards and this suggested its participation in muscle cell transformation. Further studies suggested that the 43-kDa glycoprotein was the same product as the 48-kDa one described by Silberstein and Despommier [84]. This protein has lately been obtained by recombinant DNA techniques [87] and a fusion protein with high homology to the 53-kDa immunodominant protein of the larval ES has been obtained by Zarlenga and Gamble [110].

It has been pointed out [50] that products secreted by the L1 induce muscle cell de-differentiation towards the formation of the nurse cell by direct gene regulation of the hypertrophic nuclei within the infected muscle fibres. Thus, the monoclonal antibody 7C2C5 and 1H7 [2] as well as the polyclonal sera recognise epitopes in the cytoplasm of the nurse cell and in the hypertrophic nuclei but not in the small nuclei, the cyst wall and the surrounding muscle [51], therefore suggesting that the nuclear antigens originated in the stichosome of the larvae and that they could be involved in transcription/transduction pathways. Complementary experiments have shown that ES antigens are released by encysted larvae *in vivo* [73] and when directly injected into the muscles they would induce cellular reorganisation with an increase of hypertrophic nuclei [46]. They later confirmed, however [47], that antigens were only found in the hypertrophic nuclei of the nurse cells on day 14 after infection but not after injection of ES antigens.

Despommier et al. [23] and Vassilatis et al. [100] carried out fine structural studies on the 43-kDa glycoprotein. A helix-loop-helix-like structure was found suggesting that these proteins are involved in the signal transduction process of muscle de-differentiation although they were first detected on day 11 post-larvae muscle invasion, several days after the changes in muscle fibres had started [102]. The same conclusion was reached by Jasmer et al. [40]

after injection of irradiated larvae into muscle fibres where, despite their lack of stichosome development due to irradiation, they were able to induce changes similar to those observed after infection. In spite of the co-localisation of stichocyte-derived antigens with host chromatin in infected muscle cells [108], the participation of stichosome-derived glycoproteins in the muscle cell de-differentiation process needs further investigation.

### 2.3. Somatic antigens

Somatic constitutive antigens tend to be very conserved and widely distributed molecules. They are less specific and therefore more suitable for immunological purposes than other sources of antigens. Although much more attention has been paid to the surface and ES antigens, somatic antigens must not be neglected because they could play an important role after parasite death and destruction, particularly in some pathological reactions [67].

Preliminary characterisations of somatic larval antigens have been made [5, 6, 48, 58, 97, 107]. More precise studies were conducted recently on common and stage-specific somatic antigens, some of which are of particular importance for the diagnosis of the disease [36, 44, 45, 66, 76, 82]. Despite the high rate of cross-reactivity of common antigens they appear to be very useful, especially in western blot assays for the detection of early stages of the disease. Somatic and ES antigens share common components, thus the 49-, 52-, 54-, 60- and 62-kDa *T. spiralis* antigens [86] appeared to be similar to those of the 45-, 49- and 53-kDa [31] and to those of the 48- and 50–55-kDa antigens [84] of ES origin. Most groups of antigens classified by Boireau et al. [7] share surface and ES with somatic components and only those of group 11 are restricted to the gut. A 109-kDa *T. spiralis*-specific somatic antigen was more sensitive than the ES antigens in the ELISA assay,

although the latter were more specific [54, 79].

Immunocytochemical studies demonstrated the existence of antigenic substances in the reproductive structures of adult worms suggesting that responses against them could be involved in protection by interfering with the reproductive capacity of the worms [96].

