Trichinellosis as a model of new frontier research on parasitic infection

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Introduction

Despite the fact that many parasitic infections have been studied over a century, little is known about the molecular basis of intracellular and intercellular adaptations of the causative organisms. In particular, few studies have been undertaken to elucidate the interactions between the parasitic molecules and the host cells at the nuclear level. Yet such knowledge can revolutionise the traditional concept of pathogenesis of parasitic organisms. It is also crucial for understanding the evolutionary/biological relationship between parasites and other microorganisms.

Trichinella belongs to a unique superfamily of nematodes, the trichinelloidea (Anderson & Bain, 1982). Members of this group have unconventional life histories, and are closely associated with the epithelia at least during part of their developmental cycle. They are either monoxenous or heteroxenous, and autoinfections can occur. They may not follow "the law of the third-stage larva" of secrementian nematodes in the infection of the definitive host where they can multiply in large numbers (see Wright, 1989). Depending on conditions, some females can either produce eggs or larvae (Cross *et al.*, 1978). Several species show an amazing lack of host specificity e.g. they can live alternately in distinct classes of vertebrates. Many species, which are highly pathogenic, are of great medical and veterinary importance.

The Scenario

Among the trichinellid nematodes, *Trichinella* is one of the most extensively studied genus. However, the size of the genus, and the status of its various "isolates" have remained controversial until recently. This is due to the lack of distinctive morphological characters. On the basis of the gene pools identified by allozyme analysis, Pozio *et al.* (1992) recognised five valid species i.e. *Trichinella spiralis sensu stricto*, *T. pseudospiralis*, *T. nativa*, *T. britovi*, and *T. nelsoni*. Each species can be separated by biological parameters. In this essay, however, only the main features of the first two species during their muscle phase of development will be discussed.

What are the unique characters of *T. spiralis*? This nematode can infect practically all mammals, including marine species. It is a monoxenous parasite that can disseminate its progenies directly within the vertebrate host. The infective-stage larva, which is encapsulated in host muscle, is a first-stage larva. After entrance into a mammalian host, it will undergo development in the small intestinal epithelium at an exceptional speed, moulting four times, and becoming the adult stage within 36 hours postinfection (Fig. 1). In the intestine, *Trichinella* lies within the cytoplasm of epithelial cells (Dunn & Wright, 1985). The female worms produce newborn larvae (NBL) (first-stage larvae) as soon as 5-6 days postinfection. These larvae migrate to striated muscles at various regions of the body (via blood or peritoneal cavity) where they transform the muscle cells into a new type of syncytium known as the "nurse cell" complex (Fig. 2). Each complex, which is encapsulated by collagenous fibres, can accommodate as many as four worms (Li & Ko, 1999). It represents a secluded, self-sustaining, and well-protected habitat. Within three weeks postinfection, the NBL grow into the precocious infective stage larvae without moulting. The genital primordium of the larvae is well developed, and the sexes can be separated. There is only a mild inflammatory response during the muscle phase of worm development.

T. pseudospiralis is morphologically similar to *T. spiralis*. However, this species, which was originally recovered from a racoon, *Procyon lotor*, is also infective to birds (Garkavi, 1972; Saumier *et al.*, 1988). Unlike *T. spiralis*, it does not elicit the formation of nurse cells in infected muscles. The infective stage larvae live in a non-secluded habitat i.e. the site is not encapsulated, and the worms can migrate along the myofibres while causing virtually no inflammatory response. Due to these differences, parallel experiments based on the two species may help to identify the key molecules or mechanisms involved in transforming the functions of the host muscle cells.

Although trichinellosis caused by *T. spiralis* is commonly documented, the first case of human infection by *T. pseudospiralis* was only reported in New Zealand in 1991 (Andrews *et al.*, 1994). Since then, numerous outbreaks have been reported in Thailand and Russia (Jongwutiwes *et al.*, 1998; information from the International Commission on Trichinellosis). Recently Chung & Ko (1999) successfully cloned a specific excretory/secretory antigen of *T. pseudospiralis*, which can be used in differential diagnosis.

How *Trichinella* does it? The working hypothesis

Trichinella lives in two precarious, dynamically unstable habitats i.e. the intestinal epithelium and striated muscles. The mucosal epithelium is replaced every 2-4

