Different response of satellite cells in the kinetics of myogenic regulatory factors and ultrastructural pathology after *Trichinella spiralis* and *T. pseudospiralis* infection

Z. WU, A. MATSUO, T. NAKADA, I. NAGANO *and* Y. TAKAHASHI*

Department of Parasitology, Gifu University School of Medicine, Tsukasa 40, Gifu 500-8705, Japan

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SUMMARY

Infection of an intracellular parasitic nematode, *Trichinella spiralis*, resulted in severe damage in muscle cells which was followed by activation and proliferation of satellite cells. The repairing process, shortly after the damage, histopathologically resembled those seen after mechanical injury. Resemblance was also true for kinetics of expression of myogenic regulatory factors (MyoD, myogenin and MRF4). The difference resided in the next step where the muscle cell infected with *T. spiralis* transformed to a unique cell which is parasitologically known as the nurse cell, and the proliferated satellite cells did not differentiate to the muscle cell but to the nurse cell (misdifferentiation). Thus the nurse cell was a fusion of the transformed infected muscle cell and misdifferentiated satellite cells. Infection with another species of *Trichinella*, *T. pseudospiralis*, also caused cell damage, but more extensively involving the entire length of the infected muscle cells because no septum was formed to minimize the affected area. Therefore, a large number of satellite cells were activated and proliferated. The myogenic regulatory factors such as MyoD and myogenin were activated for a longer period than in the case with *T. spiralis* infection. The infected muscle cell transformed to the nurse cell, whose cytoplasm was characterized by extensive smooth endoplasmic reticulum. Satellite cells misdifferentiated to the nurse cell, whose cytoplasm was amorphous, void of distinct cell organelles. The two kinds of cytoplasm did not fuse as examined thus far. Thus infection with *T. spiralis* and *T. pseudospiralis* caused misdifferentiation of satellite cells, but in a different way.

Key words: *Trichinella*, satellite cell, muscle differentiation, MyoD, myogenin, MRF4.

INTRODUCTION

The nematode, *Trichinella spiralis*, has 3 distinct stages, adult, larva in muscles and newborn larva. Adults in the host's intestine produce numerous newborn larvae which eventually migrate into the host's muscle cells. Upon intracellular invasion, newborn larvae mature in the muscle cells within a short time, which induces significant changes in the muscle cells. This intracellular rapid maturation accompanies reconstruction of the infected muscle cell into a completely different cell (see review by Despommier, 1998). Surprisingly, this newly-appeared cell takes care metabolically of the parasite and affords physical and immunological protection (hereby called the nurse cell). The nurse cell is surrounded by a fibrous capsule (cyst wall) produced by the nurse cell itself and fibroblasts of the host (Matsuo *et al*. 2000). This is known as the nurse cell.

Another species of nematode, *T. pseudospiralis*, dose not form a typical cyst, but establishes its parasitism in the nurse cell (Xu *et al*. 1998). Thus, *Trichinella* seems to possess an ability to remodel the host cell for the survival.

A simple but important question that comes to one's mind is why and how the muscle cell (supposedly a terminally differentiated cell) can transform to a new cell which ensures the establishment of parasitism. Recent studies suggested *T. spiralis* secretes bioactive substances that may alter gene expression of the host muscle cells (Despommier *et al*. 1990; Lee *et al*. 1991; Vassilatis *et al*. 1992; Ko *et al*. 1994; Despommier, 1998). The host cells are, however, never presumed to have genes necessary for the nurse cell whose morphology and functions are never shared by any other host cells.

Many enigmas remain unanswered as to the molecular mechanism responsible for the muscle cell transformation after *Trichinella* infection. In this paper we will demonstrate 2 types of misdifferentiation of satellite cells (myoblasts) into muscle cells after injury caused by intracellular infection of 2 species of *Trichinella*. This misdifferentiation caused by the infection may afford a good model that dissects normal differentiation of myoblasts where many steps are likely involved.

MATERIALS AND METHODS

Nematode parasites

T. spiralis (ISS413) and *T. pseudospiralis* (ISS13),

* Corresponding author. Tel: +058 267 2251. Fax: + 058 267 2960. E-mail: yu3@cc.gifu-u.ac.jp

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were maintained in mice. Infective larvae of *Trichinella* were isolated from mouse muscle by the standard pepsin digestion method (Despommier, 1974).

Infection of mice with Trichinella

Infective larvae were orally administered to 6-week-old nude mice (800 larvae per mouse). The mice were killed at 8, 13, 18, 23, 28, 33, 38 and 43 days after the infection, and muscle tissues from the hind limbs were subjected to analysis as will be mentioned in the following section. Control muscle tissues were obtained from a nude mouse without infection.

Light and electron microscopy

Muscle tissues were either fixed with neutral formalin for light microscopical analysis or half strength Karnovski solution (2·5% glutaraldehyde plus 4% paraformaldehyde) for electron microscopical analysis according to the conventional methods. Sections (4 μm thickness) for light microscopical observation were stained with H&E or toluidine blue.

