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Differences and similarities of nurse cells in cysts of *Trichinella spiralis* and *T. pseudospiralis*

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Abstract

The nurse cell in the cyst of *Trichinella spiralis* comprises at least two kinds of cytoplasm, derived from muscle or satellite cells, as indicated by the pattern of staining using regular dye (haematoxylin and eosin, or toluidine blue), alkaline phosphatase (ALP) expression, acid phosphatase (ACP) expression and immunostaining with an anti-intermediate filament protein (desmin or keratin). Muscle cells undergo basophilic changes following a *T. spiralis* infection and transform to the nurse cells, accompanied by an increase in ACP activity and the disappearance of desmin. Satellite cells are activated, transformed and joined to the nurse cells but remain eosinophilic. The eosinophilic cytoplasm is accompanied by an increase in desmin and ALP expression but not an increase in ACP activity. Differences in the staining results for ALP or ACP suggest that the two kinds of cytoplasm have different functions. *Trichinella pseudospiralis* infection results in an increase of ACP activity at a later stage than *T. spiralis*. There is also a difference in the location pattern of ACP in the cyst of *T. spiralis* compared with *T. pseudospiralis*. In *T. spiralis*, ACP is diffused within the cell, but in *T. pseudospiralis*, ACP distribution is spotty corresponding to the location of the nucleus. *Trichinella pseudospiralis* infection is accompanied by a slight increase in ALP activity. Activated satellite cells following a *T. pseudospiralis* infection exhibit an increase in desmin expression. The present study therefore reveals that nurse cell cytoplasm differs between the two *Trichinella* species and between the two origins of cytoplasm in the cyst of *T. spiralis*.

Introduction

Trichinella is a nematode which is intracellular in its muscle stage, and the larvae at this stage are infective. Following infection, larvae in infected muscles are released with the aid of host gastric juice and develop into adult worms (Despommier, 1975). The second generation is produced by the gravid female from 5 days post-infection (dpi) and migrates into the entire body of the host via the blood vessels. The final destination is striated muscle where the nematodes remodel host muscle cells and settle in the host. The resulting cell is called a nurse cell (Despommier *et al.*, 1990; Lee *et al.*, 1991).

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There are two major species in the genus *Trichinella*, namely *T. spiralis* and *T. pseudospiralis*. Both *T. spiralis* and *T. pseudospiralis* are able to form nurse cells, but only *T. spiralis* produces a typical cyst wall made up of collagen and is referred to as an encapsulated species. In *T. pseudospiralis*, on the other hand, the collagen capsule is poorly developed and cannot be identified under the light microscope (Xu *et al.*, 1997), and this has led to *T. pseudospiralis* being referred to as a non-encapsulated species. The nurse cell is the result of the transformation of the infected muscle cell itself, and this is clearly the case in *T. pseudospiralis* infections, where the infected muscle cell becomes the nurse cell, and satellite cells are activated but never fuse to the infected muscle cell.

Trichinella spiralis infection, however, is a different story. In the cyst of *T. spiralis* there are at least two kinds of cytoplasm, one originating from infected and transformed muscle cells, and the other from satellite cells

(Wu *et al.*, 2001). In the early phase of infection, the cytoplasm from muscle cells is dominant but the cytoplasm of satellite cells increases in size with time. As a consequence, the nurse cell of the *T. spiralis* cyst is replaced with cytoplasm derived from satellite cells.

The present study was undertaken to identify any cytochemical differences between the two types of cytoplasm (infected muscle cell origin and satellite cell origin) and between the two kinds of *Trichinella* nurse cells and with the aim of identifying the functional role of each cytoplasm.

Materials and methods

Tissue preparation

Each BALB/c mouse was orally infected with either 200 larvae of *T. spiralis* (ISS413) or *T. pseudospiralis* (ISS13). Skeletal muscles were processed for histopathological studies at certain time points, including at 18 days post-infection (dpi) to represent early larval stages or 23 and 28 dpi as transient stages or 35 and 38 dpi as chronic stages.

Cryosections (4 μm in thickness) of infected abdominal muscles were processed for the staining of alkaline phosphatase (ALP), acid phosphatase (ACP) or peroxidase (POX), and adjacent sections were stained with haematoxylin and eosin (H&E) for the purpose of histological orientation, which allowed the identification of the precise location of the enzyme activity in tissue.

For the immunolocalization study, muscles (normal or infected abdominal muscles) were fixed with 10% formalin, dehydrated and embedded in paraffin according to standard methods. Sections were immunostained with an anti-desmin or anti-keratin antibody. Following photography, the same slides were stained with toluidine blue and H&E for the purpose of histological orientation.

Detection of ALP

The staining solution was made by dissolving substrate in the AP substrate buffer (R&D system, Minneapolis, Minnesota, USA). The substrate used was nitro-blue tetrazolium (2-2′-di-p-nitophenyl-5, 5′-diphenyl-3, 3′[3,3′dimethoxy-4, 4′-diphenylene] ditetrazoliumchloride (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and 5-bromo-4-chloro-3-phosphate p-toludine salt (Nacalai Tesque, Inc. Kyoto, Japan) in N,N-dimethylformamide (Sigma Chemical Co., St Louis, Missouri, USA). Sample cryosections were left at room temperature for 1 h, fixed with cold acetone for 5 min and treated with the staining solution for 25–35 min. The sections were washed with distilled water and mounted in GVA (ZYMED Laboratories Inc., San Francisco, California, USA).