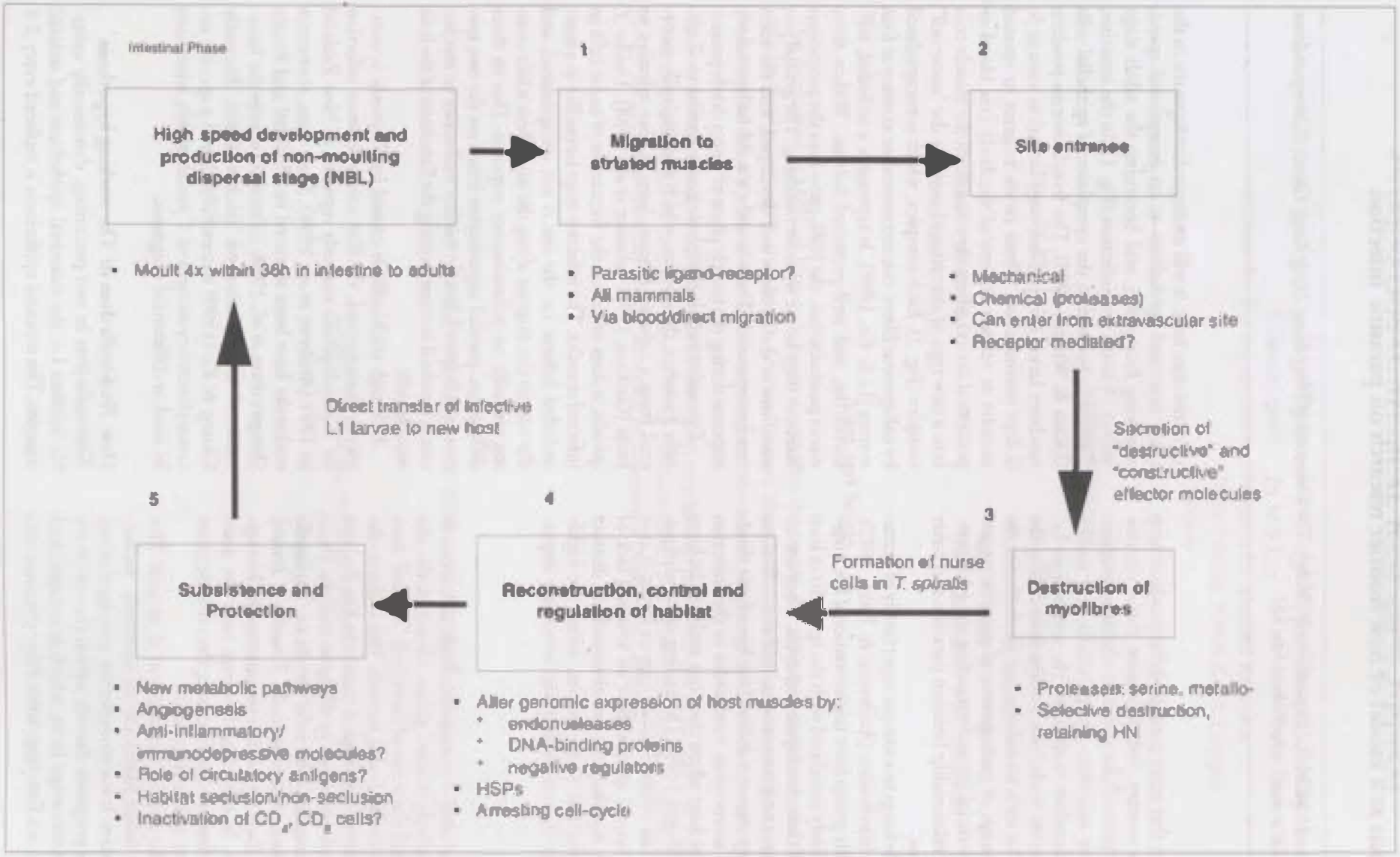

Fig. 1. Summary of the major adaptive events in the muscle phase of *Trichinella spiralis* infection.

