Review Article

Trypanosoma cruzi-Derived Neurotrophic Factor: Role in Neural Repair and Neuroprotection

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Abstract Some patients infected with the parasite *Trypanosoma cruzi* develop chronic Chagas' disease, while others remain asymptomatic for life. Although pathological mechanisms that govern disease progression remain unclear, the balance between degeneration and regeneration in the peripheral nervous system seems to contribute to the different clinical outcomes. This review focuses on certain new aspects of host-parasite interactions related to regeneration in the host nervous system induced by the trans-sialidase of *T. cruzi*, also known as a parasite-derived neurotrophic factor (PDNF). PDNF plays multiple roles in *T. cruzi* infection, ranging from immunosuppression to functional mimicry of mammalian neurotrophic factors and inhibition of apoptosis. PDNF affinity to neurotrophin Trk receptors provide sustained activation of cellular survival mechanisms resulting in neuroprotection and neuronal repair, resistance to cytotoxic insults and enhancement of neuritogenesis. Such unique PDNF-elicited regenerative responses likely prolong parasite persistence in infected tissues while reducing neuropathology in Chagas' disease.

Keywords chagas disease; *Trypanosoma cruzi*; peripheral nervous system; parasite-derived neurotrophic factor

1 Introduction

Despite recent progress in the control of Chagas' disease, it is estimated that approximately 8 million people remain chronically infected with *Trypanosoma cruzi*, the etiological agent of the disease. Many of these infected individuals (30–40%) will develop life-threatening heart or gastrointestinal pathology. Given that an additional 20% of the population is at risk of infection in endemic countries [57], Chagas' disease (CD) remains a significant health problem in Latin America.

T. cruzi is transmitted to humans by hematophagous insects of the *Reduviidae* family through the deposition of feces at bite sites. In addition, nonvectorial routes like blood transfusion, as well as mother-infant and oral ingestion are also important, particularly in nonendemic areas [57].

The initial infection causes transient flu-like symptoms and thus often escapes medical attention. Young children rarely ($<$ 5%) develop severe inflammation of the heart or central nervous system (CNS). Survivors of the acute disease do not develop clinical symptoms for many years, and approximately 60–70% of them remain in the chronic indeterminate (asymptomatic) stage of the disease for life, while the other 30–40% will exhibit chronic symptomatic CD affecting peripheral nervous system (PNS) in the heart and digestive tract several years or even decades after the initial infection [6,66].

Symptomatic CD can be fatal, but a vaccine for preventing *T. cruzi* infection has not been developed yet, and trypanocidal drugs (benznidazole and nifurtimox) are effective only in acute stage and can cause serious side effects [72].

The reason for a long latent period between initial infection and clinical manifestations, and high proportion of asymptomatic patients, is not known; there is also no explanation for the absence of chronic disease in CNS. Analysis of *T. cruzi* interaction with CNS and PNS in the heart and gastrointestinal tract (GI) can provide some clues for the understanding of CD progression from benign to pathological form and ideas for possible therapeutic intervention.

2 Neuropathology in Chagas' disease

Molecular mechanisms underlying pathogenesis in CD remain unclear. It is thought that direct cell parasitism, acute inflammation, autoimmune reactions, or neuronal damage are primary causes of disease pathogenesis [22, 37,67]. The neurogenic hypothesis states that the severe neuronal loss in the heart and GI of Chagasic patients defines the transition from asymptomatic to symptomatic CD. The foundation to neurogenic theory was laid by the pioneering work of Köberle who detected striking

neuronal depopulation in autonomous nervous system of CD patients with cardiomyopathy, megaesophagus, and megacolon [35]. Multiple pathological studies in humans and experimental animals have confirmed this finding by demonstrating extensive destruction of cardiac parasympathetic ganglia [9, 22, 23, 34, 47, 58], which is thought to allow unopposed sympathetic activation resulting in progressive myocardial damage [22, 23]. However, lesions in sympathetic postganglionic fibers in the sinus node and the myocardium have also been detected in patients with cardiac form of CD and in experimental animals [34, 59] and it was shown that reduction in both cholinergic and noradrenergic cardiac nerves paralleled development of the acute myocarditis at the end of the acute phase of experimental CD [47, 58].

In Chagasic GI disease, neuronal destruction is even more drastic [48]. Loss of 50 to 90% of nerve fibers in myenteric and submucosal plexuses and reduction in enteric glial cell population correspond to striking luminal enlargement and muscular hypertrophy of the esophagus and colon [20, 36, 46, 53]. The aperistalsis thus developed leads to organ obstruction and stagnated food passage resulting in megasyndromes (megaesophagus and megacolon) and weight loss [49].