RNA isolation

Total RNA was isolated using TRIZOL (GIBCOBRL, Life Technologies, Inc., USA) according to the manufacturer's instructions. In order to diminish DNA contamination, the isolated RNA was treated with DNase (5 units RQ1 RNase-Free DNase (Promega, Madison, USA), and 119 units of Ribonuclease Inhibitor (TakaraShuzo, Co., Ltd, Japan) in a buffer (400 mM Tris–HCl, 100 mM NaCl, 60 mM $MgCl_2$ and 20 mM DTT, pH 7·5). The treated RNA was extracted with phenol/chloroform, precipitated with ethanol, and dissolved in RNase-free water.

Primers for quantitative RT–PCR

The primer pair MyoDF2R1 (sense primer: cgcctgagcaaagtgaatg, anti-sense primer: tgtgctatgaggaaaaggaagag, product length: 933 bp) was designed based on the published mRNA sequence encoding mouse MyoD (Accession: M18779, Davis, Weintraub & Lassar, 1987). The primer pair MYGF1R4 (sense primer: tctaccggagccccacttc, anti-sense primer: catcaggacagccccactta, product length: 744 bp) was designed based on the published mRNA sequence encoding mouse myogenin (Accession: X15784, Edmondson & Olson, 1989). The primer pair MRF4F1R1 (sense primer: gccttgaagcgtagaactgtgg, anti-sense primer: acttgaggtggggagaggtttc, product length: 529 bp) was designed based on the published mRNA sequence encoding MRF4 (Accession: M27151, Rhodes & Konieczny, 1989).

Quantitative competitive PCR (QC–PCR)

QC–PCR, a sensitive and accurate method for quantifying gene expression, was performed according to the method described by Kostenuik *et al.* (1997) with some modification.

The competitor DNA (1133 bp for MyoD, 924 bp for myogenin and 589 bp for MRF4) was constructed as shown in Fig. 1, and serially diluted. For MyoD, the competitor DNA was diluted to 2·0, 1·0, 0·5, 0·2, 0·08, 0·032, 0·0128 and 0·00512 pg/μl, and for myogenin and MRF4 the competitor DNA was diluted to 0·2, 0·08, 0·032, 0·0128, 0·00512, 0·002, 0·00082 and 0·00033 pg/μl.

Reverse transcription was performed using the Ready-To-Go Kit (Pharmacia Biotech, USA) according to the manufacturer's instructions. Then 3 μl of the sample RNA and 1 μl of 0·5 μg/μl Oligo(dT)12–18 (Pharmacia Biotech, USA) were added to the Ready-To-Go tube, followed by RNase-free water to the final volume of 33 μl. The tube was incubated at 37 °C for 60 min, and then 90 °C for 5 min.

A PCR reaction mixture was comprised of 1 μl of reverse transcription products, 1 μl of the competitor DNA, 1·5 μl of 10 × PCR buffer, 1·5 μl of dNTP (2·5 mM each), 3 μl of sense and anti-sense primers (10 μM), 0·06 μl of *Taq* polymerase (5 U/μl, TakaraShuzo, Co., Ltd, Japan), and distilled water to give the final volume of 15 μl. PCR amplification was performed using the condition of 1 cycle at 95 °C for 3 min; 35 cycles of 95 °C for 30 sec, 56 °C for 30 sec and 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. Ten μl of PCR product was electrophoresed in 1·5% agarose gel stained with 1 μg/ml ethidium bromide, and photographed under UV light.

The photographs were analysed densitometrically using NIH Image 1.95 software. The optical densities of the bands of the competitor and wild-type PCR products were measured, and the ratio of both the densities was plotted against the amount of competitor cDNA template added. Finally, the mRNA concentration of wild-type was calculated by linear regression analysis.

Immunohistochemistry

Infected muscle tissues were fixed with neutral formalin and frozen in OCT compound. Cryosections (4 μm thickness) were prepared and processed for immunoperoxidase staining according to conventional methods. The sections were blocked with 5% skim milk (DIFCO Laboratories, USA), incubated with the first antibody (rabbit polyclonal

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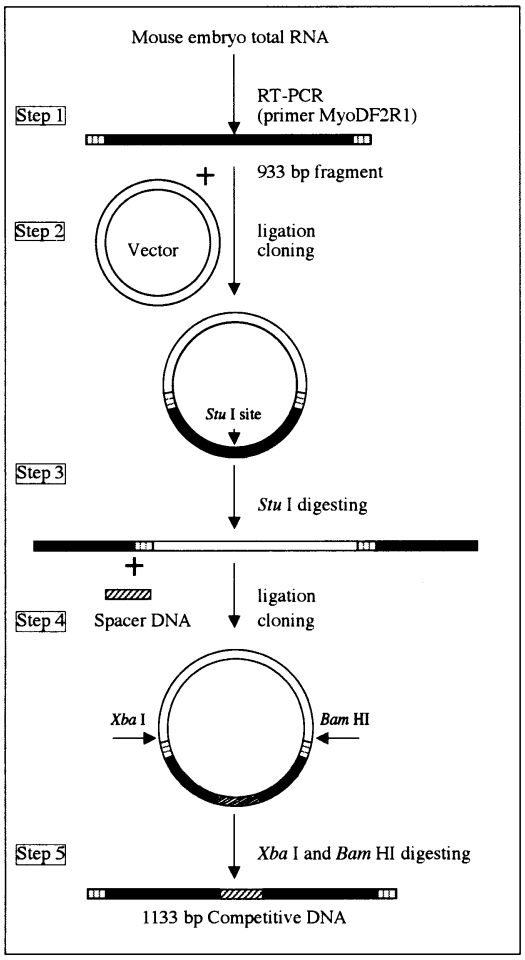