NBL = new born larvae; HN = hypertrophic myonuclei; HSPs = heat shock proteins

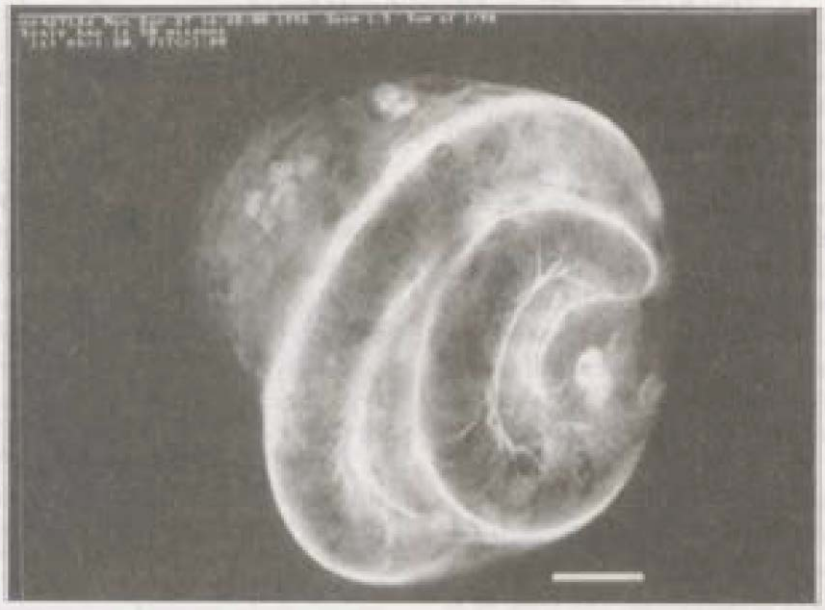

Fig. 2. Photograph of an intact nurse cell released from muscles by protease digestion, showing an infective larva of *Trichinella spiralis in situ*. The image was obtained by computer restruction of 40 optical serial sections (2.5 µm each). The cell had been reacted with mouse antiserum against ES antigens of *T. spiralis* and was stained with FITC. The preparation was examined using a laser confocal microscope. The scale bar denotes 50µm.

days. Worms within it must regularly migrate to new cells or risk being passed out. With the atrophy and hyperplasia of the epithelium, the quality and conditions of the development-nursery site are actively subjected to drastic fluctuations (Wright, 1989). In the striated muscles, the worms are also constantly subjected to traumatic changes due to muscular contractions/relaxations. This is despite the fact that infection with *Trichinella* can lead to major reductions in mechanical stress, work, power output, and fatigue resistance of muscles (Harwood *et al.*, 1996). In addition, in both the intestinal and muscular habitats, the parasite is easily exposed to the host's defence system.

Like other parasites, each phase of development of *Trichinella* is precisely compartmentalised, efficiently controlled, regulated, and secured (Fig. 1). This strategy can overcome the tremendous odds imposed by the negative host factors. Many fascinating adaptations have been evolved to manipulate the hostile host environment, thus ensuring a highly successful rate of worm survival.

In our earlier studies, Lee *et al.* (1991) and Ko *et al.* (1994) proposed a working hypothesis on the possible mechanisms of muscle cell reorganisation by *Trichinella*. The hypothesis was initially based on observing the presence of parasitic epitopes inside the hypertrophic myonuclei, and the marked lesions in muscles after exposure to the excretory/secretory (ES) products of the infective stage larvae. However, with more recent findings, the hypothesis is modified as follows:

The invasion of muscles by *T. spiralis* elicits two major independent events i.e. a general degenerative/regenerative response in muscles and specific change(s) in the genomic expression of myonuclei. The two events are mediated by a wide spectrum of specific effector molecules, which initiate the infectious process, destroying the existing host environment, and constructing a parasitic habitat. The effector molecules occur in the ES products of the first-stage larvae. One event leads to selective dissolution of myofibres, myotube formation, mitosis, migration of cells into muscle bundles and angiogenesis. The other involves folding, and translocating molecules through the nuclear membrane into hypertrophic myonuclei (HN) to control the functions of the host cells. The parasitic molecules may break/bind host DNA, alter, and regulate genomic expression, arrest the cell cycle, and to induce the synthesis of a protective capsule. The secretion of the effector molecules is regulated as the worm grows. However, the ability to reorganise host muscles is retained throughout the life of the larvae, even after development to the infective stage.

Features which can serve as models

The key to the *Trichinella*'s Pandora box lies in the analysis of the composition the ES products. They contain the adaptive molecules that can modulate the host's environment. Unfortunately, the nature of the ES products of trichinellids is poorly understood, and different molecules may be secreted at various phases of worm development. Ko & Wong (1992) found that the ES antigens from pre-encapsulated and encapsulated lar-

vae showed differences in SDS-PAGE profiles and in cross-reactivities with heterologous antisera in Triple-Antibody ELISA. Low molecular mass proteins dominate the former ES products, and the antigenic profile resembles that of the adult worms reported by Ko & Yeung (1989). Recently, we have identified and characterised several important molecules, which may be closely associated with the complicated process of host cell reorganisation. Although their actual biological functions have not been documented, the possible roles are discussed below. This provides the framework for further studies. Hopefully this brief review will stimulate more interest on the mechanisms of tissue colonisation by nematodes.

Site-targeting and entrance

Nematodes are very successful in "homing-in" their different preselected tissues/organs along their migratory path at various phases of development. The NBL of *T. spiralis* is myotrophic, and they reach the striated muscles mainly via the circulatory system. However, they can also arrive at the site by direct migration via the peritoneal cavity. NBL could easily be recovered from the peritoneal cavity by washing with saline, followed by centrifugation. Subcutaneously injected NBL can also penetrate, and enter mammalian or avian muscles. The NBL is morphologically primitive, and the internal structures are poorly differentiated. Without any obvious sensory structure at the cephalic or other regions of the body, what is the guidance system used by NBL for site finding?