In contrast to the prominent damage of the PNS, *T. cruzi* invades CNS without noticeable deleterious effects. Trypomastigotes are frequently found in the cerebrospinal fluid, brain, and spinal cord in the absence of neurological symptoms [32, 56], unless patients are severely immunocompromised [26]. In experimental CD, even in younger animals, which are usually very susceptible to *T. cruzi* infection, CNS is largely preserved regardless of the parasite load, presence of amastigote nests inside astrocytes, and strong inflammatory response [9, 10, 45]. Such silent infection is contrary to that in the heart, colon, and esophagus, and it does not produce chronic symptoms [56]. The molecular mechanisms underlying opposite effects of *T. cruzi* invasion of CNS and PNS remain unresolved.

PNS is most vulnerable to damage in the acute stage of the infection characterized by high parasitemia and tissue parasitism. Parasites found in neuronal ganglia, Schwann cells, and enteric glia [47, 73] destroy cells directly and induce cytotoxic immune responses, mediated by NO production and oxidative burst resulting in myocardial damage and lesions in enteric nervous plexuses [55, 64].

Although acute disease symptoms could in some cases be dangerous, they normally resolve, and Chagasic individuals advance to an indeterminate, asymptomatic stage, with many of them showing normal electrocardiogram and X-rays of the heart, esophagus, and colon [20, 53, 70]. In fact, it was demonstrated that the rate of age-related neuronal degeneration in the colon and heart of Chagasic patients was decreased compared to that of noninfected age-matched individuals [35], suggesting enhanced neuronal survival. In experimental CD functional improvement in the heart and colon was associated with reinnervation of muscle fibers, collateral sproutings of damaged nerves, and axonal regrowth, and with an increase in the number of enteric glia [43, 45, 53]. For most of infected patients the extent of recovery is such that they remain free of symptoms for the rest of their lives, despite retaining pathogenic *T. cruzi*. It is thus likely that neuroregeneration is involved in the mechanisms that prevent manifestations of chronic disease.

It is also possible that *T. cruzi* infection, traditionally viewed as an entirely detrimental process to the host, can elicit specific reparative/survival responses in neuronal tissues. This possibility received experimental support with the findings with the *T. cruzi* trans-sialidase.

3 *T. cruzi* trans-sialidase/parasite-derived neurotrophic factor (PDNF)

3.1 Catalytic and immunologic properties

A *T. cruzi* surface antigen, trans-sialidase/PDNF, plays multiple roles in parasite invasion of mammalian host. As an enzyme it catalyzes the transfer of α-2,3-linked sialic acid to terminal β-Gal residues [54, 62], which can protect bloodstream trypomastigotes from complement lysis [7] and promote parasite invasion [50, 68].

PDNF is attached to trypomastigote surface by GPI anchor and is copiously shed as a soluble factor into the extracellular space and bloodstream [1, 60, 63]. Soluble PDNF was shown to remodel surface of immune cells and augment *T. cruzi* immunosuppresion in the acute phase of CD by inhibiting CD8+ lymphocytes cytotoxicity and promoting apoptosis of T cells [29, 51].

PDNF consists of N-terminal catalytic domain, connected through a lectin-like region to a C-terminus with variable number of 12 amino acids repeats in tandem (long tandem repeat, LTR) [8], also called SAPA (shed acute phase antigen) [28]. LTR/SAPA is not required for sialic acid transfer, but it is highly immunogenic and contributes to parasite immune evasion by inducing abnormal polyclonal B cell activation and nonspecific Ig secretion characteristic for the acute phase of Chagas' disease [30, 31]; furthermore, it upregulates IL-6 production in various cell types [61]. Consistent with PDNF functioning as an immunosuppressor, heterologous expression of PDNF in *Leishmania major* enhanced parasite virulence [5].

However, a relatively small subset of TS/PDNF proteins has enzymatic activity—of 1400 PDNF gene family members, only 15, produced by invasive trypomastigotes, function as trans-sialidases [4, 27]. Trypomastigotes also express PDNF molecules without catalytic activity, due to a single mutation of Tyr342 to His [19].

Unique subsets of PDNF molecules lacking catalytic activity and LTR/SAPA domain belong to intracellular amastigotes, while no TS was detected for insect epimastigote forms [4].

Such structural diversity among multiple members of TS/PDNF family possibly resulted in some of them exhibiting biological activities unrelated to the release and acquisition of sialyl residues.

3.2 Interaction with Trk receptor tyrosine kinases

The discovery of PDNF specific affinity to Trk receptors of mammalian neurotrophins pointed to a new direction in *T. cruzi* research, suggesting a possible mechanism for the parasite involvement in regeneration of mammalian nervous tissue.

