THE VASCULATURE OF NURSE CELLS INFECTED WITH NON-ENCAPSULATED *TRICHINELLA* SPECIES

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Abstract. The vasculature surrounding the nurse cells of encapsulated *Trichinella spiralis* has been described previously. It has been postulated the function of these vessels is to support the growth of the parasite. We describe here for the first time the vasculature surrounding the nurse cells of non-encapsulated *T. pseudospiralis* and *T. papuae*. Similar to the vasculature of uninfected muscle cells, the vessels surrounding non-encapsulated *Trichinella* nurse cells are dense and branched longitudinally along the long axis of the muscle cells; they also appear to be similar in diameter. The netting pattern of enlarged vessels found around *T. spiralis* (encapsulated) nurse cells is not present in non-encapsulated *Trichinella* infections. The vessels surrounding non-encapsulated *Trichinella* nurse cells seem to exist prior to parasite invasion of the muscle cell.

Keywords: *Trichinella,* nurse cell, vasculature

INTRODUCTION

Trichinella is an intracellular parasitic nematode that infects skeletal muscle. Infection occurs by ingesting meat contaminated with the infective stage of *Trichinella* larvae. After the larvae develop into adults in the host intestine, mating occurs and gravid females release newborn larvae into the blood stream resulting in the spread of the larvae throughout the body. The larva infects muscle cells and transforms them into nurse cells which support the growth of the parasite.

There are many changes in the formation of the nurse cell infected with *T. spiralis,* including loss of myofilaments and replacement by rough endoplasmic reticulum (Despommier, 1976; Chang *et al,* 1988; Jasmer, 1993), enlargement and division of nurse cell nuclei (Chang *et al,* 1988; Despommier, 1993; Jasmer, 1993), mitochondrial damage by vacuolation (Despommier, 1975), and formation of collagen capsule surrounding the nurse cell (Polvere *et al,* 1997). The collagen capsule of the *T. spiralis* nurse cell is lemon-shaped and composed of collagen types IV and VI (Polvere *et al,* 1997; Nareaho, 2006). The *T. spiralis* larva is coiled and firmly enclosed in the collagen capsule of the nurse cell (Al Karmi and Faubert, 1981). The presence or absence of a collagen capsule is used to distinguish the species of *Trichinella* as either encapsulated or non-encapsulated species, respectively (Zarlenga *et al*, 2006).

Non-encapsulated species of *T. pseudospiralis* and *T. papuae* are able to form nurse cells. The obvious differences

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between the nurse cell of *T. spiralis* and the non-encapsulated *Trichinella* species are the latter do not form a collagen capsule and the cytoplasmic changes occur throughout the length of the infected muscle cell which is more elongated than that of *T. spiralis* (Al Karmi and Faubert, 1981; Chang *et al*, 1988; Pozio *et al*, 1999; Wu *et al*, 2001; Boonmars *et al*, 2005). A feature of the cytoplasm of the *T. pseudospiralis* nurse cell is eosinophilic staining with H&E stain, in contrast to the basophilic staining of the *T. spiralis* nurse cell (Matsuo *et al*, 2000; Wu *et al*, 2001; Boonmars *et al*, 2004). More acid phosphatase activity is observed in the nurse cells of *T. spiralis* than in those of *T. pseudospiralis* and is probably associated with muscle cell damage (Boonmars *et al*, 2004). Loss of myofilaments, increased number of endoplasmic reticulum and mitochondrial damage are found in the nurse cells of both *T. pseudospiralis* and *T. papuae* (Pozio *et al*, 1999). The nuclei of non-encapsulated *Trichinella* nurse cells are smaller than those of *T. spiralis*-infected nurse cells. Non-encapsulated larva appears both coiled and uncoiled and moves freely in the nurse cell (Al Karmi and Faubert, 1981).