To confirm enzyme specificity, control samples were pretreated to destroy ALP activity by incubation with phosphate buffered saline (PBS) at 70°C in a humidity chamber for 30 min (Posen, 1967), and then treated in the same way for ALP detection.

Detection of ACP

Acid phosphatase activity in the tissue was detected using the method of Stevens & Palmer (1996). Cryosection samples were left at room temperature for 1 h, fixed with cold acetone, washed with PBS three times and incubated in the developing solution at 37°C for 40–60 min. The substrate used was napththol AS-BI phosphate-pararosaniline hydrochloride (Nacalai Tesque).

To confirm enzyme specificity, control sample tissues were pretreated with sodium tartrate (0.5 M) to inactivate ACP activity, and treated in the same way for the ACP detection. This resulted in a significant decrease in ACP activity.

Detection of POX

Cryosections were fixed in cold acetone and incubated with diaminobenzidine (DAB) (ZYMED Laboratories) for 5 min at room temperature. To confirm enzyme activity, control samples were pretreated with 3% H_2O_2 in 100% methanol, and then processed in the same way for POX detection.

Immunohistochemical staining for desmin or keratin

Deparaffinized sections were treated with 3% H_2O_2 in 100% methanol to destroy endogenous POX at room temperature for 10 min, blocked with 0.5% skim milk and 0.5% Tween20 in PBS, incubated with rabbit anti-desmin serum (Biomarkor, Inc. Rehovot, Israel) or rabbit anti-keratin serum (Biomeda Inc., Froster, California, USA) as the primary antibody (1:20 dilution) for 1 h at 37°C, and then with biotinylated anti-rabbit IgG as the second antibody. The third layer, streptavidin-peroxidase (ZYMED Laboratories), was reacted for 20 min at 37°C in a humidity chamber, and finally DAB was used to develop peroxidase.

Results

Histopathology of T. spiralis *infected tissues*

Results with H&E staining (table 1) were similar to those reported by Wu *et al.* (2001). Basophilic cytoplasm of infected muscle cell origin (ICO in fig. 1A) appeared from an early stage (18 dpi) and decreased in size at a chronic stage (table 1, fig. 1G). The eosinophilic cytoplasm, of satellite cell origin (SCO in fig. 1A), first appeared at the periphery of the cyst and prevailed throughout the entire cyst.

The basophilic cytoplasm was positive for ACP activity (fig. 1C), and the staining intensity per area increased as cyst formation proceeded (table 2, fig. 1F). The eosinophilic cytoplasm showed a lower degree of ACP activity than the basophilic cytoplasm (table 2). Sodium tartrate treatment destroyed ACP activity in the polymorphic neutrophils and in the infected cell of *T. spiralis*, which suggested a specific reaction.

Negative or faint staining of the basophilic cytoplasm indicated a low level of ALP activity whereas more distinct staining of the eosinophilic cytoplasm indicated high levels of ALP activity, which increased with time (table 3, fig. 1B,E,H). Preheating at 70°C reduced the ALP activity, which suggested a specific reaction.

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Table 1. The appearance and disappearance of eosinophilic and basophilic cytoplasm in nurse cells of *Trichinella spiralis* and *T. pseudospiralis* using haematoxylin and eosin (H&E) staining.

	T. spiralis				*T. pseudospiralis*	
	Infected cell origin		Satellite cell origin		Infected cell origin	Satellite cell origin
dpi*	H&E staining	Size**	H&E staining	Size**	H&E staining	H&E staining
18	Basophilic	+++	Eosinophilic	+	Eosinophilic	Eosinophilic
23	Basophilic	++	Eosinophilic	++	Eosinophilic	Eosinophilic
35	Basophilic	+	Eosinophilic	+++	Eosinophilic	Eosinophilic

* dpi, days post-infection.
** Basophilic or eosinophilic staining area score: −0% of cytoplasm; +25% of cytoplasm; ++26–50% of cytoplasm; +++51–75% of cytoplasm; ++++76–100% of cytoplasm.

Histopathology of T. pseudospiralis *infected tissues*

Results with H&E staining (table 1) were similar to those reported by Wu *et al.* (2001). The entire cytoplasm of the infected muscle cell was affected but remained eosinophilic (table 1, fig. 2A,D,G).

The infected muscle cell exhibited low ACP activity at an early stage (table 2, fig. 2C) but ACP activity increased at a later phase (table 2, fig. 2F,I). The nucleus in particular exhibited high ACP activity (fig. 2I). The ACP activity in the nurse cells of *T. pseudospiralis* was always much lower than that in the nurse cells of *T. spiralis*. The infected