Although a small percentage of NBL may enter other tissues by accident (in case of heavy infections), most will reach the skeletal muscles. In view of this, the worms must follow an efficient and specific marker-site recognition system. One possibility is using a ligand-receptor system that has been adopted by some protozoa. For example, *Cryptosporidium parvum* uses a glycoprotein of > 900 kDa as a ligand for a host receptor involved in attachment/invasion (Barnes *et al.*, 1998). Glycoprotein (GP) 63 is another well-known receptor involved in the invasion of *Leishmania* into macrophages (Chakraborty *et al.*, 1998). A 175 kDa erythrocyte-binding protein (EBA 175) mediates the invasion of *Plasmodium falciparum* into erythrocytes (Sim *et al.*, 1994; Dolan *et al.*, 1994). Liver-specific heparin sulphate proteoglycans (HSPGs) have been implicated in the targeting of sporozoites of malaria into the liver (Shakibaei & Frevert, 1996). *Trypanosoma cruzi* attaches and binds to mammalian cells by binding parasite trans-sialidase to host sialyl receptors (Herrera *et al.*, 1994). Major surface glycoproteins, including the SA85-1, have been identified as *T. cruzi* ligands of the mannose-binding protein. The binding of mannose-binding protein to microorganisms facilitates their uptake into phagocytic cells (Kahn *et al.*, 1996).

Although stage-specific surface antigens are well known to occur in many nematodes, most previous studies only dealt with immunological aspects. However, there is a possibility that some surface molecules may serve as a ligand in the initial invasion process. Antigens with molecular masses of 28-64 kDa have been reported on the cuticle of NBL (Philipp *et al.*, 1980,1981). It would be worthwhile to isolate and identify the surface molecules of NBL, and to determine their ligand serving potentials. There are numerous specific receptors associated with skeletal muscles e.g. nicotinic acetylcholine, alpha-dystroglycan, dihydropyridine, ryanodine, bradykinin B2 etc. (Dennis & Prody, 1997; Ervasti *et al.*, 1997; Aymard *et al.*, 1993; Schmoelzl *et al.*, 1996; Figueroa *et al.*, 1996). There is a possibility that some may serve as a marker molecule. Any positive finding would represent a major step forward in Parasitology.

Destruction of host tissues

Once the target has been located, NBL probably enter the myofibres by a combination of mechanical and chemical methods. If injected subcutaneously, NBL can penetrate the sarcolemma quickly. Although the enzymes secreted by NBL have not been studied (probably due to technical constraint), proteases have been found in the ES products of both the infective stage larvae and adults (Criado-Fornelio *et al.*, 1992; Lai & Ko, 1994; de Armas-Serra *et al.*, 1995a,b; Todorova *et al.*, 1995; Lai, 1996). Using gelatin-substrate electrophoresis, discontinuous protease assays, affinity chromatography, and *in vitro* cultures of different durations, Lai & Ko (1994) and Lai (1996) noted the presence of alkaline serine proteases of 49 and 57 kDa and a 47 kDa metallo-protease in the ES products of the infective stage larvae of *T. spiralis*. Serine proteases of 60 and 51 kDa were also observed in *T. pseudospiralis*. Other authors e.g. Criado-Fornelio *et al.* (1992) and de Armas-Serra *et al.* (1995a) also reported three proteases of 35, 62 and 230 kDa in *T. spiralis*. Armas-Serra *et al.* (1995a) purified the 35 kDa protease using continuous elution electrophoresis. Mocson & Wranicz (1999) reported the presence of cysteine proteinase.

The proteases may be involved in the breaking down of the sarcolemma. Some may also act as mediators for the entrance into host muscles as the metallo-protease, GP63, of *Leishmania* which is well known to play a major role in the intracellular invasion of macrophages (Chang *et al.*, 1989). After the NBL have entered the muscle cells, the proteases can also act as the "destructive molecules" to clear the site of host tissues. But one remarkable phenomenon is that the "cleaning up" mechanism is highly selective. The myonuclei, which by now have become hypertrophic (HN), are not destroyed but are retained to control the functioning of the new parasitic habitat. The HN are concentrated in

the form of a myotube. An exciting question is how can the nuclear membrane of the HN withstand the action of the proteases. If the underlying mechanism is known, it would serve as an effective chemotherapeutic method by blocking the formation of nurse cells at the beginning of its development.

In vitro and *in vivo* experiments (based on intramuscular injections, implantation of mini-osmotic pump, electron microscopy, and cell cultures) have shown that the ES products of the first-stage larvae of *Trichinella* can elicit marked lesions and tissue reorganisation in muscles (Ko *et al.*, 1994; Leung & Ko, 1997). The ES products were pyrogenic. They could induce the mobilisation of a wide range of mononuclear polymorphonuclear cells. Some cells were observed to enter muscle bundles in a cluster. The dissolution of myofibres in many muscle bundles was extensive. The addition of ES products to day 5 culture of primary myocytes induced drastic morphological and structural changes in myotubes. Distinct nodular structures were formed at intervals along the slender myotubes. Each node bore a prominent nucleus. Histological and immunocytolocalisation studies have revealed that the node contained large cavities enclosed by an intact sarcolemma. The cavities indicated the result of proteolytic digestion. Coculturing myocytes with NBL could also induce similar lesions. The sarcoplasm and sarcolemma both showed a strong fluorescence when tested by IFA for the presence of *Trichinella* ES antigens.