The development and maintenance of mammalian PNS and CNS critically depends on the neurotrophins (NTs) nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (NT3). NTs activate their respective Trk tyrosine kinase receptors TrkA, B, and C, and downstream pathways of PI3kinase/Akt kinase, PLCγ, and mitogen-activated protein kinases (MAPK) to control survival, axon, and dendrite outgrowth, plasticity and neurotransmission. As a result, they protect neurons from toxic insults and facilitate neural repair [33]. Deficit of endogenously generated NTs causes neuronal apoptosis and degeneration, and is implicated in stroke, trauma, and neurodegenerative conditions such as Parkinson, Alzheimer, and Huntington diseases, and amyotrophic lateral sclerosis [25].

3.2.1 Activation of Trk signaling and survival of neurons

Similar to neurotrophic factors, PDNF affinity to Trk receptors was shown to support DRG, hippocampal, and cerebellar neurons and a variety of neuronal cells through trophic deficiency, oxidative stress, and neurotoxic insults—pathological conditions that typically lead to neurodegeneration and apoptosis [11, 12, 14, 15, 75].

Resistance to apoptotic death induced by PDNF in neuronal cells is a direct result of activating Trk downstream signaling via PI3K/Akt and Ras/MAPK/Erk (Figure 1). Thus TrkA-dependent activation of Akt kinase by PDNF is followed by inhibition of pro-apoptotic GSK-3 kinase, upregulation of mitochondrial anti-apoptotic Bcl-2, and reduced ROS formation [12, 14], while induction of MAPK/Erk signaling prevented activation of caspase-3 and cleavage of caspase-3 substrate PARP (poly-ADP-ribose polymerase), a DNA repair enzyme [18]. This chain of events leads to enhanced neuronal survival and resistance to exogenous insults, such as neurotoxin MPTP that causes symptoms and pathology in nigrostriatal neurons analogous to Parkinson disease [14].
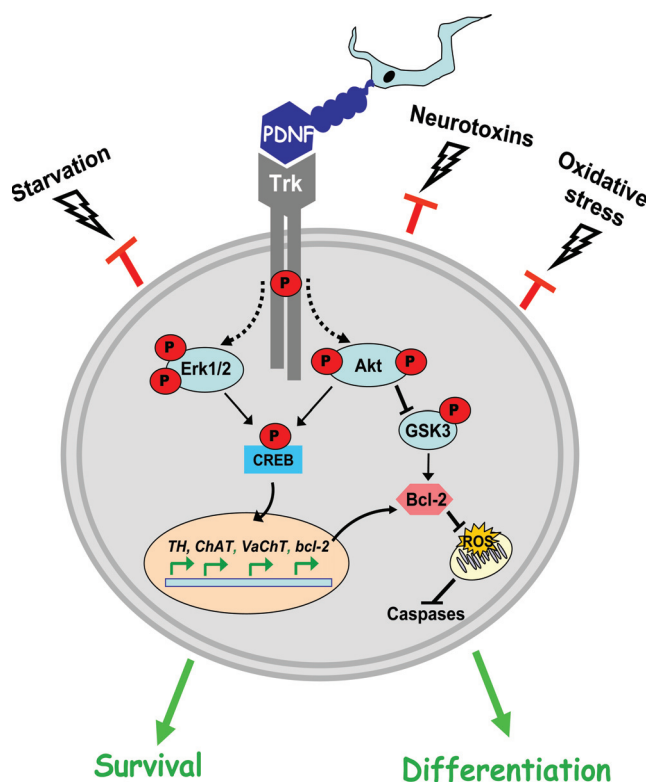

Figure 1: PDNF regulation of host cell signaling during *T. cruzi* infection. *T. cruzi* binding to Trk receptors mediated by PDNF activates Trk-dependent signaling of MAPK/Erk1/2 and PI3K/Akt, resulting in phosphorylation of transcription factor CREB and induction of CRE-dependent transcription, upregulation of anti-apoptotic Bcl-2 gene, and cholinergic (ChAT and VAChT) and adrenergic (TH) cascades. Activated Akt phosphorylates and inhibits activity of pro-apoptotic GSK3 kinase, resulting in stabilization of Bcl-2 transcripts and inhibition of oxidative stress (ROS) and caspase cascade. Cumulatively, these signaling events result in cell resistance to cytotoxic stimuli and lead to enhanced survival and differentiation in nervous tissue.