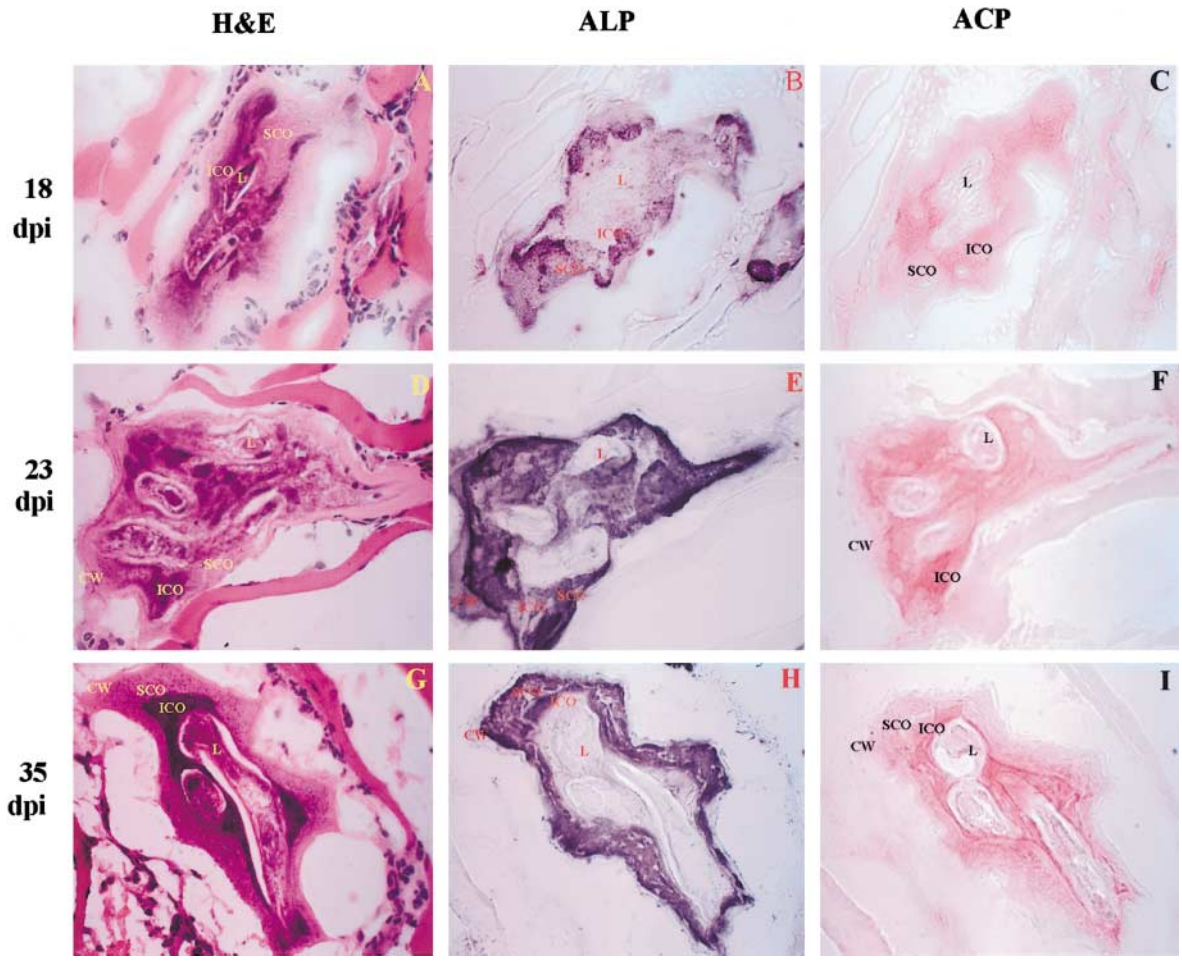

Fig. 1. Demonstration of alkaline phosphatase (ALP) activity (18 dpi in B, 23 dpi in E and 35 dpi in H) and acid phosphatase (ACP) activity (18 dpi in C, 23 dpi in F and 35 dpi in I) in cryosections of muscles following a *Trichinella spiralis* infection. L, larva; ICO, infected cell origin cytoplasm; SCO, satellite cell origin cytoplasm; CW, cyst wall. Original magnification, 400 ×.

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Table 2. The level and distribution pattern of acid phosphatase activity in nurse cells of *Trichinella spiralis* and *T. pseudospiralis*.

	T. spiralis				*T. pseudospiralis*			
	Infected cell origin		Satellite cell origin		Infected cell origin		Satellite cell origin	
dpi*	Level**	Pattern	Level**	Pattern	Level**	Pattern	Level**	Pattern
18	+	Diffuse	+/−	Diffuse	−	−	+/−	Diffuse
23	++	Diffuse	+/−	Diffuse	++	Dot-like (Nucleus)	+	Diffuse
35	+++	Diffuse	+/−	Diffuse	+++	Dot-like (Nucleus)	++	Diffuse

* dpi, days post-infection.
** Acid phosphatase activity level: − negative; +/− very weakly positive and some regions were negative; + weakly positive; ++ positive; +++ strongly positive.

Table 3. The level and distribution pattern of alkaline phosphatase activity in nurse cells of *Trichinella spiralis* and *T. pseudospiralis*.

	T. spiralis			*T. pseudospiralis*			
	Infected cell origin	Satellite cell origin		Infected cell origin		Satellite cell origin	
dpi*	Level**	Level**	Pattern	Level**	Pattern	Level**	Pattern
18	−	+	Diffuse	+/−	Diffuse	+/−	Diffuse
23	−	++	Diffuse	+/−	Diffuse	+	Diffuse
35	−	+++	Diffuse	+/−	Diffuse	+	Diffuse

* dpi, days post-infection.
** Alkaline phosphatase activity level: − negative; +/− very weakly positive and some regions were negative; + weakly positive; ++ positive; +++ strongly positive.

muscle cells exhibited low ALP activity at the early phase (table 3, fig. 2B) with increasing activity at the late phase (table 3, fig. 2E,H). The nucleus showed no ALP activity.

