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EXPRESSION OF MyoD AND MYOGENIN IN MUSCLES OF MICE EXPERIMENTALLY INFECTED WITH *TRICHINELLA SPIRALIS* OR *TRICHINELLA PSEUDOSPIRALIS*

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Summary :

We developed a detection system for myogenic regulatory factors such as MyoD and myogenin. Adapting the method we performed a longitudinal analysis of such regulatory factors after infection with *T. spiralis* and *T. pseudospiralis*. MyoD and myogenin were expressed from the early phase of cystogenesis in *T. spiralis* infection. The expression returned to the normal level after 18 days from the infection when the cyst was complete. In *T. pseudospiralis* infection, they were also expressed from the early phase of cystogenesis, but continuously expressed at least up to 43 days post infection.

KEY WORDS : *Trichinella spiralis, Trichinella pseudospiralis,* nurse cell, satellite cell, differentiation, MyoD, myogenin.

Establishment of parasitism of *Trichinella* results in transformation of muscle cells leading to the nurse cell formation (see review by Despommier, 1998). An essential question that immediately comes to one's mind is how terminally differentiated muscle cells can transform into another. In this paper we will show how the satellite cell is involved in the nurse cell formation.

MATERIALS AND METHODS

INFECTION

Nude mice were orally infected with muscle larvae of *T. spiralis* or *T. pseudospiralis,* and at the certain time points up to 43 days post infection, host muscle tissues were processed for kinetic study of the gene expression.

TOTAL RNA ISOLATION

The muscle sample (100 mg) was sliced into small pieces and homogenized in 1 ml of TRIZOL (Life Technologies, Inc., NY). The suspension was transferred to a 1.5 ml tube and incubated for five min at room temperature. The insoluble material was removed by centrifugation at 12,000 g for 10 min at 4° C, and the supernatant was mixed with the 200 µl of chloroform. After the centrifugation, the aqueous phase was transferred to a new 1.5 ml tube, and mixed with 0.5 ml of isopropyl alcohol. Thus total RNA was precipitated and dissolved in 80 µl of RNase-free water, and incubated for 10 min at 60° C.

RT-PCR

Reverse transcription (RT) was carried out using Ready-To-Go kit (Pharmacia Biotech). Three ul of sample RNA and 1 µl of 0.5 µg/µl Oligo(dT)12-18 (Pharmacia Biotech) were added to a Ready-To-Go tube, and RNase-free water to give the final volume of 33 µl. The tube was incubated at 37° C for 60 min, and then at 90° C for five min. PCR primers were developed based on the reported sequence of MyoD (Davis *et al.*, 1987) and myogenin (Edmondson & Olson, 1989). The oligonucleotide sequence for MyoD primers were CGCCTGAGCAAAGTGAATG and TGTGCTATGAGGAAAGGAAGAG. The expected size of PCR product was 933 bp. The oligonucleotide sequence for myogenin primers were TCTACCGGAGCCCCACTTC and CATCAGGACAGCCCCACTTA. The expected size of PCR product was 744 bp.

PCR was performed by mixing the following reagents: 1 µl of reverse transcription product, 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, TAKARA), and 7.94 µl of distilled water to give the final volume of 15 µl.

PCR condition: step 1: one cycle of 94° C for three min; step 2: 35 cycles at 94° C for 30 sec, 56° C for 30 sec and 72° C for two min; and step 3: one cycle at 72° C for 10 min for the final extension. Ten ul of PCR product was loaded for agarose gel electrophoresis. The gel was stained with ethidium bromide.