One of the remarkable features of *T. spiralis* nurse cells is the vascular network surrounding the collagen capsule. The vascular network is a net of vessels surrounding the nurse cell which can be seen by injection of China ink (Ogielski, 1949) and by the intravascular precipitation of lead chromate (Humes and Akers, 1952). Blood vessel studies using an anatomical plastic cast in muscles infected with *T. spiralis* show details of the vascular network structure similar to a randomly woven basket or rete (Baruch and Despommier, 1991). These vessels have been postulated to function in the transportation of nutrients and disposal of waste products (Despommier, 1998). Until now, there have been no studies demonstrating the presence of this vascular network surrounding non-encapsulated species of *Trichinella* nurse cells. In this study, we attempted to delineate the vasculature of muscle cells infected with non-encapsulated *T. pseudospiralis* and *T. papuae*.

MATERIALS AND METHODS

Trichinella infected mice

Imprinting control region (ICR) mice were orally infected with 250 L1 stage *T. spiralis* or *T. pseudospiralis* larvae. The *T. spiralis* used in this study was isolated from a human in Mae Hong Son, Thailand, in 1986 (Pozio and Khamboonruang, 1989). The laboratory strain of *T. pseudospiralis* used in this study was generously provided by Professor Yuzo Takahashi, Gifu University, Japan. These parasites were maintained in ICR mice. The mice were raised at Animal Laboratory House, Faculty of Medicine, Chiang Mai University, Thailand. Swiss mice orally infected with 40 L1 *T. papuae* larvae were kindly provided by Professor Pewpan Maleewong, Khon Kaen University, Thailand. This study was approved by the Animal Research Ethics Committee, Faculty of Medicine, Chiang Mai University.

Muscle perfusion with ink

Ink perfusion for the observation of blood vessels surrounding *Trichinella* nurse cells was conducted using the modified method of Baruch and Despommier (1991). The mice were infected with *Trichinella* at least 3 months prior to the study. The *Trichinella*-infected mice were euthanized by intraperitoneal injection with 0.3 ml of sodium pentothal at a concentration of 65 mg/ml. The chest cavity was opened and a hole was made in the right atrium.

Heparinized saline (500 U of heparin/ml) was then immediately instilled into the left ventricle and flushing was continued until the solution flowing from the right atrium was clear when the 50% Rotring drawing ink (black) in saline was injected until the ears, feet and tail turned gray. The muscles infused with ink were surgically removed and the vasculature of the *Trichinella*-infected muscles was studied using a muscle compression technique, under an Olympus AX70TF differential interference contrast (DIC) microscope or an Olympus BX51 DIC microscope. The blood vessels adjacent to the infected muscle surface in the same focal plane were then measured.

Histological study

Trichinella-infected gastrocnemius muscles perfused with drawing ink were fixed with 10% buffered formalin and dehydrated using increasing concentrations of alcohol: 80% ethanol for 30 minutes twice, 95% ethanol for 20 minutes three times and 100% ethanol for 20 minutes three times. The muscles were cleared with xylene for 20 minutes three times. The specimens were then placed in melted paraffin at 60°C for 2 hours. The paraffin-embedded muscles were sectioned at 5 m thick and placed on silanized slides. The sections were stained with H&E stain according to conventional method and examined under the microscope (Olympus AX70TF DIC).

RESULTS

The normal vasculature of skeletal muscle sections is shown in Fig 1A. The vessels of uninfected muscle are dense and longitudinally branched along the long axis of the muscle fiber. The vessels supplying the muscle cells are branches of the larger vessels. The sizes of the vessels range from 4 to 6 m in width.

T. spiralis-infected nurse cells are found to have blood vessels surrounding the collagen capsule in a netting pattern or rete (Figs 1B, 2A and 2B). Some vessels are seen in the collagen layer (Fig 2A). The diameters of the vessels surrounding the nurse cells are generally larger than those in the uninfected muscles (Figs 1B, 2A and 2B). The sizes of the vessels in the rete range from 4 to 12 m.

Examination of *T. pseudospiralis* and *T. papuae* infected muscles reveals that the vessels supplying the nurse cells are dense and run along the length of the muscle (Fig 1C and 1D). The sizes of the vessels are 4 to 6 m in diameter and their branching patterns appear to be similar to those of adjacent uninfected muscle cells. Unlike *T. spiralis*-infected muscles, no netting patterns of vessels are seen around *T. pseudospiralis* and *T. papuae* infected muscle cells. Paraffin sections confirm that the vessels surrounding muscle cells infected with *T. pseudospiralis* seem to be not different from those surrounding uninfected muscle cells (Fig 2C and 2D).