Endogenous peroxidase

Infected cells of both *Trichinella* species did not exhibit endogeneous peroxidase activity except for the polymorphic neutrophils (PMN) (fig. 3).

Immunohistochemical analysis

Positive staining for desmin occurred in the cyst of *T. spiralis* (table 4, fig. 4C,F,I). These sections were directly compared to adjacent sections with toluidine blue staining and H&E staining for orientation purposes. Desmin was localized in the cytoplasm of the cyst (fig. 4C,F,I) that was eosinophilic in H&E staining (fig. 4A,D,G). This eosinophilic cytoplasm is reportedly derived from satellite cells (Wu *et al.*, 2001), and was very light blue in toluidine blue staining (fig. 4B,E,H). Positive staining for desmin looked dot-like and diffuse. No desmin was localized in the basophilic cytoplasm (compare fig. 4C and 4A, fig. 4F and 4D, fig, 4I and 4G), which is reportedly derived from infected muscle cells (Wu *et al.*, 2001), and was dark blue in toluidine blue staining (fig. 4B,E,H). The nucleus was always negative for desmin staining (fig. 4C).

Strong positive staining for desmin was also seen inside the body of *T. spiralis* corresponding to the location of muscles (fig. 4C,F,I). Staining for desmin in *T. pseudospiralis* infected tissues (fig. 5C,F,I) resembled that in *T. spiralis* infected tissues (table 4). In *T. pseudospiralis*, staining with toluidine blue was more useful than H&E in differentiating infected cells from activated satellite cells. Strong positive staining for desmin was also seen inside the body of *T. pseudospiralis* corresponding to the location of the hypodermal muscles (fig. 5C,F,I).

The anti-keratin antibody positively stained epithelial cells of the host tissue but failed to stain nurse cells of both species of *Trichinella* (fig. 6A,B). This antibody also positively stained the pseudocoelom of *Trichinella* although the exact location within the parasite was not determined.

Discussion

Matsuo *et al.* (2000) showed that the nurse cell of the *T. spiralis* cyst is complex, and comprises at least five types of nuclei and two kinds of cytoplasm, including basophilic and eosinophilic cytoplasm (Wu *et al.*, 2001). Muscle cells infected with *T. spiralis* separate the affected cytoplasm from unaffected cytoplasm (Wu *et al.*, 2001). This affected cytoplasm transforms to the nurse cell (basophilic change) and later disappears. Satellite cells are activated and transformed to the nurse cell (eosinophilic with H&E staining), which fuse to the basophilic cytoplasm.

In infections with *T. pseudospiralis*, the separation following a *T. spiralis* infection does not occur, thus the entire length of the infected muscle cell is affected and is

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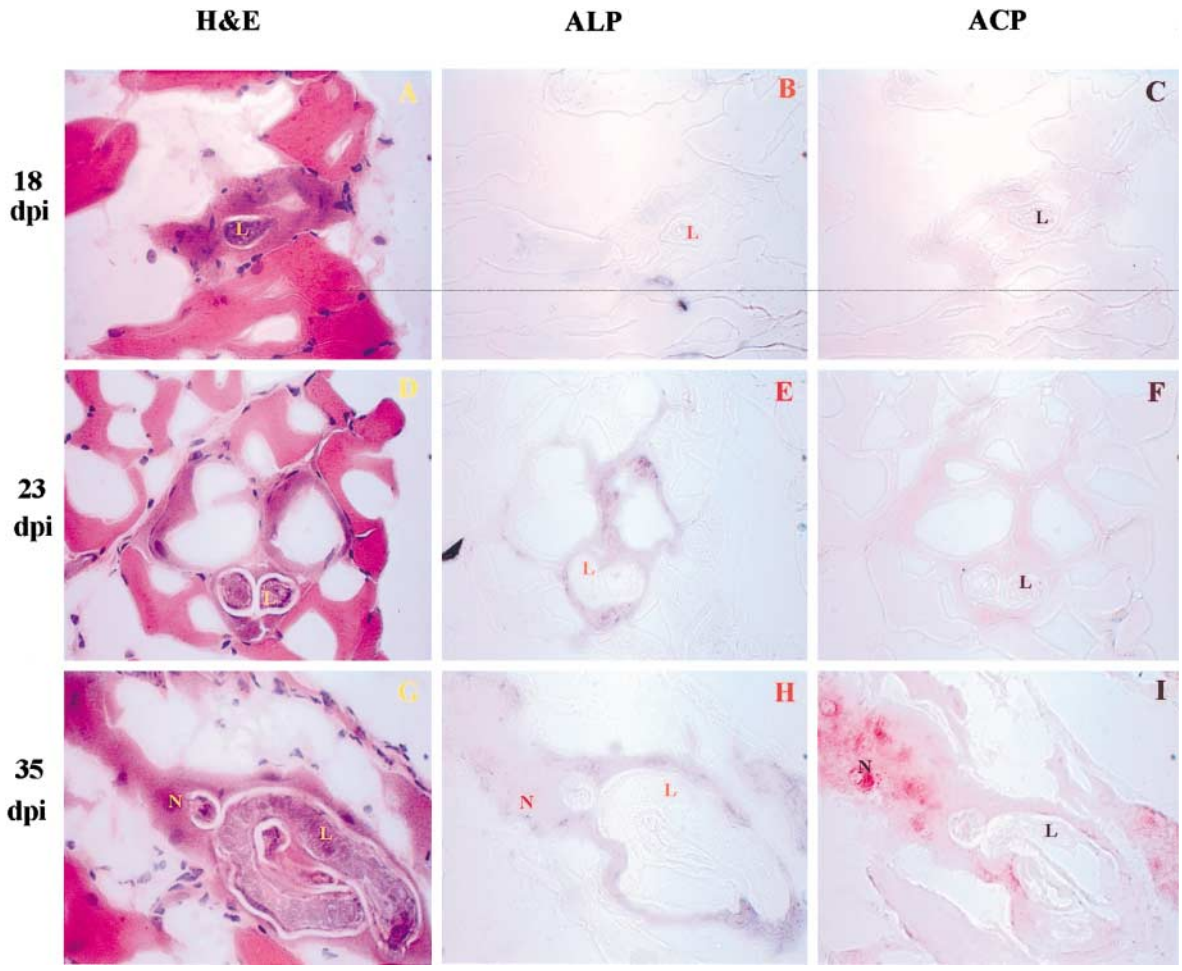

