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Impact of trypanosomes on infected tissues of the mammalian host (Review)

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The protozoan parasite *Trypanosoma* infects various warm-blooded animals, usually via an insect vector. In humans, *T. brucei* causes sleeping sickness in Africa, and *T. cruzi* causes Chagas disease mostly in South America. *Trypanosoma brucei* is traditionally observed in the blood and later in the central nervous system but new data prove additional localization in other organs including skin, lungs, liver, heart and kidneys. *Trypanosoma cruzi* is found in multiple organs and tissues during the acute form of the disease and predominantly in the skin, heart and gastrointestinal tract during the chronic form. Both species inflict specific histopathological changes in the organs depending on disease progression. *Trypanosoma equiperdum* is an equine parasite causing the sexually transmitted disease dourine. It causes inflammation of the genital tract of both sexes, and in males also degeneration of seminiferous tubules. In addition, it causes skin plaques and spinal cord lesions resulting in neurological damage.

Key words: Chagas disease, *Trypanosoma sp.*, sleeping sickness

Medical importance of *Trypanosoma*

Protozoan parasites of the kinetoplastid genus *Trypanosoma* are dangerous pathogens causing sleeping sickness and Chagas disease in the human, as well as diseases in domestic and wild animals. Comprehensive knowledge of the histopathological changes caused by their presence is needed to understand the pathogenesis of trypanosomiases and to improve the methods of diagnosis and treatment. In this respect, the most clear-cut case is the New World species *Trypanosoma cruzi* causing Chagas disease. In its mammalian hosts, including humans, T. cruzi switches to an intracellular amastigote form which causes damage by using cardiomyocytes as host cells and provoking an inflammatory response [16]. Unlike it, the African species *Trypanosoma brucei* exists in the mammalian host solely in the form of an extracellular flagellated trypomastigote which evades the antibody response by periodically changing its major surface antigen, the so-called variant surface glycoprotein [11]. However, despite the fact that *T. brucei*

does not destroy host cells directly by intracellular parasitism, it is even more virulent than its American counterpart: its invasion of the central nervous system leads to meningoencephalitis known as sleeping sickness, which is fatal if untreated [9].

Histopathological changes caused by *Trypanosoma cruzi*

Chagas disease is a sickness typically associated with Latin America, although in recent years there have been many reported cases around the world including North America and Europe [12, 21]. According CDC more than 8 million people worldwide suffer from the disease and numbers in the USA alone have reached 280 thousand [6]. *Trypanosoma cruzi* has three forms – trypomastigote, epimastigote, and amastigote (intercellular in the vertebrate host). It relies mostly on vector-borne transmission by bedbugs belonging to genus *Triatoma* (kissing bug), but other significant pathways have also been reported – blood-borne, congenital, organ-derived, salivary - mostly among reservoir hosts, and even oral transmission by food and drinks contaminated with bugs or their droppings [4]. The disease has two stages – acute and chronic. In most cases the acute form has mild or no symptoms while in other cases flu-like symptoms are reported. Chancre around the bite site, and inflammation with partial closing of one of the eyes (Romana's sign) could be observed. Eye inflammation could be attributed to either direct inoculation of the eye with the parasite or an allergic reaction to the parasite/vector. Severe symptoms as myocarditis or meningoencephalitis are rare but pose a significant risk for the patient's life. The severity of the acute form is correlated with the type of transmission. The oral pathway is more likely to cause life-threatening conditions, likely due to the low pH value in the human stomach which results in surface protein changes in the parasites [2]. The chronic stage could initially be asymptomatic (indeterminate form) but progressively leads to development of severe abnormalities in the cardiovascular system and gastrointestinal tract such as ventricular arrhythmias, bradycardia, complete heart block, an apical aneurysm, thromboembolic phenomena, dysphagia, odynophagia, bowel ischemia, etc. [4].