### 3. IDENTIFIED ANTIGENS AND/OR EPITOPES

#### 3.1. Phosphorylcholine (PC)

The presence of PC in micro-organisms and parasites has been determined by the reactivity of their protein extracts with either monoclonal or polyclonal anti-PC antibodies. PC has been found in bacteria [12] and in fungi [3]. It has been found in *Ascaris suum* [15], *Nippostrongylus brasiliensis* [68], *Toxocara canis* [88], *Trichuris suis* [14], *Anisakis simplex* [37] and *T. spiralis* [98, 99] Nematoda species. Apart from being an abundant hapten in the larval and adult stages [95] it is a dominant epitope. PC-carrying *T. spiralis* antigens are included in the TSL-4 and TSL-8 groups [2, 65]. As an ubiquitous substance, it is responsible for many cross-reactions, thus limiting its use in diagnostic tests, although it has proved to be useful as a tracer for some parasite infections [49]. Anti-PC antibodies have been measured throughout the life cycle of the parasite in experimentally infected mice [1, 11, 15]. Anti-PC antibody responses increase sharply following effective treatment of muscle larvae with mebendazole, thus revealing the existence of PC as an internal component. A PC-enriched fraction from the whole extract of muscle larvae was obtained by differential centrifugation which contained T-cell epitopes capable of inducing a great number of IgM- but not IgG-producing cells during the secondary response [99] in normal mice. Infection by *T. spiralis* down-regulates this specific IgM

response by inducing anti hapten IgG1 antibodies [4].

#### 3.2. Glycoproteins

Glycoproteins are present in all forms of life [60]. In parasites, they are represented by the phosphoinositolglycan of the surface glycoprotein of *Trypanosoma* [28], polysaccharide-containing antigens from *Schistosoma* [24] and oligosaccharides present on the surface and ES products of *Toxocara canis* [55], among others. Many glycoproteins have already been described in somatic as well as in surface and ES parasite products. Nevertheless, the role that the glucidic part plays as an immunogen agent has acquired real relevance since the discovery of its main presence in TSL-1 antigens of the *Trichinella* larvae. Antibody responses against crude extract, surface and ES antigens were found to be biphasic allowing antigen classification into groups I and II [17–19, 91]. Group I antigens are mainly composed of somatic components in the crude extract, whereas antigens from group II are mainly made up of cuticular and ES products sharing a major immunodominant carbohydrate restricted to the larval stage of the parasite. Six major bands within the 43–68-kDa and 5.0–6.0-isoelectric point range were detected in group II by two-dimensional analysis. All bands reacted with the monoclonal antibody Tsp 130 [2, 65]. By unidimensional SDS-PAGE analysis, group II was resolved into six bands of 43, 47, 54, 57, 63 and 68 kDa under reducing conditions or into three bands of 166, 158 and 141 kDa under non-reducing conditions. These non-reduced antigens, which may represent heterodimeric forms of those obtained under reducing conditions, still reacted with the Tsp 130 monoclonal antibody. Group II antigens probably included some of those described by Silberstein and Despommier [84, 85] and by Gamble [30]. Glycosylation of the group II antigens was demonstrated by the lack of recognition by

the Tsp 130 monoclonal antibody after chemical treatment with trifluoromethanesulphonic (TFMS) acid, but not after enzymatic treatment with N-glycosidases. This group of proteins sharing a common carbohydrate was able to induce a strong antibody response of the IgG1 subclass and, based on recognition profiles by monoclonal antibodies, they were included in the TSL-1 antigens [2, 65].

The role of carbohydrate epitopes in generating protective immunity against *T. spiralis* has been widely investigated. Treatment of somatic, surface cationic detergent extracts and ES antigens with sodium periodate, an oxidising agent that cleaves hexose rings, completely abrogated the antigen binding capacity to lectins. The recognition by immune sera against the 43–55-kDa region of the TSL-1 antigens was, however, only partially affected, thus suggesting that carbohydrates do not play a main role in generating protection against reinfection [39]. The fine structure of carbohydrates within this antigenic group has been elucidated [105, 106]. By using gas chromatography associated with mass spectrometry, the authors were able to identify 3,6-dideoxyhexose in the TSL-1 antigens. This unusual sugar has been found previously in gram-negative bacteria such as *Salmonella* and *Escherichia coli*, and in the ascaroside alcohols of the *Ascaris* eggs. Fucose, the other sugar present in a significant amount in *Trichinella* antigens has also been reported in *Toxocara canis* and *Schistosoma mansoni* [43, 52]. The total amount of 3,6-dideoxyhexose and 83 % of fucose appeared as non-reducing terminal residues suggesting that both sugars are associated with the immunodominant epitope of TSL-1 antigens. Fucose constitutes 36 % molar, whereas 3,6-dideoxyhexose was 24 % molar in the TSL-1 fraction isolated from the crude extract by affinity chromatography with the monoclonal antibody Tsp 130. In ES products, the percentage of fucose was 19 % molar and that of 3,6-dideoxyhexose was 21 % molar together with high levels of hex-