Fig. 2. Demonstration of alkaline phosphatase (ALP) activity (18 dpi in B, 23 dpi in E and 35 dpi in H) and acid phosphatase (ACP) activity (18 dpi in C, 23 dpi in F and 35 dpi in I) in cryosections of muscles after *Trichinella pseudospiralis* infection. L, larva; ICO, infected cell origin cytoplasm; SCO, satellite cell origin cytoplasm. Original magnification, 400 × .

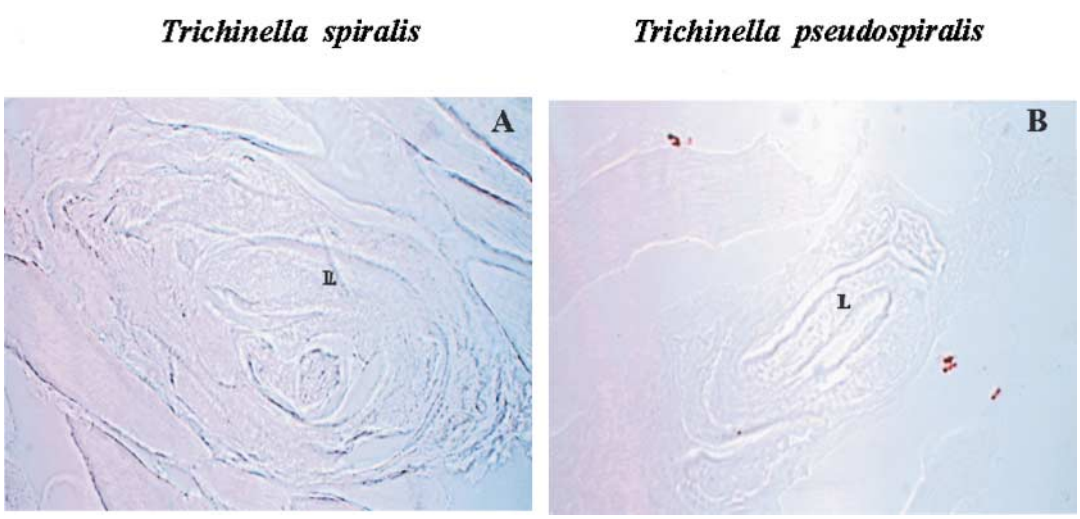

Fig. 3. Demonstration of peroxidase activity in cryosections of infected muscles of *Trichinella spiralis* (A) and *T. pseudospiralis* (B) at 35 dpi. Infected muscles of *T. spiralis* (A) and *T. pseudospiralis* (B) showed no peroxidase activity. White blood cells showed peroxidase activity (B). L, larva; NC, the nurse cell. Original magnification, 400 × .

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Table 4. The expression and distribution pattern of desmin in the nurse cells of *Trichinella spiralis* and *T. pseudospiralis*.

	T. spiralis				*T. pseudospiralis*			
	Infected cell origin		Satellite cell origin		Infected cell origin		Satellite cell origin	
dpi*	Level**	Pattern	Level**	Pattern	Level**	Pattern	Level**	Pattern
18	+	Diffuse & dot-like	+/−	Diffuse & dot-like	+	Diffuse	++	Diffuse & dot-like
28	−	−	++	Diffuse & dot-like	+	Diffuse	++	Diffuse & dot like
38	−	−	++	Diffuse & dot-like	±	Diffuse	++	Diffuse & dot like

*dpi, days post-infection.
** Desmin level: ++ strongly positive; +/− positive in some areas but negative in other areas; ± weakly positive.

transformed to the nurse cell (eosinophilic with H&E staining). Satellite cells are activated and transformed, but they do not fuse to the nurse cell (Wu *et al.*, 2001).