The intriguing question is how the protease can selectively digest the sarcoplasm but not the sarcolemma of the myotube. One speculation is that the protease recognises a specific receptor on the surface of the membrane. Alternatively, it may be highly substrate-specific towards the sarcoplasm of the myotubes. Such proteases have been reported in *Schistosoma mansoni* (Mckerrow *et al.*, 1985) and *Plasmodium falciparum* (Cooper & Bujard, 1992). The former parasite produces a specific collagenase that only cleaves collagens of the basal membrane but not those of the interstitial structures.

Besides proteases, *n*-butylamine has also been found in the ES products of *T. pseudospiralis*. Injection of this amine into the gluteal muscle of mice resulted in degenerative and regenerative changes. Thus, it was also implicated as one of the factors that could induce muscle reorganisation (Zenka *et al.*, 1980).

Constructing, controlling, regulating parasitic habitat

The construction of a parasitic habitat involves complicated changes to the original host muscles. This is initiated and mediated by a heterogenous mixture of "constructive" effector molecules secreted and regulated at various stages of larval development. In the ES products of both *T. spiralis* and *T. pseudospiralis*, we have documented for the first time the presence of heat shock proteins (HSPs), endonucleases, and DNA-binding proteins. These may directly or indirectly be involved in the process of nurse cell formation, and in affecting the host cell cycle.

Heat shock proteins

Using L-[^{35}S] methionine labelling and SDS-PAGE autoradiography, Ko & Fan (1996) discovered that at 43°C, the major HSPs in the somatic extracts of *T. spiralis* were 20, 47, 50, 70, 86 kDa, and in the ES products, 11, 45, 53, 64 kD. In *T. pseudospiralis*, the major HSPs in the somatic extracts were 20, 26, 31, 50, 53 and 86 kDa and in the ES products, 11, 35, 37, 41 and 64 kDa.

HSP 70 is one of the most common conserved HSPs in eukaryotes. Its substantial synthesis in *Trichinella* may have a distinct implication. HSP 70 has been incriminated as a "molecular chaperone" which can escort and influence the conformation taken by proteins, thus facilitating their transport across membranes. This is supposedly carried out by hydrophobic interactions between HSP and its charge (Newport *et al.*, 1988; Pelham, 1988). HSP 70 may play a role during the early phase of tissue invasion by folding DNA-binding effector molecules to facilitate their transport (via other mechanisms) into the target myonuclei.

DNA-binding proteins

In a preliminary study in our laboratory, Leung (1995) has observed that the ES products binds to the calf genomic DNA in South-western blotting. Band-shift assay using a random 20 mer oligonucleotide has shown that a single DNA-binding species is present. However, the DNA-binding specificity of the protein is low as the non-specific competitor poly (dI/dc) can effectively abolish the DNA-binding of ES protein in band shift assay. A DNA-binding of similar mobility was also detected in the ES products of *T. pseudospiralis*. Recently, using a modified technique, we have successfully documented the presence of DNA-binding proteins in the ES products of the two trichinellids (paper in preparation). The presence of DNA-binding proteins in the ES products strongly suggest that they may play a major role in modulating the expression of muscle genes, which is the key step required for constructing and maintaining the parasitic habitat.

Endonucleases

Recently, single- and double-stranded endonucleases have been demonstrated for the first time in the ES products of *Trichinella* (Mak & Ko, 1999). The finding of endonucleases in the ES products of a parasitic nematode may have broad biological implications, especially in the pathogenesis of intracellular invasion by multicellular organisms. It provides a new perspective of host-parasite interactions at the nuclear level.

The double-stranded endonuclease(s) of *Trichinella* is sequence non-specific, with a pH optimum below 6, and requires divalent cations as a cofactor. Its activity can be inhibited by the Zn^{2+} ion. It was detected mainly in the ES products of the infective-stage of *T. spiralis* collected at 37°C and was present in much smaller amounts in samples collected at 43°C and in the ES products of *T. pseudospiralis*.

The properties of the double-stranded endonuclease of *T. spiralis* differ from those of many well-documented endonucleases. Dnase I-like enzymes, which degrade chromosomal DNA during apoptosis, are divalent cation dependent and are mostly active at neutral pH (Peitsch *et al.*, 1993). DNase II enzymes, which play an important role in apoptosis, show acidic pH optimum and are divalent cation independent (Appierto *et al.*, 1997).