RESULTS

Figure 1 shows RT-PCR results with samples of the *T. spiralis* infected muscles. The bands with the expected bp length for MyoD and myogenin

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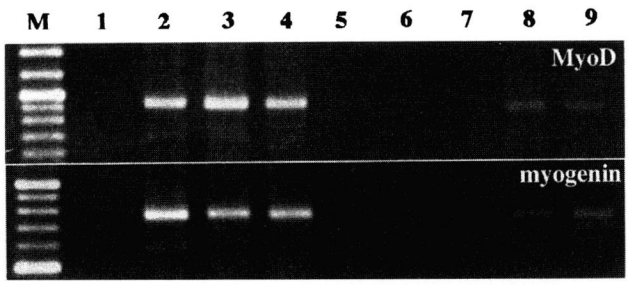

Fig. 1. – RT-PCR results for MyoD and myogenin expression in *T. spiralis* infection. Lane 1 shows the result from a normal muscle. Lanes 2 to 9 show the results from infected muscles at 8, 13, 18, 23, 28, 33, 38 and 43 days post infection, respectively. M: 100 base pair ladder of molecular weight marker.

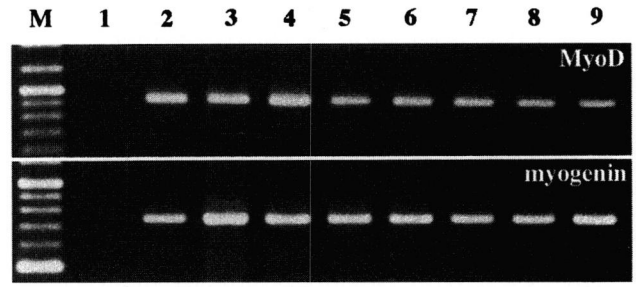

Fig. 2. – RT-PCR results for MyoD and myogenin expression in *T. pseudospiralis* infection. Lane 1 shows the result from a normal muscle. Lanes 2 to 9 show the results from infected muscles at 8, 13, 18, 23, 28, 33, 38 and 43 days post infection, respectively. M: 100 base pair ladder of molecular weight marker.

were detected in the lanes 2 to 4, but not in the lanes 1 and 5-9. This implied these regulatory factors were expressed at least between 8 dpi to 18 dpi. Normal muscle tissues did not express these factors at a detectable level. No bands were detected between 23 and 43 dpi, which meant the expression returned to the normal level.

In the *T. pseudospiralis* infection, the positive bands with the expected bp length for MyoD and myogenin were detected in the lanes from 2 to 9, but not in the lanes 1 (Fig. 2), which revealed the expression of MyoD and myogenin began at least from 8 dpi and remained high as far as analyzed in this experiment. The experiment was repeated three times and resulted in the same banding pattern.

DISCUSSION

Changes of muscle cells after *Trichinella* infection is known as nurse cell formation and biologically known as transformation of muscle cells (see review by Despommier, 1998). This study revealed the kinetics of myogenic regulatory factors during the nurse cell formation by RT PCR method for MyoD and myogenin. The result revealed that the kinetics resembled those seen in myogenesis, embryogenesis, and in muscle cell repair after injury (Fuchtbauer & Westphal, 1992). These factors, MyoD and myogenin, are derived from the satellite cells, therefore it is conceivable to conclude that the satellite cell activation is associated to the nurse cell formation after *Trichinella* infection. Satellite cells are to be activated after muscle cell injury and differentiate to muscle cells (see review by Grounds, 1991; Carlson & Faulkner, 1983). In the mature cyst, however, no structures as a muscle cell are seen (Matsuo *et al.*, 2000). This means the satellite cells do not differentiate to muscle cells but differentiate to the nurse cell whose cytoplasm is completely different from those of any other cells in the host tissue.

There is notable difference in the pattern of expression of the regulatory factors between *T. spiralis* and *T. pseudospiralis* infection. The expression was just transiently increased in the early phase of the infection in *T. spiralis* infection, but the expression remained high in *T. pseudospiralis* infection. This likely implies some difference, probably duration, in satellite cell activation among two kinds of infection.

Some peptides in the excretory and secretory products are known to be transported to the nurse cell nucleus, which leads to the assumption that the transformation of muscle cell is triggered by such peptides (Lee *et al.*, 1991; Vassiliatis *et al.*, 1992). But our preliminary results showed that 43 kD peptides are localized hypertrophied nucleus in the cyst but not in the nucleus of the satellite cells (to be published elsewhere). Satellite cells are likely involved in the nurse cell formation, but no definite evidences are available what and how triggers such change.

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