Fig. 1. Cloning strategy of competitor DNA for quantitative RT–PCR. Step 1: production of a 933 bp MyoD cDNA. Step 2: ligation and cloning. The fragment was ligated into plasmid vector (pT7Blue T-Vector). Step 3: linearizing of the vector. The vector containing the MyoD fragment with a *Stu* I site was linearized with *Stu* I digestion. Step 4: spacer DNA inserting and cloning. A 180 bp spacer DNA was ligated into the linearized vector. The positive clone was screened using PCR with the primer MyoDF2/R1, which produced an 1133 bp product. Step 5: isolation and quantification of competitor DNA. The insert DNA was isolated from the plasmid by digestion with *Xba* I and *Bam* HI. Using the same method, competitor DNA for myogenin and MRF4 was prepared. The unique sites in the insert of myogenin and MRF4 are *Nco* I and *Sty* I, respectively.

IgG against mouse MyoD, Santa Cruz Biotechnology, Inc., Japan; or normal rabbit serum), biotinylated anti-rabbit IgG and streptavidin-peroxidase (ZYMED Laboratories Inc., USA), and finally developed with diaminobenzidine (DAB).

RESULTS

Histopathology of muscles after T. spiralis *infection*

This is a repetitive observation that has been published elsewhere (Matsuo *et al*. 2000). Therefore, only a short summary is described here. Shortly after the entrance by a newborn larva, the muscle cell transformed to the nurse cell which was stained basophilic by H&E staining. The area damaged by infection was sealed off from the unaffected area by the septum formation in the muscle cell.

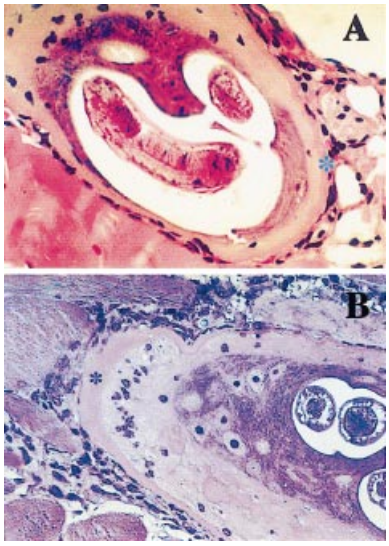

Fig. 2. Histopathology of muscles at 33 days after *Trichinella spiralis* infection. (A) H&E staining, within the cyst wall (*), there are 2 kinds of cytoplasm, eosinophilic and basophilic. (B) Toluidine blue O staining, the 2 kinds of cytoplasm are more discriminated.

A linear alignment of nuclei of proliferated satellite cells was observed along the damaged area. The satellite cells did not differentiate to the muscle cells but misdifferentiated to the nurse cell, which was stained eosinophilic in H&E staining specimens. This cytoplasm of the nurse cell consisted of mitochondria, vacuoles, and rough endoplasmic reticulum, and produced the inner layer of the cyst wall. The basophilic cytoplasm decreased in size as time proceeded and was replaced with eosinophilic cytoplasm when the cyst was completed (Fig. 2).

Histopathology of muscles after T. pseudospiralis *infection*

Newborn larvae were found in the muscle cell at 8 days after the infection. Electron microscopical observation showed initial morphological changes including myofibril disturbance and swelling of mitochondria. No basophilic change was observed in H&E stained specimens at 8 days post-infection (p.i.).

At a later stage such as 13 days p.i. (panel A in Fig. 3), striation of the muscle cell began to disappear, and the extent of the damage differed place by place. The mildly damaged and more damaged areas were aligned alternatively in a tandem manner, resulting in a zigzag appearance of the infected muscle cell (panel G in Fig. 3). A linear alignment of the nuclei