In both *T. spiralis* and *T. pseudospiralis* infections, the host muscle cell changes its phenotype so that the unexpected invader (*Trichinella*) can survive, but the underlying mechanisms as to how the nurse cell helps with the establishment of *Trichinella* are still unknown. No reports have dealt with functional differences of the nurse cell between *T. spiralis* and *T. pseudospiralis*, or between basophilic cytoplasm and eosinophilic cytoplasm of the *T. spiralis* cyst. As the first step towards this

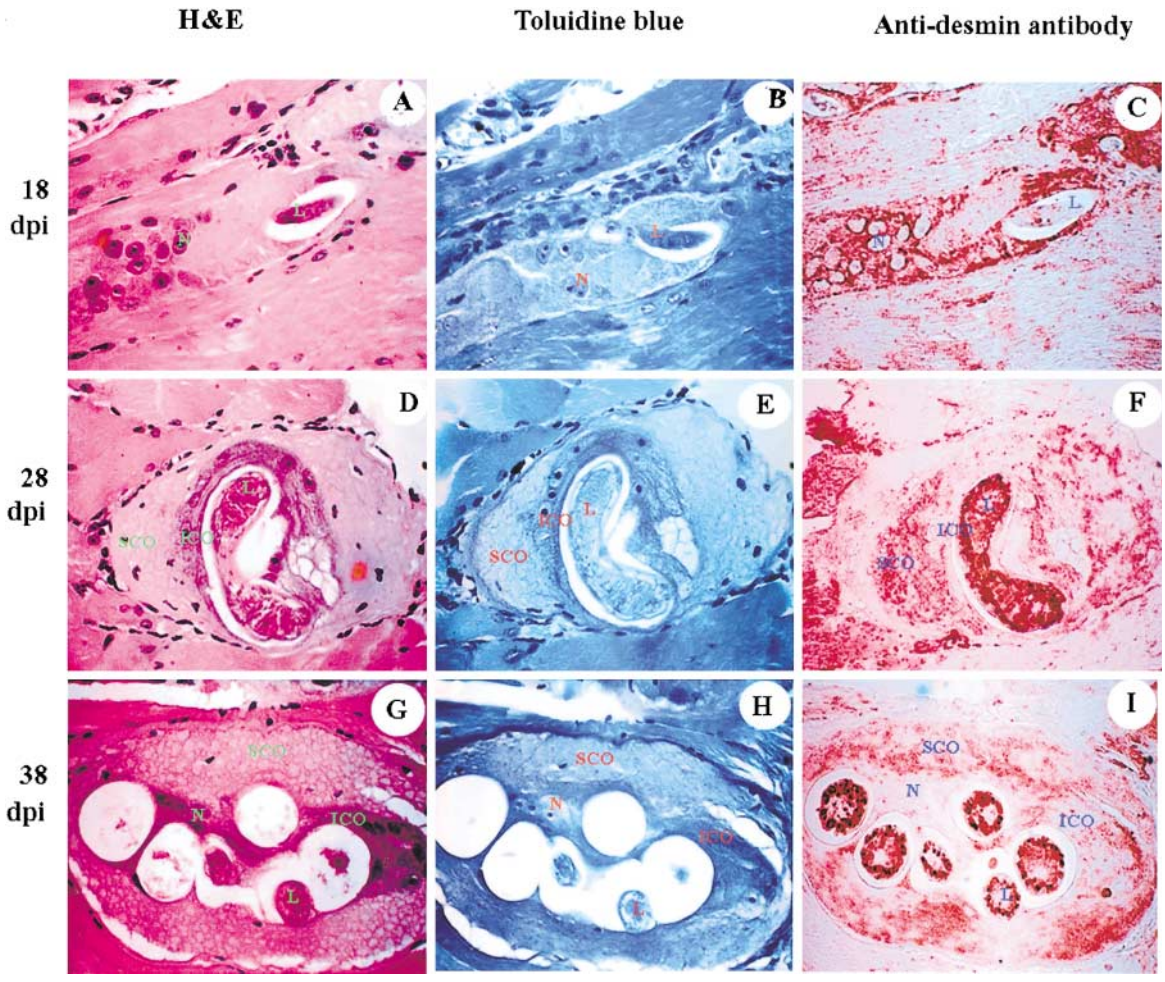

Fig. 4. Demonstration of desmin in deparaffinized sections of muscles following a *Trichinella spiralis* infection. After anti-desmin staining (18 dpi in C, 28 dpi in F and 38 dpi in I) the same section was subjected to two staining techniques for histological orientation, toluidine blue staining (18 dpi in B, 28 dpi in E and 38 dpi in H) and H&E staining (18 dpi in A, 28 dpi in D and 38 dpi in G). L, larva; N, nucleus; ICO, infected cell origin; SCO, satellite cell origin. Original magnification, 400 ×.