Sustained activation of Erk by PDNF also correlated with increased neuritogenesis in DRG neurons and differentiation of neuronal PC12 cells to sympathetic phenotype [13], underlined by activation of cAMP-response-element- (CRE-) binding protein CREB and CRE-dependent transcription [16, 17].

Recent data demonstrated that PDNF also mediates *T. cruzi* interaction with TrkC receptor. PDNF similar to natural TrkC ligand NT3 induces activation of MAPK/Erk signaling and promotes differentiation and survival of neuronal and glial cells [71]. TrkC-expressing Schwann and enteric glial cells, which myelinate neurons in PNS and modulate neuronal homeostasis and neurotransmission in the gut [65, 76], undergo regeneration in the course of *T. cruzi* infection in humans and animals [21, 53]. Thus PDNF activation of TrkC widens the scope for possible role of *T. cruzi* in the repair of infected nervous tissues. It would be broadened even further by *T. cruzi* activation of TrkB [74]; however, live trypomastigotes and parasite-isolated

PDNF did not recognize TrkB receptor in the conditions they bind TrkA and TrkC [71].

3.2.2 Induction of cholinergic and adrenergic phenotypes in neuronal cells

T. cruzi residence in PNS and affinity to TrkA and TrkC could underlie such an important aspect of nervous tissue functional integrity as neurotransmitter release, which is imbalanced in acute CD [22, 38, 42, 45]. PDNF activation of TrkA and downstream Akt and Erk1/2 signaling increased expression of cholinergic locus genes, choline acetyltransferase (ChAT), and vesicular acetylcholine (ACh) transporter (VAChT)—two key components of ACh synthetic cascade that define cholinergic phenotype in neurons [2].

Such PDNF activity also stimulated acquisition of adrenergic phenotype in ventral mesenchephalic neurons and neuronal PC12 cells [17], mimicking NGF and other neurotrophic factors, which can restore both cholinergic and adrenergic neuronal populations in NS [41]. PDNF, via upregulation of MAPK/Erk and CRE-dependent transcription, induced activation of tyrosine hydroxylase (TH)—the rate-limiting enzyme in the biosynthesis of catecholamines, increasing TH-catalyzed conversion of tyrosine to dopamine precursor L-DOPA [17] (Figure 1).

Such PDNF activity could be instrumental in restoring ChAT expression, ACh and catecholamine levels in later stages of *T. cruzi* infection [23, 42, 44], and regeneration of sympathetic neurons.

3.2.3 Promotion of cell invasion

PDNF interaction with TrkA induced endocytosis of receptor-PDNF complexes [75], and *T. cruzi* successfully exploits this mechanism for cell invasion. Trypomastigotes enter neuronal cells, Schwann cells, and other cells in Trk-dependable manner, which in addition requires receptor-mediated signaling, as inhibition of Trk-dependent signal transduction abrogated parasite invasion, reduced parasite load and inflammatory responses, and attenuated experimental CD [24].

The dependence of *T. cruzi* invasion on intact TrkA-mediated signaling is in line with the other data describing signaling pathways downstream of Trk receptor tyrosine kinase as critical checkpoints in the invasion process. These include activation of the MAPK and PKC pathways that enhanced infection of macrophages, endothelial and vascular smooth muscle cells [52, 69], and PI3K-mediated signaling, which induced accumulation of membrane PIP3, mobilization of intracellular Ca2+ stores, and formation of parasitophorous vacuole [3]. Therefore, *T. cruzi* activation of TrkA tyrosine kinase prior to using it as a vehicle for cell entry is a powerful mechanism to provide conditions for efficient cell invasion.

3.2.4 Anti-infective anti-Trk receptor antibody

The importance of Trk receptors in *T. cruzi* invasion is further underlined by the discovery that patients with asymptomatic CD produce specific antibody against Trk receptors, which blocked *T. cruzi* cell invasion [39, 40]. Passive immunization of mice with these autoantibodies reduced parasitemia and inflammation in the heart and protected from lethal *T. cruzi* infection [39]. Isolated from asymptomatic patients these autoantibodies might function as a defense mechanism to control *T. cruzi* invasion of nervous tissues.

4 Conclusion

Growth factors play important roles as intercellular signaling molecules throughout mammalian nervous system, taking part in numerous functions, including neuronal regeneration. As a parasite-derived growth factor, the *T. cruzi* TS/PDNF induces survival and resistance to cytotoxic stimuli in neuronal cells, possibly subserving neural repair and structural and functional recovery in the PNS of the heart and GI in Chagas' disease. The realization that *T. cruzi* can promote along with destructive, regenerative processes in infected tissues offers a new framework for studies of molecular pathogenesis that may suggest future therapeutic opportunities to prevent progression from asymptomatic to pathological CD.

References

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