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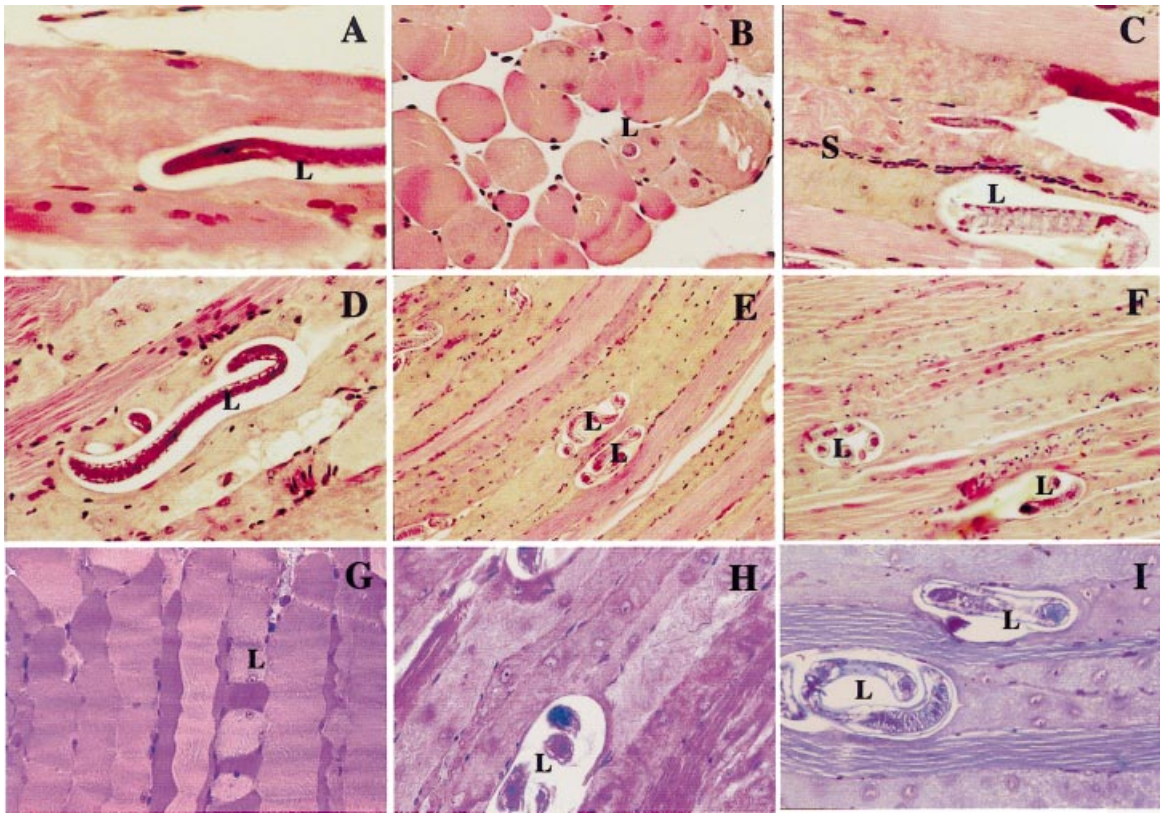

Fig. 3. Histopathology of muscles after *Trichinella pseudospiralis* infection. (A–F) H&E staining of the section of infected muscle cells at 13, 18, 23, 28, 33 and 38 days after infection, respectively. (G–I) Toluidine blue O staining of the section of infected muscle cells at 8, 33 and 38 days after infection, respectively. L, larva; S, satellite cells. The infected muscle lost its morphological characteristics as a muscle cell, but still stained eosinophilic (A). Many cells are infected with the parasites (B). The infected muscle cells are surrounded with a line of satellite cells (C). There are hypertrophic nuclei within infected muscle cells (D). The infected cells are lined with satellite cells, and the whole length of the infected cells are affected (E). The cytoplasm staining is still eosinophilic but can be discriminated from uninfected muscle cells, and the infected cells are stained less eosinophilic (F). The infected cells exhibit zig-zag appearance in the early phase of infection (G). The cells with amorphous appearance can be seen adjacent to the infected cells (H). The infected cells have hypertrophic nuclei (I).

of the proliferated satellite cell was observed in the periphery of the infected cell. Basophilic change was still not observed in the infected muscle cells at 13 days p.i.

Septum formation, which seals off the damaged area in the normal repair process, was never observed in *T. pseudospiralis*-infected muscle cells. The disintegration of myofibrils spread over the whole length of infected muscle cell at 18 days p.i. This change was most severe in the vicinity of the worm, and less severe in the periphery region. Although the infected cells resembled those of *T. spiralis* infected muscle cells ultrastructurally, still no basophilic change was observed (panel B in Fig. 3).

At 23 and 28 days p.i., a linear alignment of the nuclei of satellite cells was still observed in the periphery of the infected cells along their long axis.

Interestingly, 2 kinds of cytoplasm were coexisting. Although both were eosinophilic by H&E staining (panels C and D in Fig. 3), separation of the two kinds of cytoplasm was depicted by toluidine blue staining (panel H in Fig. 3). One was infected muscle cell cytoplasm which was already transformed to the nurse cell, and the other was satellite cell origin cytoplasm. Electron microscopical observation revealed the presence of 2 plasma membranes at the junction of the 2 cytoplasms (panel D in Fig. 4).