During the acute stage T. cruzi is believed to invade a diversity of nucleated cells in the spleen, gut, adipose tissue, heart endothelium, striated muscles, immune cells etc. The same results were demonstrated using animal models, mostly rodents. Interestingly, no parasites were found in the brain or liver during the initial stages, and no parasites were observed in the spleen and striated muscles during the chronic form [20]. This is a direct result of the *T. cruzi* tissue tropism. The way the parasite recognizes the target tissue is still debatable but galectins are presumed to play a major role in the process, especially Galectin-1 for cardyomiocyte recognition. After recognizing the host cell, the parasite penetrates it in a Ca^{2+} dependent manner, using its tc85, gp35/50, gp 82, gp 80, and other surface proteins [19]. During the acute form of the disease inflammatory and immune response changes can be observed in heart tissue (**Fig. 1**). In the amplification stage multiple parasites could be seen in the cytoplasm of the cells. Activated phagocytes and lymphocytes infiltrate the area, which leads to an increased production of proinflamatory proteins, and a histological picture similar to many forms of myocarditis. After the acute stage ends there are fewer infected cells and usually no significant damage to the organ. During the chronic form cardiomyocytes start to die out mostly due to local inflammation and are replaced by connective or fat tissue. The heart becomes enlarged, but the miocard becomes thinner with hypertrophic muscle cells [5]. The arrhythmia observed in such

patients is believed to be caused by either direct or indirect neural damage caused by the presence of *Trypanosoma cruzi* [14].

Damage to the gastrointestinal tract caused by *T. cruzi* is not observed as frequently as other symptoms, and could be attributed to the destruction of neural cells and damage caused by inflammation in regions in the lower or upper part of the gastrointestinal tract. The symptoms may vary from constipation to dysphagia and megacolon. Many factors are considered to play a role in the manifestation and severity of the symptoms including the genetic variant of *T. cruzi*, the microbiome, and the presence of mutations affecting epithelial receptors such as NOD2 [13].

Histopathological changes caused by *Trypanosoma brucei*

Sleeping sickness, or human african trypanosomiasis, develops in two stages: 1) early or hemolymphatic stage, characterized by non-specific symptoms and presence of trypanosomes in the blood plasma, and 2) late or meningoencephalitic stage, when the parasite spreads to the central nervous system and causes neurological impairment [15]. In other mammals, the disease follows a similar course though the predominant symptom is weight loss [7].

Trypanosomes in the blood and central nervous system have understandably attracted most attention due to their importance for diagnosis and pathogenesis, respectively, and also because of a key treatment criterion: after the disease has progressed to the late stage, treatment options are narrowed down to drugs able to pass the blood-brain barrier, which are few, not very efficient, and highly toxic [9]. However, *T. brucei* is by no means limited to the blood and the central nervous system. Recent studies have revealed presence of the parasite in extravascular spaces of other organs such as the skin, adipose tissue, eyes, and testis; a cathepsin-like protease secreted by it, called brucipain, is presumed to help its extravasation [7]. There are data that these tissue-resident trypanosomes are the main reproducing pool and a proportion of their daughter cells, carrying new versions of the variant surface glycoprotein, are "seeded" to the bloodstream where they are accessible to the vector – the tse-tse fly *Glossina* [11]. In studies on experimentally infected animals, trypanosomes have been found also in the lungs, liver, heart and kidneys [15]. They tend to adhere to collagen fibers in the skin and other tissues [7]. This behavior of the parasite may be related to the fact that the vector bite injects it into the collagen-rich dermis, which is thus the first tissue of the mammalian host contacted by the trypanosome. Moreover, extravascular trypanosomes may persist in the skin of infected hosts that present no symptoms and no detectable blood forms. Such latent infections, usually omitted from textbook descriptions of African trypanosomiasis, can be transmitted to tsetse flies and therefore will hinder any effort to eradicate the parasite [1].