DISCUSSION

It has been known for some time angiogenesis leads to the vascular network around nurse cells infected with *T. spiralis*; occurs during nurse cell formation (Ogielski, 1949; Humes and Akers, 1952; Baruch and Despommier, 1991). In this study, we describe the vasculature of nurse cells infected with non-encapsulated *T. pseudospiralis* and *T. papuae*. There are differences between the vasculature of encapsulated and non-encapsulated *Trichinella*-infected muscles. Nurse cells in *T. spiralis* infection are surrounded by a network of blood vessels with an average diameter larger than those of uninfected muscles. Humes

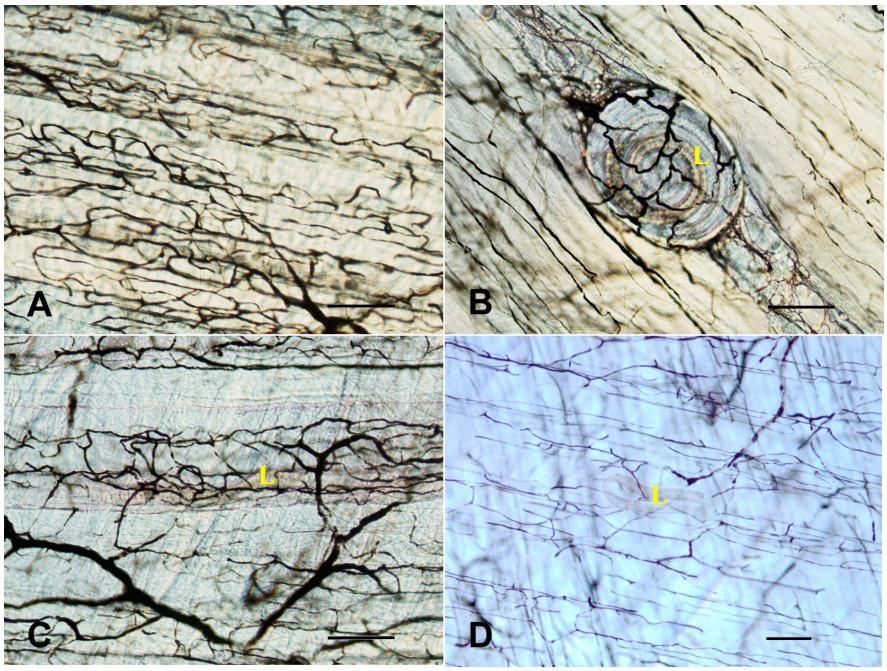

Fig 1–Photomicrograph of the vasculature perfused with drawing ink in an uninfected muscle cell (A), *T. spiralis* (B), *T. pseudospiralis* (C) and *T. papuae* (D) nurse cells. A, B and C were captured by an Olympus AX70TF DIC microscope whereas D was captured by an Olympus BX51 DIC microscope. The yellow L indicates the first stage larva of *Trichinella* (B). Scale bar = 100 m.