The endonucleases of *Trichinella* may have multiple functions that await further studies. The difference in the occurrence of endonuclease between *T. spiralis* and *T. pseudospiralis* may be correlated with the difference in pathogenesis between the worms; *T. spiralis* causes a drastic reorganisation of muscle cells whereas *T. pseudospiralis* does not. Jasmer (1993) suggested that muscles infected with *T. spiralis* are associated with a reduction in muscle-specific gene expression, including fibrillar proteins, and muscle transcriptional factors. Therefore, the occurrence of endonuclease only in a worm that can form nurse cells suggests that the molecule may play a crucial role in host cell transformation.

The endonucleases may also be involved in the arrest of the infected muscles at the G2/M phase of the cell cycle. According to Jasmer (1993), the hypertrophic nuclei of nurse cells are stably maintained at 4N complement of DNA. Muscle cells infected with *T. spiralis* are suspended at the G2/M phase of the cell cycle. There are numerous reports in the literature showing that persistent double-stranded breaks in chromosomal DNA, would not only destabilise DNA (accounting for genomic changes), but could also lead to the arrest of the G2 cell cycle, and eventually cell death (Bennett *et al.*, 1997; Barratt *et al.*, 1998). Further discussions on the cell cycle are given below.

Besides the above, the endonucleases of *Trichinella* may also act as a pathogenic determinant as in *Mycoplasma* and *Acholeplasm*, or play a role in degrading the host genome, dysregulating nucleic acid metabolism, and creating chromosomal alterations (Roganti & Rosenthal, 1983; Rice & Roberts, 1983; Minion & Goguen, 1986; Bendjennat *et al.*, 1997). They may behave like the endonuclease of Epstein-Barr virus, which provides nucleotides for DNA synthesis, or involve a host shut-off function as in herpes simplex virus (Feighny *et al.*, 1981; Krikorian & Read, 1991). Further studies on the biological functions of the endonucleases of *Trichinella* may yield some significant findings.

Effect on host cell cycle

Trichinella may affect muscle cell dedifferentiation. Besides modulating the architecture of the host cell, muscles infected with *T. spiralis* show an increase in lysosomal acid phosphatase (ACP) activity (Jasmer *et al.*, 1990). Hypertrophy of the myonuclei is associated with an increase in such activity. Transcriptional active HN are stably maintained at an approximate 4N complement of DNA. This suggests that terminally infected muscles reenter the cell cycle with DNA synthesis, and the nurse cells are suspended at the G2/M phase of the cell cycle (Jasmer, 1993).

The withdrawal of the cell cycle and the terminal differentiation of muscle cells are mutually exclusive. Retinoblastoma (Rb) has been suggested to play a central role in muscle differentiation. This is probably due to the close correlation between the high level of hypophosphorylated Rb and muscle differentiation (Kiess *et al.*, 1995; Martelli *et al.*, 1994). Skeletal muscle cells lacking Rb, which are arrested at the S/G2 phase of the cell cycle, are defective in differentiation. In Rb deficient muscles, the expression of myogenin and cyclin inhibitor (p21) is unaffected at the early stage of differentiation. However, the late differentiation markers (e.g. myosin heavy chain) are altered, and the cells fail to exit the cell cycle (Novitch *et al.*, 1996). Cyclin-dependent kinases, which regulate the progression of cell cycle via phosphorylation of specific cycle regulators (e.g. Rb), also modulate the activities of myogenic factors (Skapek *et al.*, 1995). It is possible that the serine/threonine kinase in the ES products of *T. spiralis* may be involved in the re-entry of the host cell cycle (Arden *et al.*, 1997).

Recently, Yao & Jasmer (1998) detected nuclear antigens (NA) with 79, 86 and 96 kDa (localized to host cell nuclei) by using antibodies against *T. spiralis* proteins. A monoclonal antibody against a defined epitope of glycans binded to the NA. The authors hoped to determine the role of NA in regulating the cell phenotype of the infected skeletal muscle.

Positive and negative regulators

Leung & Ko (1997) observed that ES products of NBL and infective stage larvae appeared capable of affecting the development of perfused myocytes under *in vitro* conditions. Shorter and more slender myotubes were formed when ES products were added to day 3 muscle culture. Network formation was also inhibited. A speculative explanation is that ES products contain negative regulators on muscle development. Blau (1992) suggested that positive and negative regulators modulate the differentiation of myocytes into myotubes. The former include MyoD1, myogenin, myf-5 and myf-6 which are DNA-binding proteins having a helix-loop-helix motif (Davis *et al.*, 1987). They recognise consensus sequences (e.g. E-box) in the muscle genes and en-

hance the production of muscle specific proteins in myocytes. Some negative regulators (e.g. dystrophin) are also DNA-binding proteins. Lindh *et al.* (1998) reported that a novel *Trichinella* gene, ssJ5, which shared common properties with negative regulators, was preferentially expressed in the infective stage larvae.