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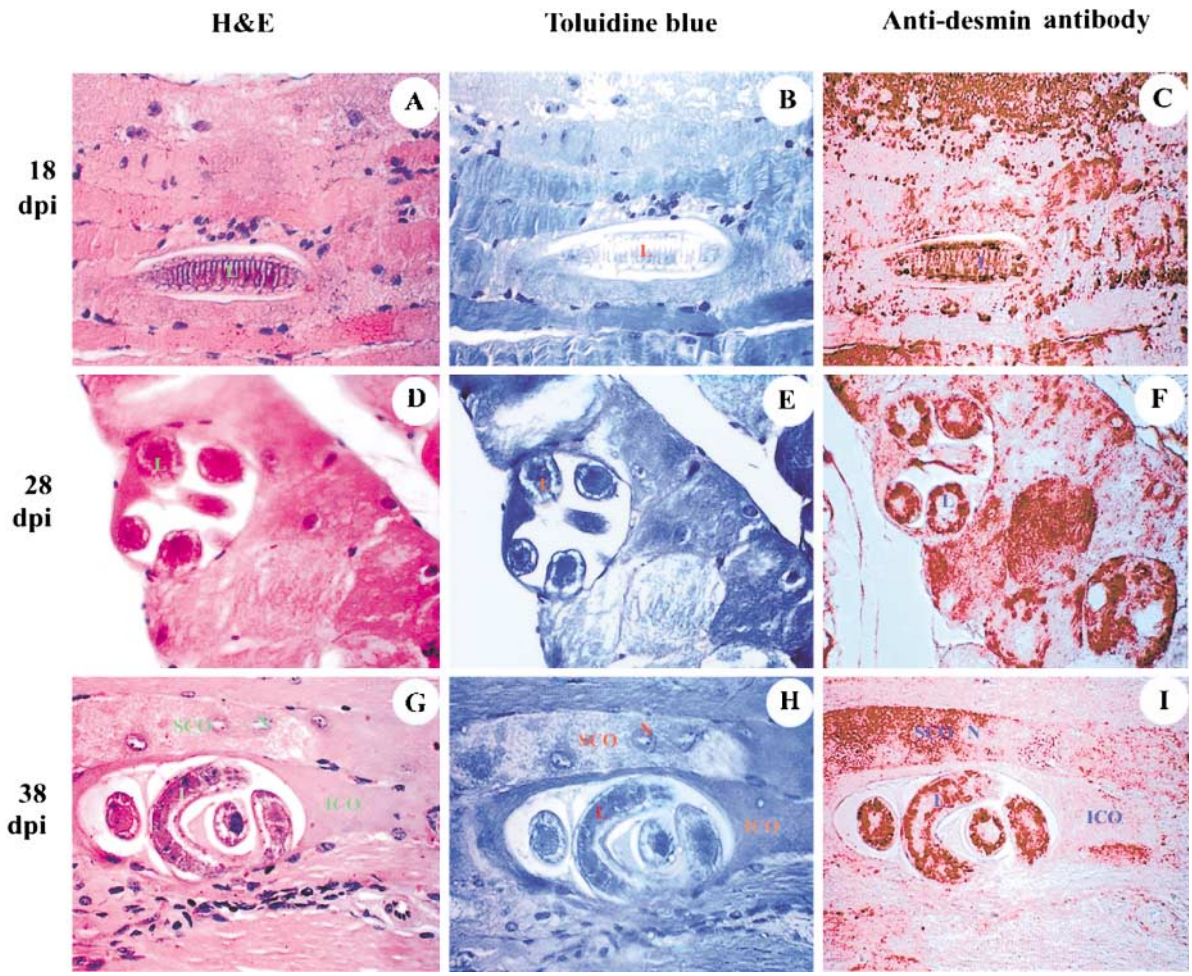

Fig. 5. Demonstration of desmin in deparaffinized sections of muscles following a *Trichinella pseudospiralis* infection. After anti-desmin staining (18 dpi in C, 28 dpi in F and 38 dpi in I) the same section was subjected to two staining techniques for histological orientation, toluidine blue staining (18 dpi in B, 28 dpi in E and 38 dpi in H) and H&E staining (18 dpi in A, 28 dpi in D and 38 dpi in G). L, larva; N, nucleus; ICO, infected cell origin; SCO, satellite cell origin. Original magnification, 400 ×.

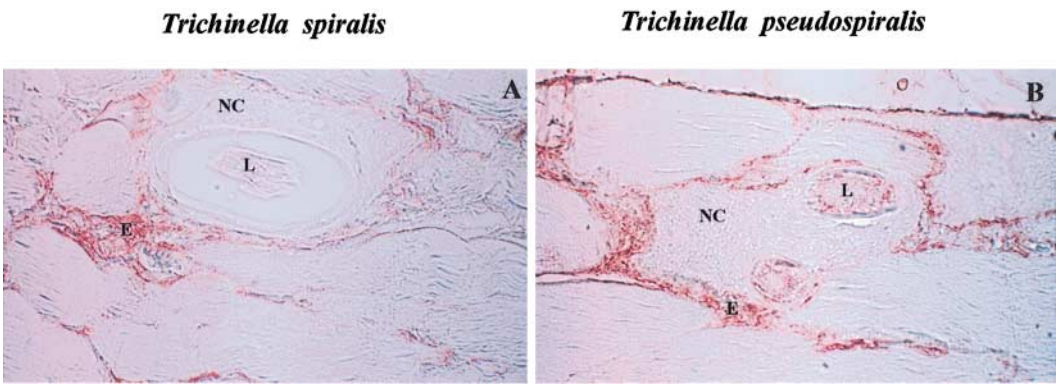

Fig. 6. Anti-keratin antibody staining on deparaffinized sections of infected of *Trichinella spiralis* (A) and *T. pseudospiralis* (B) at 35 dpi. Anti-keratin antibody stained the epithelial cells of the host but not the nurse cells in both species. E, epithelial cell of host; NC, nurse cell; L, larva. Original magnification, 400 ×.

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goal, the present study was undertaken to reveal the cytochemical nature of the nurse cells of *T. spiralis* and *T. pseudospiralis*.

The present study clearly showed there was a significant difference between basophilic cytoplasm (infected cell origin) and eosinophilic cytoplasm (satellite cell origin) in *T. spiralis* infections. Basophilic cytoplasm did not accompany the expression of ALP but eosinophilic cytoplasm expressed ALP activity from the beginning of cyst formation, and this activity increased as cyst formation proceeded (table 3). On the other hand, a *T. pseudospiralis* infection resulted in only a slight increase in ALP activity in the nurse cells and transformed satellite cells.