For obvious reasons, tissue pathology has been more extensively studied in animal models than in human patients. Both in the central nervous system and in other organs, it includes blood vessel congestion and infiltration by lymphocytes and mononuclear phagocytes. There are also organ-specific changes such as pulmonary fibrosis and renal tubular necrosis [15]. In the skin, a common finding during the early stage is the so-called trypanosomal chancre. It forms around the inoculating tse-tse fly bite and is an inflamed nodule full of proliferating parasites. Host fibroblasts and endothelial cells also proliferate. In addition to this localized lesion, there are often rashes, pruritus, and dermatitis during both the early and the late stage. Testicular infection has been studied in experimentally

infected animal models, and seems limited to connective tissue. So far, *T. brucei* has not been observed beyond the blood-testis barrier; and while experimentally infected male mice can infect their partners, sexual transmission is extremely rare in natural hosts [7].

Trypanosoma brucei is thought to invade the brain through the choroid plexus and circumventricular organs, which contain relatively permeable capillaries devoid of a blood-brain barrier [3]. Microglial cells, as resident mononuclear phagocytes of the brain, are the cell population most prominently engaged with controlling the infection. In an experiment with experimentally infected mice, activation and proliferation of microglia surprisingly started during the early, hemolymphatic stage of trypanosomiasis. In addition to increasing their number and activity, microglial cells, as in other pathological processes in the brain, decreased their structural complexity, i.e. reduced the number and length of their processes (**Fig. 2**). The microglia is involved also in the natural infection, since brain samples of patients who died of sleeping sickness reveal hyperplasia and formation of nodules by the microglia [22].

In the final stage of the above described experimental infection, peripheral immune cells massively infiltrated the brain, but this last-resort attempt to eliminate the pathogen did not seem to mitigate the pathological process [22]. One of the reasons may be the maladaptive nature of the immune response against the parasite. In another experiment on mice, *T. brucei* infection caused formation of extopic lymphoid aggregates in the meninges, making the meninges behave as a secondary lymphoid organ. Many of the B cells differentiated there, however, were autoreactive and produced antibodies against myelin basic protein and other brain components [18]. *Trypanosoma brucei* and other kinetoplastids are known to skew the host immune response towards Th2 cells, which creates permissive conditions for their survival [1].

Histopathological changes caused by *Trypanosoma equiperdum*

Trypanosoma equiperdum is an equine parasite causing a disease called dourine. It is unique among trypanosomes by being transmitted sexually rather than by a blood-sucking insect. Despite these biological differences, *T. equiperdum* is morphologically very similar to *T. brucei*, which has made researchers speculate about their evolutionary relationship. Molecular data confirmed that *T. equiperdum* has evolved from *T. brucei* relatively recently. Concomitantly with an adaptation to sexual transmission, it lost key parts of its kinetoplast – the complex mitochondrial genome which is a hallmark of the group. This made it unable to perform oxidative phosphorylatin and, hence, to survive in the energy-poor environment inside an insect body. As a result, *T. equiperdum* was restricted to its mammalian host where it could rely on glycolysis for energy metabolism. The degenerative change paradoxically gave the parasite the opportunity to greatly expand its distribution range, while the ancestral *T. brucei* remains confined within the range of its vector, the tsetse fly [8, 10]. In the light of these data, some recent sources consider *T. equiperdum* a subspecies of *T. brucei* [17].

Little is known about pathogenesis of dourine. The gross pathology includes swelling of the genitalia, skin plaques, neurological symptoms, and emaciation often leading to death. Unlike *T. brucei*, *T. equiperdum* is present in the blood only briefly and then settles in various tissues. The testes of males and the genital tract of both sexes are infiltrated by lymphocytes, plasma cells and mononuclear phagocytes. In stallions, chronic orchitis causes degeneration of seminiferous tubules and may completely

abolish spermatogenesis (**Fig. 3**). Perineal and penile skin is affected by dermatitis leading to keratinocyte and melanocyte necrosis and, as a result, depigmentation. In addition to the reproductive system, the nervous system is also heavily infected. Degenerative lesions are observed in the distal spinal cord, especially the lumbar and sacrococcygeal regions, as well as spinal nerves and ganglia. The spinal cord damage affects white matter and is manifested as axonal degeneration and demyelination. The peripheral nerves and ganglia associated with them are inflamed and infiltrated by the same populations of immune cells as described above for the reproductive system. The spinal cord and nerve damage can explain the hindleg incoordination characteristic for dourine. Neuritis including vacuolation and demyelination has also been described in the facial nerve, though the brain seems largely undamaged [23, 24].