At 33 and 38 days p.i. (panels E and F in Fig. 3), the full length of the muscle cell was affected. Satellite cell origin cytoplasm was amorphous, void of any distinct cell organelles such as rough endoplasmic reticulum and mitochondria (panels C and D in Fig. 4). Thus, when examined up to 43 days p.i., there was no basophilic change and no septum formation in the *T. pseudospiralis*-infected muscle. Cyst wall was not constructed at the periphery of the infected muscle cells.

Quantitative analysis of expression of MyoD, myogenin and MRF4

QC–PCR was used to determine the level of mRNA expression of MyoD, myogenin and MRF4. In *T.*

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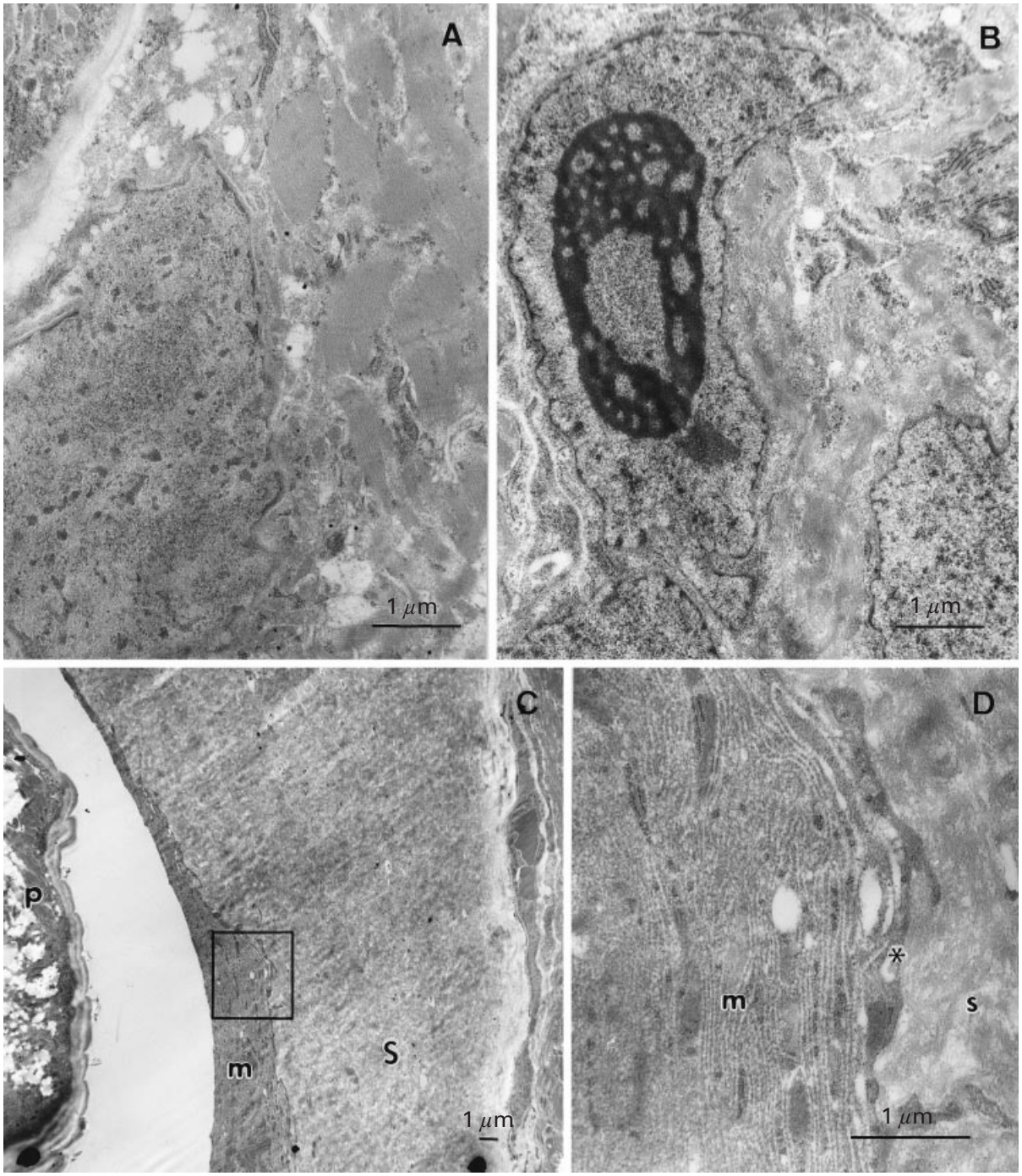

Fig. 4. Electron micrographs of muscle cells infected with *Trichinella pseudospiralis*. (A) The earliest change of muscle cells after the entrance of newborn larvae of *T. pseudospiralis*. Sarcomere is disintegrated and the nuclei are hypertrophic. (B) At 3 months after the infection, the muscle cell has a hypertrophic nucleus and prominent nucleolus. Amorphous materials occupy some area in the cytoplasm. (C and D) There are two kinds of muscle cell cytoplasm, one is the infected muscle cell origin (m) and the other is satellite cell origin (s). The former has extensive endoplasmic reticulum and the latter has amorphous material. The 2 kinds of cytoplasms are separated by the plasma membrane (*). (D) High power view of the rectangled portion in (C).

spiralis infection, as shown in Fig. 5 and Table 1, the level of expression of MyoD was quite low or undetectable before infection. The amount of mRNA significantly increased to 44·348, 97·522 and 70·925 pg per 100 mg of the muscles at 8, 13 and 18 days p.i., respectively. Then after a time-point of 23 days, the expression decreased to the level seen in the normal muscle. A similar result was obtained with myogenin expression, which showed a high expression at 8, 13 and 18 days p.i. (Fig. 5 and Table 1). On the contrary, the expression of MRF4 was essentially unchanged before and after infection (Fig. 5 and Table 1).