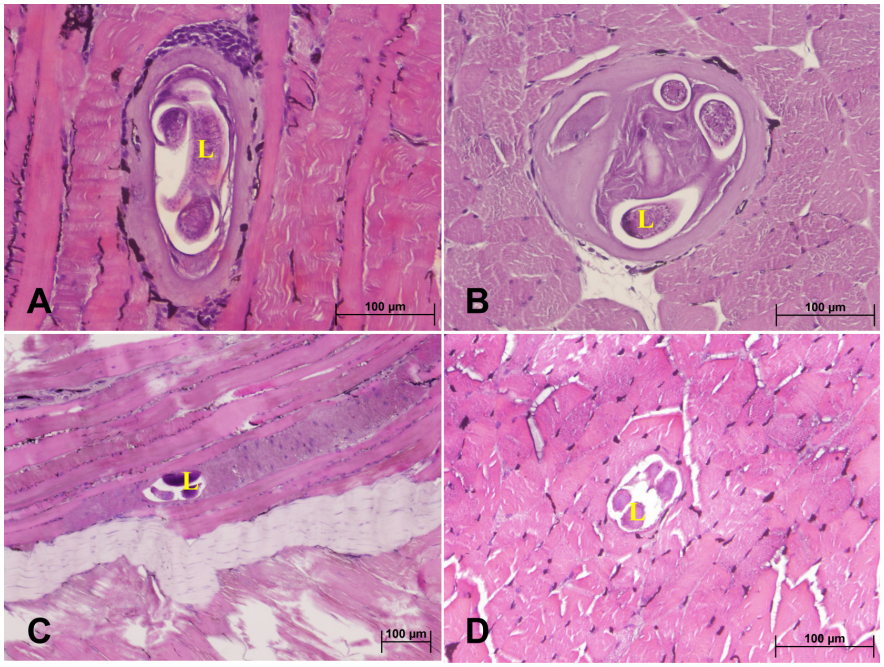

Fig 2–Longitudinal (A and C) and transverse (B and D) sections of *T. spiralis*-(A and B) and *T. pseudospiralis*-(C and D) infected muscles stained with H&E. Blood vessels appear black with perfused drawing ink. The yellow L indicates the first stage larva of *Trichinella*.

and Akers (1952) found that the vascular network around nurse cells in *T. spiralis* infection was composed of novel vessels, first visible on day 19 of infection. With non-encapsulated *Trichinella* infection the vasculature of the nurse cells does not appear to be any different from that of uninfected muscle cells in both pattern and size. The blood vessels surrounding *T. pseudospiralis* or *T. papuae* nurse cells probably existed prior to parasite invasion without any apparent neovascularization. Since the collagen capsules of *T. pseudospiralis* and *T. papuae* nurse cell are thin or unseen and the whole length of the muscle cell is affected (Xu *et al,* 1997), the preexisting blood vessels surrounding them may be sufficient to supply the nurse cells and the larvae without the need to increase vascularization. In contrast, extra blood vessels are formed around *T. spiralis* nurse cells, perhaps to overcome the thickness of the collagen capsule, which could impede the transfer of nutrients, waste products and oxygen.

Following invasion of the muscle cells by *T. spiralis*, a septum is formed enclosing the larva to limit the damage and separate it from uninfected area (Hulinska *et al,* 1985; Wu *et al,* 2001, 2008b). Subsequently, the cytoplasmic changes of infected cells occur including cell and nuclear enlargement, mitochondrial dysfunction, and thick collagen capsule formation around the nurse cell (Despommier, 1993). These phenomena may lead to a lack of nutrients and oxygen supply to the nurse cell and parasite. Hypoxia is a major stimulator of the production of vascular endothelial growth factor (VEGF) which has been known to play a key role in angiogenesis (Shweiki *et al,* 1992). There have been a few observations showing the expression of both VEGF mRNA and protein in *T. spiralis* nurse cells (Capo *et al,* 1998; Kang *et al,* 2011). It has also been shown *in vitro* that *T. spiralis* larval antigens can induce VEGF expression via an unknown mechanism (Shariati *et al,* 2009). Following VEGF expression, angiogenesis begins on approximately day 12 and ceases by day 26 after the onset of infection (Despommier, 1998).

Muscle cells infected by non-encapsulated *T. pseudospiralis* and *T. papuae* are extensively damaged probably because there is no septum or collagen capsule formation (Al Karmi and Faubert, 1981; Hulinska *et al,* 1985; Pozio *et al,* 1999). *T. pseudospiralis* has more active movement in the muscle than *T. spiralis* (Al Karmi and Faubert, 1981). These may explain why the entire length of a non-encapsulated *Trichinella*-infected muscle cell is altered resulting in an elongated shape of the nurse cell. Our data suggest angiogenesis surrounding nurse cells infected with *T. pseudospiralis* and *T. papuae* may not occur because there is no difference between the vasculature of the nurse cells and uninfected muscle cells. *T. pseudospiralis* antigens may not be able to induce the expression of VEGF, an important factor in angiogenesis (Shariati *et al,* 2009). This finding contradicts another study that showed that VEGF expression in *T. pseudospiralis* infected muscle cells was up-regulated (Wu *et al,* 2008a). However, there have been few studies on the relationship between the vasculature of *Trichinella*-infected muscle cells and VEGF expression during nurse cell formation. Further studies are needed to clarify this issue.

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