Another interesting phenomenon is that co-culturing with NBL appear to prolong substantially the survival time (about 6 more days) of the myotubes in culture (Leung & Ko, 1997). One possible explanation is that in the presence of NBL, the myocytes show a slower growth rate. The reduction in metabolic demand may, in turn, prolong the survival of the cells under culture. Alternatively, living NBL may provide some sustaining factors that help to extend the life span of myotubes. According to McLennan (1990), the histogenesis of myotubes in vertebrates is growth factor dependent. Some factors are required for maintenance and repair. In mice, they include insulin, catecholamines and prostaglandins (Platzer, 1978; Ontell *et al.*, 1988).

Subsistence and protection

Source of nutrients

T. spiralis lives in a well-secluded habitat. Under such condition, how can the larvae obtain nutrients? One of the major functions of the nurse cells of *T. spiralis* is to sustain the larvae accommodated within the complex. A unique feature of the nurse cell is that the cytoplasmic region is not separated from the cavity containing the worms by a membrane (Lee & Shivers, 1987). The cytoplasm is full of mitochondria, endoplasmic reticulum, and secretory vesicles, and contains mononuclear cells as well as two types of nuclei i.e. HN and small nuclei. The contents of the secretory vesicles are probably discharged directly into the cavity. It is not known whether the worms feed directly on the cytoplasm, or the secretions from the vesicles, or from the blood supply of the host. If the former is the method of choice, then there must be a continuous regeneration of the depleted cytoplasm. The functions of the two types of nuclei are also uncertain. HN, which are the original muscle nuclei, have been suggested to play a role in maintaining the extensive endoplasmic reticulum and its secretion into the cytoplasm (Lee *et al.* 1991). They may be involved in initiating nurse cell formation because they are the only nuclei at the site when the NBL penetrates the myofibres. However, the origin and function of the small nuclei are unknown.

Wright *et al.* (1989) and Baruch & Desportmier (1991) observed that an elaborate network of blood vessels surrounded the nurse cells of *T. spiralis*. Ko *et al.* (1994) noted that introduction of ES products into muscles of mice either by injection or implantation of a mini-osmotic pump could also induce angiogenesis. Baruch & Desportmier (1991) also suggested that the worm might induce angiogenesis directly through the secretion of unique products, or indirectly through the production of host-derived angiogenic factors. Recently, Capo *et al.* (1998) reported that the vascular growth factor was up-regulated within the nurse cell during its early stage of development. A constant state of hypoxia cell was supposedly maintained.

The circulatory rete can bring nutrients to the nurse cells and remove waste products from the worms. Li & Ko (1996) and Li *et al.* (1999) reported that in *T. spiralis*, although immunocytolocalisation studies revealed that ES antigens were localised within the nurse cell complex, substantial levels of these antigens could be detected in peripheral blood at various periods post-infection. (A "sandwich" time-resolved immunofluorescence assay (DELFIA system) was used). ES antigens probably enter the general circulation via the rete. Detection of circulatory ES antigens can serve as an accurate method for diagnosing acute trichinellosis. In contrast, the *in situ* distribution of ES antigens of *T. pseudospiralis* is widespread. ES epitopes can be detected along the infected myofibres and in adjacent non-infected areas. This is due to the non-secluded habitat selected by this trichinellid. However, it is not known whether a similar network of blood vessels encircles the site of *T. pseudospiralis*. Such network is likely absent because the worm supposedly migrates along the myofibres.

Using energy-dispersive X-ray microanalysis (EDXA) on bulk-frozen infected muscles, Wranicz *et al.* (1998) observed significant increases in phosphorus concentrations both within the nurse cells and inside the larvae. This could reflect changes in the energy metabolism and/or in the turnover of nucleic acids of the nurse cell. High phosphorus concentrations within the larval cells may correspond to high levels of thymidylate synthase, thus reflecting intensive DNA replications.

Encapsulation

Although both the first stage larvae of *T. spiralis* and *T. pseudospiralis* live in myofibres, only the former elicits the formation of a well-defined collagenous capsule. As the NBL of *T. spiralis* grows and develops into the infective stage larva, collagenous fibres eventually encapsulate the nurse cell. The capsule becomes apparent around day 9 after the arrival of the NBL. On one hand, the collagenous wall may provide a better structural support to the nurse cell for accommodating multiple larvae, and to enhance their survival in carrion. This would ensure a successful transmission. One can argue that the capsule may be analogous to the eggshell of oviparous monoxenous nematodes. On the other hand, it will also help to confine the ES products of worms within an enclosed system (Li *et al.*, 1999), and to protect the parasite from inflammatory cells and immune attack. Using scanning electron microscopy and protease digestion, Li (1996) showed that the external surface of isolated nurse cells was smooth, devoid of cellular infiltrates, except at the polar regions. At these re-

gions, about 30-50 cells including lymphocytes and fibrocytes in a fibrous matrix were attached to the surface. Therefore, protection from host attack is likely the dominant role of encapsulation of *T. spiralis*.