Conclusions

Both *T. brucei* and *T. equiperdum* invade a range of tissues and despite their strictly extracellular habitat lead to lesions, inflammation, immune cell infiltration and functional impairment. Tissue-resident forms may predominate over the blood forms even in *T. brucei*, indicating that diagnosis should be based on tissue biopsy rather than blood sample. The histopathological changes caused by the two extracellular trypanosomes are similar in many respects, which implies common features in pathogenesis. It is therefore appropriate to take advantage of the easier handling of *T. equiperdum* and use it as a model of *T. brucei* in research. An interesting finding is that both trypanosomes infect the testis but only *T. equiperdum* seems able to cross the blood-testis barrier, which presumably made possible its sexual transmission. It may be hypothesized that this ability was the first trait to evolve as *T. equiperdum* diverged from *T. brucei* and took its own independent path.

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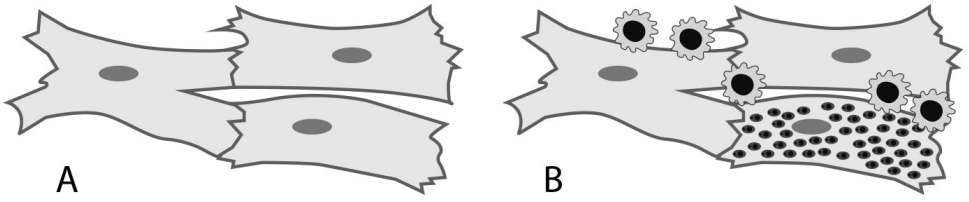

Fig. 1. A – normal muscle cells; B – muscle cells with amastigotes, and surrounded by immune cells

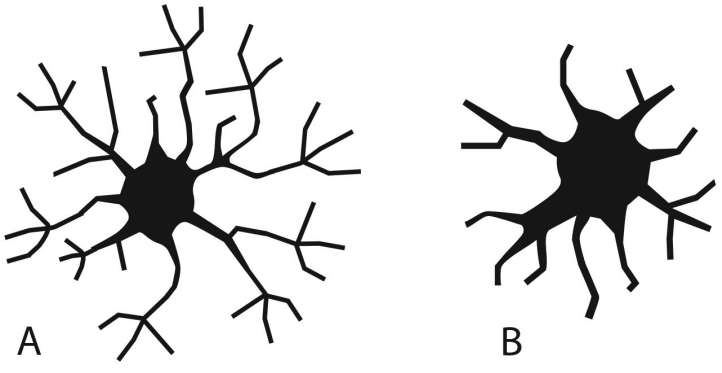

Fig. 2. A – normal glia cell; B – glia cell with changed morphology as a result of an immune reaction

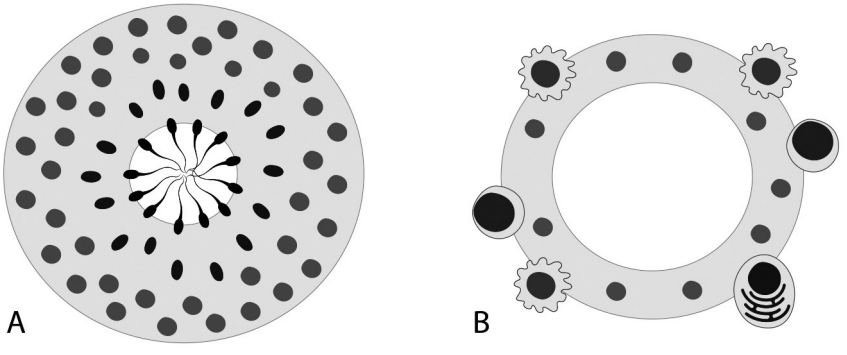

Fig. 3. A – normal seminiferous tubule; B – seminiferous tubule with abnormal morphology, and spermatogenesis due to immune cell infiltration