In *T. pseudospiralis* infection, the expression of MyoD and myogenin was significantly increased at 8, 13 and 18 days p.i. As shown in Fig. 5 and Table

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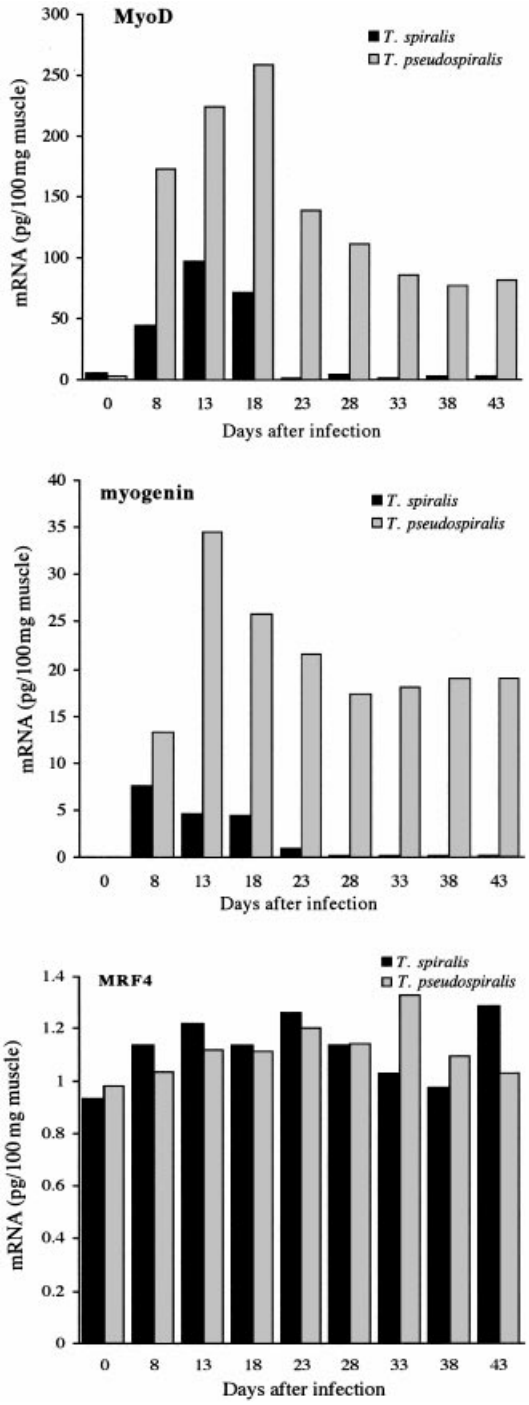

Fig. 5. Expression levels of myogenic regulatory factors in the muscle infected with *Trichinella spiralis* or *T. pseudospiralis*, quantified by the QC–PCR method. (A) MyoD; (B) myogenin; (C) MRF4.

1, the amount of mRNA of MyoD increased from 2·216 before infection to 172·390 at 8 days p.i., 224·112 at 13 days p.i. and 258·580 pg per 100 mg of the muscles at 18 days p.i. After 23 days p.i., though it was somewhat decreased, the expression still remained high. The same is true for myogenin expression. The amount of mRNA of myogenin increased from 0·086 before infection to 13·360 at 8 days p.i., 34·478 at 13 days p.i. and 25·858 pg per 100 mg of the muscles at 18 days p.i., and even at a time-point of 23 days, the expression was still at high level. The expression of MRF4 was similar to that in *T. spiralis* infection.

Immunohistochemical localization of MyoD

As shown in Fig. 6, positive reaction for MyoD expression was observed exclusively on the satellite cells of infected muscles. No reaction was observed on the infected muscle cell itself. The control serum resulted in negative staining suggesting the specificity of the immunostaining.

DISCUSSION

Histopathology associated with injury and subsequent repairing of the muscle tissue has been well documented (Grounds, 1991; Robertson, Papadimitriou & Grounds, 1993; Boding-Fowler, 1994; Chambers & McDermott, 1996), and has shown that the muscle repair process involves multiple steps including the removal of myofibrils, septum formation, satellite cell proliferation, differentiation to the muscle cell fusion, and replacement with damaged sarcoplasm. The present study shows that this normal repairing process was not followed after damage by *Trichinella* infection, that is, misdifferentiation of satellite cells occurred. Moreover, there were some differences in misdifferentiation between the two species of *Trichinella*, which suggest that *Trichinella* infection may afford an intriguing model to study muscle differentiation and regeneration.

