Nurse cell Biology of *Trichinella spiralis*

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Abstract:

Trichinella spiralis is unique among all known nematodes in being the only species to occupy an intra-multicellular niche in the body of its host. Like developing tumours they also need to recruit new blood vessels towards the developing nurse cells to meet the ever-growing demand of nutrients. Like viruses they highjack the cell machinery and physiology so as to ensure their growth. Thus, interfering with the key steps in their development of nurse cells like angiogenesis and collagen synthesis can effectively control Trichinellosis.

Key words: *Trichinella*, Trichinellosis, angiogenesis, nurse cells

Introduction:

Members of the genus *Trichinella* are able to infect a broad spectrum of mammalian hosts, making them one of the world's most widely-distributed groups of medically important helminths. These roundworms constitute an unusual group of organisms in the phylum Nematoda, in that they all live a part of their life cycle as intracellular parasites. The diseases that *Trichinella sp.* cause are collectively referred to as trichinellosis. Currently, prevalence of trichinellosis is low within the United States, occurring mostly as scattered outbreaks[1]and the majority of human cases are due to *Trichinella spiralis*. The domestic pig is the main reservoir host for *T. spiralis*. This species is significantly higher in prevalence in people living in certain parts of Europe, Asia, and Southeast Asia than in the United States. It is now considered endemic in Japan and China. A large outbreak of trichinellosis occurred in Lebanon in 1997, infecting over 200 people.[2] *Trichinella spiralis* infection in humans has been reported from Korea for the first time.[3]

Infection is initiated by ingesting raw or undercooked meats harbouring the **Nurse cell-larva complex**. Larvae are released from muscle tissue by digestive enzymes in the stomach, and then locate to the upper two-thirds of the small intestine. The outermost cuticular layer (epicuticle) becomes partially digested.[4,5] The parasite on receiving environmental cues in the form of acidic stomach juice[6] select an infection site within the small intestine. The immature parasites penetrate the columnar epithelium at the base of the villus. They live within a row of these columnar epithelial cells and are considered **intra-multi-cellular** organisms.[7] Larvae moult four times in rapid succession over a 30-hour period, developing into adults. After mating of male and female larvae, females produce live

offspring — newborn larvae. These new born larvae migrate into cell leaving capillaries. After invasion, they induce a remarkable series of cell physiological changes causing the fully differentiated muscle cell to transform into one that supports the growth and development of the larva. This process is termed **Nurse cell formation**.[8]

After formation of Nurse cell (one week after infection) it may cause periorbital and facial edema, conjunctivitis, fever, myalgias, splinter haemorrhages, rashes, and peripheral eosinophilia. Occasional life-threatening manifestations include myocarditis, central nervous system involvement, and pneumonitis.

How does *T. spiralis make itself at home?*

Genetic Reprogramming of Host Muscle Cell

Four fundamental changes induced in host cells are:

(i) infection-induced cell cycle re-entry;

(ii) suspension of host cells in apparent G2/M;

(iii) repression of host muscle gene expression; and

(iv) further induction of the infected cell phenotype.

Capsule collagen synthesis

The nurse cell–parasite complex is surrounded by a collagen capsule[13] and consists predominantly of two collagen types, IV and VI , both of which are synthesized by the nurse cell.[14] Parasite secretion of proteins within the matrix of the infected host cell begins on Day 7 after infection.[13] The onset of host collagen type IV and type VI mRNA synthesis is between Days 7 and 8. By Day 8, parasite peptides localize to the nucleoplasm of all enlarged nurse cell nuclei.[15,16] Hence, upregulation of these two host genes is temporally coincident with peptide secretion. Throughout the period of collagen synthesis, all enlarged nuclei remain transcriptionally active, resulting in the over-expression of these two collagen proteins. Collagen type IV synthesis then ceases on about Day 26, while synthesis of type VI collagen continues throughout the infection at a low level. [17] Thus, each of these two host genes appears to be under separate regulatory control mechanisms.
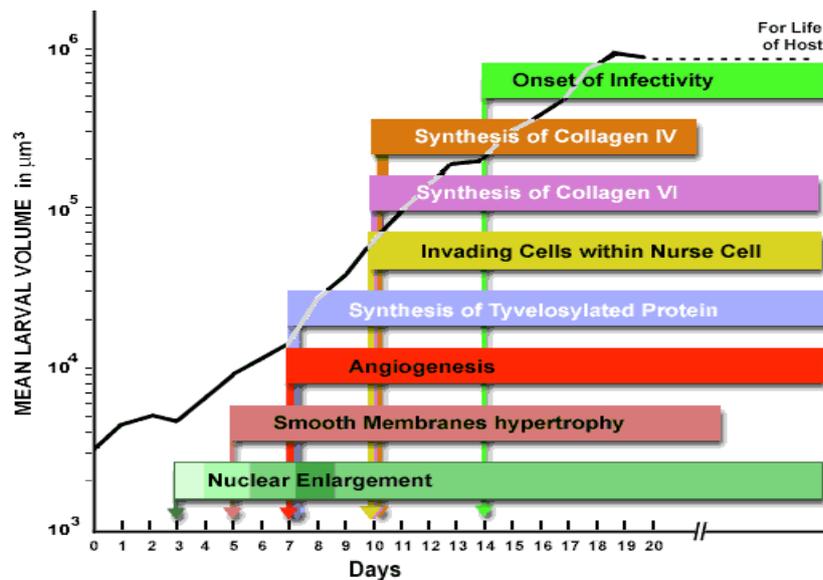
Fig.1- Events of host-parasite interaction in the life history of *Trichinella spiralis.*