osamines (GalNac 15 % molar, GlcNAc 25 % molar). The molar percentages of 3,6-dideoxyhexose, fucose and hexosamines (GalNAC, GlcNAc) were lower in the crude extract than in other preparations (8, 12, 13 and 25 %, respectively). Finally, the relative and absolute configuration of 3,6-dideoxyhexose was determined by alditol acetate derivation and chiral octyl-glucoside acetylation, respectively, resulting in 3,6-dideoxy-D-arabino-hexose or tyvelose.

A family of tri- and tetra-antennary glycans was obtained by using fast atom bombardment mass spectrometry (FAB-MS) [75]. Their antennae are compressed in the tyvelose capped structure Tyv 1,3 GalNac  $\beta$  1,4 (Fuc $\alpha$ 1,3) GlcNAc $\beta$ 1. In order to determine the anomeric configuration of tyvelose existing in *T. spiralis*, the monosaccharides methyl-3,6-dideoxy- $\alpha$ -D-arabino-hexopyranoside and the methyl-dideoxy- $\beta$ -D-arabino-hexapyranoside, as well as the 2-(trimethylsilyl) ethyl disaccharide  $\alpha$ - and  $\beta$ -Tyv-(1-3)- $\beta$ -D-GalNac, were synthesised and the corresponding glycoconjugates were prepared [74]. Further analysis of the glycoconjugates by immunochemical inhibition or H1 RMN suggested that the  $\beta$ -tyvelose epitopes are more active than the  $\alpha$ -glucosides. This was the first time that these molecules were reported in nature under such a form. These authors also demonstrated that some protective monoclonal antibodies (9D and 18H), recognising *T. spiralis*-derived N-glycans, were also able to recognise synthetic saccharides and their glycoconjugates.

Further characterisation of *Trichinella* glycans was achieved by combining the western blotting immunorecognition patterns of a panel of protective monoclonal antibodies with antigens after deglycosylation with non-selective (TFMS) or with selective (PNGase F or O-glycosidases) agents [26, 27].

The participation of L1 surface tyveloses in antibody-mediated protection of epithelial cells against *Trichinella spiralis* was shown



in an 'in vitro' assay of epithelial cell invasion [57].

### 3.3. The 43-kDa glycoprotein

Among the TSL-1 group of *Trichinella* antigens, the 43-kDa glycoprotein is one of the most intensely investigated. In 1990 Gold et al. [33], were able to separate a 43-kDa protein by chromatographic techniques that, after deglycosylation, gave rise to a 32-kDa polypeptide which was not recognised by antibodies generated against the native protein. Studies were conducted by Vassilatis et al. [100–102] who cloned the cDNA of the protein secreted by *T. spiralis* muscle larvae. Further analysis of the 344-aminoacid-deduced sequence revealed: i) the presence of two potential N-glycosylation links at positions 24 and 117; and ii) the presence of two strongly amphipathic helices confirming a helix–loop–helix motif. Antibodies raised against the recombinant protein recognised the native purified form and two additional bands under both glycosylated and deglycosylated forms. It was concluded that either different variants of the 43-kDa proteins with a different C-terminal exist or that different proteins share the same immunological epitope. Antibodies against the 43-kDa purified glycoprotein recognised the 81–120- and 121–160-mer non-protective synthetic peptides, whereas it failed to recognise the 40–80-mer protective peptide, again suggesting that the epitopes of this region in the native molecule may be masked by carbohydrates [77].