Morphological changes after infection

Soon after the entrance by newborn larvae of *T. spiralis*, the muscle cell loses its characteristic myofibrils and transforms to the nurse cell (Despommier, Symmans & Dell, 1991; Jasmer, 1993; Matsuo *et al.* 2000). *T. pseudospiralis* infection also caused disintegration of myofibrils, although it did so more slowly.

In general, the damaged sarcoplasm of the muscle fibre is rapidly sealed off from the nearby undamaged sarcoplasm to minimize necrosis after injury (Grounds, 1991). This sealing also occurred after *T. spiralis* infection, and the sealed sarcoplasm became the nurse cell where the worm parasitized. This sealing, however, never happened after *T. pseudospiralis* infection. Consequently, the whole length of the muscle cell was affected by the intracellular infection of *T. pseudospiralis*, and a large number of satellite cells were activated.

Satellite cells are the source of post-natal growth and repair in adult skeletal muscle (reviewed by Bischoff, 1994). When activated through injury,

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Table 1. The expression levels of myogenic regulatory factors in the muscle infected with *Trichinella spiralis* and *T. pseudospiralis*

	MyoD*		myogenin*		MRF4*	
Days p.i.	*T. spiralis*	*T. pseudo*†	*T. spiralis*	*T. pseudo*	*T. spiralis*	*T. pseudo*
0	5·541	2·216	0·089	0·086	0·931	0·981
8	44·328	172·390	7·520	13·360	1·135	1·035
13	97·522	224·112	4·655	34·478	1·219	1·120
18	70·925	258·580	4·433	25·858	1·135	1·109
23	1·773	137·904	0·887	21·549	1·263	1·200
28	3·989	112·053	0·222	17·239	1·135	1·140
33	1·108	86·190	0·244	18·100	1·025	1·330
38	2·837	77·587	0·188	18·929	0·975	1·096
43	2·837	81·887	0·111	19·002	1·285	1·027

* pg/100 mg muscle.
† *T. pseudospiralis*.

satellite cells initiate stem cell activity and gene expression that leads to the regeneration, replacement and hypertrophy of skeletal muscle fibres (Bischoff, 1994; Yablonka-Reuveni, 1995; Cornelison & Wold, 1997). In regenerating muscle, these myoblasts fuse with each other or with existing myofibres to form new myofibres (Robertson *et al*. 1993; Chambers & McDermott, 1996; Luque *et al*. 1996). The satellite cells proliferate and fuse with the damaged muscle cell in *T. spiralis* infection following the same process as normal healing, but does not differentiate to a normal muscle cell. The satellite cell misdifferentiated to the nurse cell (our present results; Matsuo *et al*. 2000).

T. pseudospiralis infection also caused satellite cell proliferation more extensively than did *T. spiralis* infection. But the satellite cell developed amorphous cytoplasm which does not fuse with the infected muscle cell. The two kinds of cytoplasm coexisted when examined up to 43 days p.i.

Kinetics of myogenic regulatory genes

Recent molecular studies indicated that some genes involved in normal muscle differentiation are also involved in the repairing process. MyoD and myogenin expression is high during embryonic development, but low in mature muscle (Eftimie, Brenner & Buonanno, 1991; Hughes *et al*. 1993; Voytik *et al*. 1993). The expression level changes after cross-reinnervation or hormone treatment (Hughes *et al*. 1993), denervation (Voytik *et al*. 1993), increase of functional load (Adams, Haddad & Baldwin, 1999; Sakuma *et al*. 1999), or pharmacologically and surgically induced hypertrophy (Mendler *et al*. 1998; Mozdziak, Greaser & Schultz, 1998). Adams *et al*. (1999) showed that kinetics of myogenin expression increased at 6 h after overload stimulus, reached a peak at a 24 h time-point and then decreased.

Our present study using quantitative RT–PCR revealed that the kinetics of such regulatory genes after *Trichinella* infection resembled those after muscle damage and those during muscle differentiation and regenerating. There were, however, some differences in the kinetics between *T. spiralis* and *T. pseudospiralis* infection. In *T. spiralis* infection, MyoD and myogenin were expressed transiently in the early phase from 8 days p.i. (that is, 2 days after newborn larva entrance) to 23 days p.i. when the nurse cell had been formed. In *T. pseudospiralis* infection there was also an initial increase of expression, but it remained high until the end of the observation (43 days p.i.). The difference in kinetics seems to reflect the difference in the size of the affected area in the infected muscle cell, because in *T. spiralis* infection, the affected area is sealed off with the cyst wall by 18–23 days p.i. while in *T. pseudospiralis*, the full length of the infected cell remained affected and the satellite cell was proliferated even at 43 days p.i.