Angiogenesis in nurse cell formation and maintenance

Two essential requirements of any long-term host–parasite relationship in which the parasite remains metabolically active are nutrient acquisition and waste disposal. It is likely that *T. spiralis* accomplishes these two tasks in one operation; namely by attracting a highly permeable set of blood vessels (i.e. the circulatory rete) to the surface of the outer collagen capsule. In this way, the larva could assure a constant source of small molecular weight metabolites for itself, while ridding its living space of metabolic by-products. The mechanism(s) by which the worm accomplishes this is by initiation of the angiogenic program. [18] This may involve an initial hypoxic event early on within the nurse cell. Hypoxia in many situations (e.g. wound healing and tumorigenesis) leads to upregulation of vascular endothelial growth factor (VEGF), which in turn elicits the construction of new vessels. VEGF mRNA by *in situ* hybridization in the cytoplasm of the developing nurse cell beginning on Day 7, up to eight months after initial infection of the muscle cell.[19] The presence of VEGF peptide was observed shortly thereafter, beginning on Day 9 using immunohistochemical methods, and was demonstrable within the nurse cell from that point on. Thus, the VEGF gene remains upregulated throughout the infection period, while the mRNA signal appears to be strongest at Day 15. A constant, low level of production of VEGF peptide (also known as vascular permeability factor) after circulatory rete formation is complete implies a permanently heightened state of vascular permeability, and would present obvious advantages to the parasite for maintaining itself within the host for long periods of time. The vessels of the circulatory rete are now known to be derived from adjacent venules, not arterioles as was thought previously, and they have the diameter of sinusoids, thus facilitating the rapid flow of formed elements through them. The large diameter of the vessels, compared with capillaries, also favours rapid exchange of nutrients and wastes, but

offers less than optimal conditions for the efficient exchange of gasses between the nurse cell and the red blood cells that circulate past it.   These observations are consistent with data collected from a variety of experimental approaches indicating that larval and nurse cell energy metabolism are anaerobic. This metabolic strategy explains how the parasite remains infectious for another host (ie. scavengers) from days up to weeks after the death of the infected host (depending upon the ambient temperature) in its decaying muscle tissue – the ultimate in anaerobic environment.
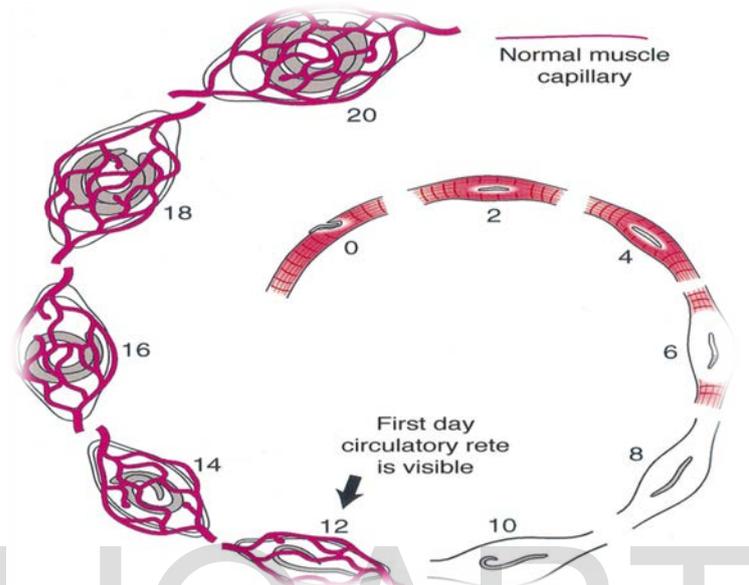

Fig 2. Angiogenesis and nurse cell formation. Angiogenesis begins on about Day 12 after the larva invades the muscle cell and ceases by Day 26.

Fig 3. -   Circulatory rete around nurse cell

Information exchange

Intravital microscopy has revealed that *Trichinella* constantly moves about within its nurse cell[20], slowly rocking back and forth and probing its immediate environment with its anterior end, expending energy as it does so. The worm is anything but quiescent. Thus, *Trichinella* almost certainly depends upon a common communication

system to construct and maintain its nurse cell. Mammalian intercellular communication systems depend upon a wide range of secreted signalling molecules [21,22] (i.e. cytokines), which direct specific cellular behaviour. Presumably, *T. spiralis* uses similar molecules to carry out its own developmental programs. In addition, however, it must instruct the host, most probably using its secreted signalling molecules termed 'parakines'.[12] The host cell then responds to those signalling molecules enabling the nurse cell to form. *T. spiralis* produces an unusual glycan which is added to multiple excretory-secretory proteins of muscle stage larvae. The glycan has a tri- and tetrantenary structures with a terminal tyvelose, which is a dideoxy arabinohexose.[9] The structure conferred by the tyvelose moiety creates an antibody epitope, which occurs on multiple excretory-secretory proteins of *T. spiralis* muscle stage larvae. From an immunological perspective, antibodies against his epitope can protect against intestinal invasion by the parasite [10]. The collagen capsule that envelopes infected cells of encapsulated *Trichinella* spp. appears to be host derived, yet conveys phylogenetic information that distinguishes major clades within the parasite genus. Debate continues on the origin of the collagen capsule. For instance, collagen gene expression is elevated in infected cells. [11]

Conclusion:

Carcinogenesis and Trichinellosis have one thing in common, that is, in both cases elevated need of nutrient supply for new growth in neoplastic tissues and nurse cells respectively, angiogenesis (formation of new blood vessels) is absolutely essential. So, anti-angiogenesis based therapies should be equally effective as it is in cancer therapy, in treating trichinellosis without affecting the homeostatic physiology of the host. Temporal and spatial interference with the collagen biosynthesis may also prove to be promising in thwarting the growth and development of nurse cells in particular. But for an effective and long lasting cure of Trichinellosis a detailed understanding of the disease pathogenesis is of paramount importance.

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