The fact that the anti-43-kDa antibody recognised the 40–80 peptide only in its deglycosylated form suggests that the carbohydrates could act by capping some of the epitopes and therefore glycosylation would constitute an evasion strategy from the immune system by hiding functionally relevant epitopes.

## 4. ANTIGENIC VARIATION IN THE *TRICHINELLA* SPECIES

Recently, the *Trichinella* genus has been resolved into five species (*T. spiralis*, *T. britovi*, *T. nativa*, *T. nelsoni* and *T. pseudospiralis*), and three related gene pools (T5, T6 and T8) [71], of various distributions and host range. Biochemical differences between these species involving relevant antigens have been shown [1, 89]. A correlation between surface antigenic variation and geographical origin has been found in *T. nativa* and *T. spiralis* that influences infectivity [8]. Similarly, marked differences were observed between the infectivity of *T. spiralis* and T5 that correlated with variations in complementary DNA sequences of the 46-, 49/43- or 53-kDa antigens from both species [109]. Antigenic variation was also observed at the infra-specific level as shown by the differences in antigen recognition patterns by immune sera against infections by different geographical isolates of *T. spiralis* [9, 25, 34]. These specific and infra-specific variations have important implications in the serodiagnosis of trichinellosis [10, 29, 32, 72].

Monoclonal antibodies have been very useful not only for antigen purification but also for species and isolate identification. Monoclonal antibodies have been generated that recognise antigens present in *T. pseudospiralis* but not in *T. spiralis* [104] or that recognise seven components (between 45 and 105 kDa) of the *T. spiralis* larval crude extract and only five in *T. pseudospiralis*. The close relationships between *T. britovi* and T5 and between *T. spiralis* and *T. nativa* were confirmed by Boireau et al. [7] using a large panel of monoclonal antibodies. Three groups of *T. spiralis* isolates from quite different geographical origins were distinguished according to the recognition patterns of larval antigens of these isolates by two monoclonal antibodies [80]. A 53-kDa glycoprotein was purified from *T. spiralis* larval crude extracts by affinity chromatography using monoclonal antibodies

(Romaris et al., unpublished results) that resulted in a 99.5 % homologue with that cloned by Zarlenga and Gamble [110]. This 53-kDa protein was found to be restricted to the cyst-forming species (Romaris et al. and Dea-Ayuela et al., unpublished results).

## 5. CONCLUSION

A large number of antigens, some of which are similar, have been described in the cuticle, ES products and somatic extracts of the various stages of the *Trichinella* life cycle. Glycoproteins seem to be the most important targets for the immune system and carbohydrate epitopes have been described as immunodominant in *Trichinella*. Various procedures of preparation by different laboratories have been applied and this could lead to some variation in MW and biochemical properties. Attempts have been made in order to standardise *Trichinella* antigens. Three main classifications have been adopted on the basis of chronology and recognition patterns by monoclonal and polyclonal antibodies. Antigens can be classified into groups I and II according to the chronology of antibody recognition during the course of *Trichinella* infection. Antigens included in group I bear phosphorylcholine, are known as rapid-responders and induce humoral response about 2 weeks after infection. Group II antigens are the slow-responding group inducing humoral responses about 4–5 weeks after infection and they share a fucose- and tyvelose-containing immunodominant epitope. They are mainly present in the ES.

The recognition patterns in western blot analysis by monoclonal and polyclonal antibodies allows the establishment of nine antigen families known as TSL-1 to TSL-8 and TSA-1. A more complete analysis based on localisation, size, immuno-reactivity with ES and soluble products and stage-species specificities has led to the establishment of 11 antigen groups.

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