Different views have been given on the origin of the collagenous capsule. According to Stewart (1995), three different hypotheses have been proposed: (1) the capsule is formed by the altered muscle fibre alone (Teppema *et al.*, 1973); (2) the capsule is formed by surrounding fibroblasts (Frothingham, 1906); (3) the capsule is formed by both the redifferentiated myofibre and surrounding fibroblasts (Bruce, 1970a, b). The first two hypotheses were based on structural studies using electron and light microscopy. Bruce noted that deposition of collagen occurred from both outside and inside the nurse cell. On this basis, he suggested that the capsule was formed both by fibroblasts and the altered muscle cell. Kwiatkiewicz-Pauw (1994) demonstrated that the muscle cell produced collagen fibres and glycoproteins plus proteolglycans. The products formed the inner part of the capsule. The outer part of the capsule contained collagen fibres and matrix, synthesised by fibroblasts and vascular endothelium cells in particular.

Other studies have suggested that both type IV and type VI collagen are synthesised within the nurse cell itself (Kwiatkiewicz-Pauw *et al.*, 1994). Haehling *et al.* (1995) observed that the ES products of *T. spiralis* and *T. pseudospiralis* could induce extensive synthesis of exclusively type IV collagen by 3T3 murine fibroblasts *in vitro*. Polvere *et al.* (1997) reported that periodic acid Schiff reagent staining revealed at least two distinct layers in the capsule. RNA analysis showed that mRNA specific for type IV and VI collagen occurred in muscles only on Days 9 and 15 following intracellular infection. Most mRNA was within the nurse cell. The HN were transcriptionally active for these messages. The authors suggested that *T. spiralis* could directly or indirectly influence the synthesis of the two types of collagens throughout its developmental cycle in the nurse cell.

Anti-inflammation/immunodepression

One of the distinct features between *T. spiralis* and *T. pseudospiralis* infections is that the latter elicits practically no inflammatory response despite a non-secluded muscular habitat, be it in mammals or birds. How can this parasite suppress or evade the host's response? What is the advantage of adopting a non-secluded habitat that is constantly exposed to cellular/immune attack by the host? Despite extensive studies by many authors, the answer remains elusive. Immunogenesis of *Trichinella* is a huge subject. However, due to the constraint of this paper, only a few ideas are discussed.

The evasive/suppressive action is probably highly specific. In an attempt to determine whether the anti-inflammatory action of *T. pseudospiralis* is localised and specific, Li (1996) injected NBL of *T. spiralis* and *T. pseudospiralis* into the left and right thighs of the same BALB/c mouse respectively. Surprisingly, substantially stronger granulomatous reactions were observed around the sites of *T. spiralis* in the heterologous than homologous infections. There was little cellular infiltration around *T. pseudospiralis* in both heterologous and homologous infections. Therefore, the anti-inflammatory action undertaken by *T. pseudospiralis* appears to be highly specific and localised.

According to Stewart (1995), two different strategies for survival were evolved in *Trichinella*. One approach is a defensive strategy and this involves building a wall around the worm for protection against host attack. The second approach, exemplified by *T. pseudospiralis*, is an offensive approach. The parasite inserts its own message into the molecular communications passing between the immune and neuroendocrine systems of the host. This message supposedly triggers increased release of corticosteroids by the adrenals, leading to a suppression of host immune response. It was further suggested that the increase in corticosterone be related to a dramatic increase in interlukin 2 (IL2). IL2 can participate in the communication link between the endocrine and immune systems. The worm also employs molecular mimicry by expressing host-like antigen, asialo ganglio-N- tetraosylceramide, on its surface (Stewart *et al.*, 1978; Stewart & Larsen, 1989).

Using monoclonal antibodies and laser confocal microscopy, Li (1996) observed the presence of CD4 and CD8 cells in the cytoplasmic region of the nurse of *T. spiralis*. Why are these cells coexisting with the parasite inside an enclosed complex, and with no apparent ill effect on the latter? It is possible that these cells are simply trapped inside with the worms during encapsulation. However, the finding can imply that the worms are well protected against the cytotoxicity of CD8 cells.

Epilogue

The above brief review highlights the importance of adopting an integrated approach in studying parasitic infections. Molecular and immunological studies must be undertaken with special reference to the unique developmental/transmission biology of a given species. By virtue of their wonderful adaptations for survival in a hostile environment, many intracellular or tissue dwelling species can serve as excellent models for studies on the mechanisms of host cell reprogrammine, immunodulation, cell targeting, tissue restructuring etc. Many parasitic molecules have distinct properties and some may have important medical applications. Such studies may also help to expand the "time and space" of Parasitology in the new millennium. We truly believe that there is a pot of gold at the end of the rainbow.

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