These results are largely in good agreement to those of Borgers *et al.* (1975) and Hulinska *et al.* (1984) but not those of Bruce (1970), which showed no change of ALP activity with time after infection with *T. spiralis*. Unfortunately, these earlier studies did not recognize the presence of two kinds of cytoplasm in the cyst of *T. spiralis*, and they probably referred to the eosinophilic cytoplasm only. Although the exact function of ALP in the cell is unclear, it is likely to be involved in the transport of metabolites across the cell membrane (Borgers *et al.*, 1975; Bennington, 1984). Therefore, the basophilic cytoplasm of *T. spiralis* may play a different role from eosinophilic cytoplasm or the nurse cell of the *T. pseudospiralis* cyst.

Microbial infections have been shown to increase ACP activity (Michejda & Boczon, 1972), which in turn engages in the digestion of cell debris (Bowen *et al.*, 1982). The same is true for *T. spiralis* or *T. pseudospiralis* infections, which are likely to cause cell damage. A large increase in ACP activity, and thus more damage, was observed in the cytoplasm of infected cells compared with that in satellite cells (table 2), suggesting that the two kinds of cytoplasm in the nurse cells have distinct differences, especially in deciding the fate of the cell.

Peroxidase is a haemoprotein enzyme, which plays a role in host defence mechanisms through detoxification of free radicals by converting peroxide to water. Peroxidase activity is low in normal tissues including leucocytes and milk (Burstone, 1962), but this activity increases during pathological processes (Brown *et al.*, 2001; Morimoto *et al.*, 2001; Visser *et al.*, 2002). The process of nurse cell formation may not have needed a peroxidase enzyme because its activity is absent from infected cells of both *Trichinella* species, and this is in good agreement with the work of Hadas *et al.* (1995).

Desmin is an intermediate filament protein that can serve as a marker of muscle cells (Lodish *et al.*, 2000; Banwell, 2001). It is expressed around the Z-bands in a normal muscle cell (Lodish *et al.*, 2000; Banwell, 2001), probably playing an important role in the maintenance of myofibril, muscle tissue structure and the functional integrity of the muscle cell (Milner *et al.*, 1996; Li *et al.*, 1997). In the present study, the distribution pattern of desmin changed following infection with *Trichinella* (figs 4 and 5). The infected cell itself tended to lose the expression of desmin with time, but desmin expression was increased in satellite cell origin cytoplasm (table 4). The positive area was dot-like and diffusely distributed in the cytoplasm (but not in the nucleus) of the nurse cell.

The *T. pseudospiralis* infected muscle cell expressed less desmin but activated satellite cells expressed more desmin. Differences in the two kinds of cytoplasm at the level of desmin expression suggest that they may be different in terms of muscle cell differentiation. The abnormal expression of desmin has been reported in different types of myopathy (Pellissier *et al.*, 1989; Telerman-Toppet *et al.*, 1991; Vajsar *et al.*, 1993; Baeta *et al.*, 1996; Nakano *et al.*, 1996; Fidzianska *et al.*, 1999) and cardiomyopathy (Goudeau *et al.*, 2001; Wang *et al.*, 2001). Keratin is another type of intermediate filament that is used as a marker for epithelial cells (Lodish *et al.*, 2000). The absence of keratin and the presence of desmin in the nurse cells suggest that nurse cells, despite having a hepato cell-like morphology, have characteristics similar to those of muscle cells.

Thus the present study provided convincing evidence that the two species of *Trichinella* influence host muscle cells in a different way. These differences are likely to be linked to the excretory-secretory (ES) products of each *Trichinella*, because ES products have the ability to partially mimic the process of nurse cell formation (Ko *et al.*, 1994). Wu *et al.* (1998) have already shown the differences and similarities of the two kinds of *Trichinella* using a peptide map. The differences and similarities of cysts of *T. spiralis* and *T. pseudospiralis* are summarized in table 5.

In summary, the present data have shown that the two kinds of cytoplasm in the *T. spiralis* cyst are different in origin as well as in function, and the cysts of *T. spiralis* and *T. pseudospiralis* are different not only in the formation of the cyst wall but also in its function. Thus the effect of *Trichinella* infections seems to be diverse in muscle cell transformation which in turn leads to nurse cell formation.

Table 5. A review of the differences and similarities of cysts of *Trichinella spiralis* and *T. pseudospiralis*.

Content	*T. spiralis*	*T. pseudospiralis*	Reference
Location of parasites	Intracellular	Intracellular	
Cyst wall	Thick	Very thin	Xu *et al.*, 1997
Septum formation in muscle cell	+	−	Matsuo *et al.*, 2000
Basophilic change	+	−	Matsuo *et al.*, 2000
Satellite cell proliferation	+	+	Wu *et al.*, 2001
Satellite cell fusion	+	−	Wu *et al.*, 2001
43 kDa ES products	+	+	Wu *et al.*, 1998; Vassilatis *et al.*, 1992
53 kDa ES products	+	−	Wu *et al.*, 1998; Zarlenga & Gamble, 1995
23 kDa ES products	−	+	Chung & Ko, 1999

ES, excretory–secretory.

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