Reportedly, satellite cells express MyoD and myogenin, which is confirmed in injured muscle (Koishi *et al*. 1995; Cooper *et al*. 1999), proliferating myoblasts (Yablonka-Reuveni & Rivera, 1994) and newly formed myotubes (Fuchtbauer & Westphal, 1992). Also, in *Trichinella* infection, MyoD was exclusively expressed in satellite cells adjacent to infected muscle cells.

From the present results demonstrating increased population of the satellite cells and increased expression of myogenic regulatory factor in the satellite cell, it is not unreasonable to conclude that *Trichinella* infection causes satellite cell activation resembling that in muscle differentiation and/or after muscle damage.

MRF4 is present in adult muscle and is responsible for maintenance of muscle phenotype (Walters, Stickland & Loughna, 2000). *Trichinella* infection did not alter the expression level of MRF4, even if half the muscle cells were infected with *T. pseudospiralis*.

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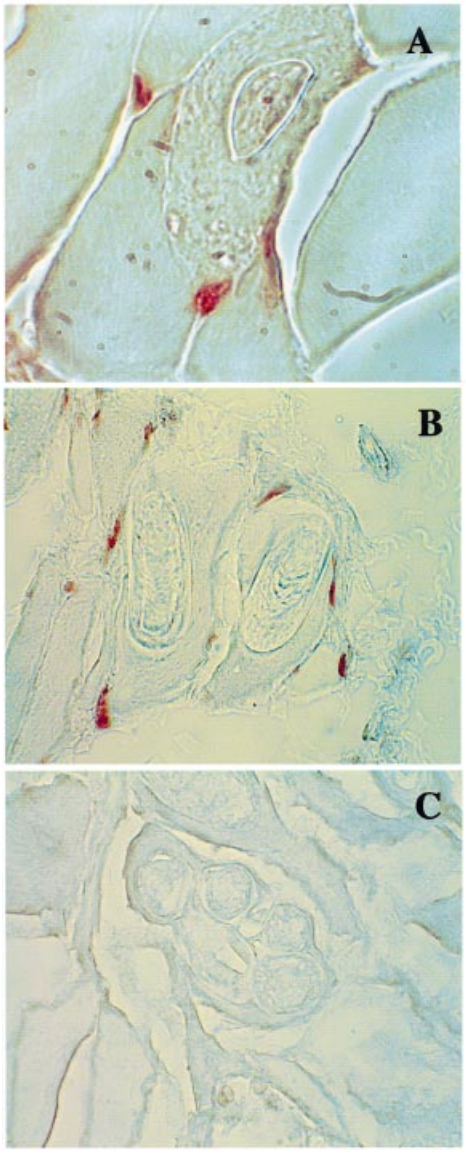

Fig. 6. Immunohistochemical staining of the hindlimb muscle infected with *Trichinella pseudospiralis*. The cross-section of the muscle at 18 days after infection, stained with anti-MyoD antibody(A); at 38 days after infection, stained with anti-MyoD (B); and 38 days after infection, stained with a normal rabbit serum (C). Brown colour indicates positive reaction.

Difference between T. spiralis *and* T. pseudospiralis

The two species of *Trichinella* are similar but different in the effect against host muscle cells. The present study showed a clear observable difference in satellite cell response among infection of the two species.

To explain molecular mechanisms underlying their similarities and differences, a closer examination of excretory and secretory (ES) products are intriguing because they may alter gene expression of infected muscles (Despommier *et al*. 1990; Lee *et al*. 1991; Vassilatis *et al*. 1992; Jasmer, 1993; Ko *et al*. 1994; Despommier, 1998; Yao & Jasmer, 1998*a*; Yao, Bohnet & Jasmer, 1998*b*). An example is the 43 kDa glycoprotein in ES products. They have the HLH motif and are transported to the nucleus of the nurse cell after secretion from the parasite. It is supposed that the ES products initiate or induce the transformation of the infected cell. This hypothesis is supported by a recent finding which demonstrated that 2 kinds of proteins from *Trichinella* larva might be related to myogenic factors. One protein (Tsmyd-1) contains a highly conserved bHLH (Connolly, Trenholme & Smith, 1996), and another (TsJ5) shares functional properties with negative regulators of the myogenic bHLH family of proteins (Lindh *et al*. 1998). The ES products of the two species of *Trichinella* are similar but different (Zhang, Lee & Smith, 1993; Wu, Nagano & Takahashi, 1998, 1999). Therefore, shared ES products may be responsible for satellite proliferation, and unique ES products may be responsible for differences in interfering with satellite cell differentiation.

Some ES products are currently produced by gene engineering technology (Vassilatis *et al*. 1992; Chung & Ko, 1999). These fusion proteins may be used as a tool to analyse this unique host–parasite interaction at the molecular level, because many regulatory genes are likely involved in different steps during satellite cell differentiation, including specific muscle regulatory genes, actins, myosins, tropomyosins, desmin and M creatine phosphokinase (see review by Grounds, 1991). And each ES product may have a specific site for interfering with satellite cell differentiation. Thus, *Trichinella* infection in muscle cell likely provides an excellent model to dissect the sequence of steps leading to successful satellite cell differentiation.

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