Review

CellPress

Determinants of disease phenotype in trypanosomatid parasites

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Trypanosomatid parasites infect over 21 million people worldwide, with a range of disease phenotypes. *Trypanosoma cruzi* causes American trypanosomiasis, wherein 30–40% of infected individuals develop disease manifestations, most commonly cardiomyopathy but also digestive megasyndromes. In the case of *Trypanosoma brucei*, the etiological agent of African trypanosomiasis, disease progression can be rapid or slow, with early or late central nervous system involvement. Finally, *Leishmania* species cause leishmaniasis, a disease that ranges from self-healing but scarring cutaneous lesions to fatal visceral leishmaniasis in which parasites disseminate to the liver, spleen, and bone marrow. This review highlights parasite factors involved in disease phenotype in all three trypanosomatid diseases, with a particular focus on recent advances using large-scale 'omics' techniques.

Trypanosomatid parasites: spectrum of disease

Leishmania spp, *Trypanosoma cruzi*, and *Trypanosoma brucei* are protozoan parasites transmitted to mammalian hosts via their insect vectors, sandflies, triatomines, and tsetse flies, respectively. During their lifecycles *Leishmania* and *T. cruzi* have an intracellular mammalian stage, whereas *T. brucei* remains extracellular. *T. cruzi* causes American trypanosomiasis, commonly referred to as Chagas disease, in Central and South America (see Glossary) [1]. *T. brucei* causes African trypanosomiasis, also known as sleeping sickness [2]. *Leishmania* are the causative agents of leishmaniasis and are found worldwide in tropical and subtropical regions [3].

Jointly, these diseases cause over 4 million disability-adjusted life years (DALYs). Twenty-one million people are currently infected, with over 2 million new cases per year [4]. Chagas disease is divided into an acute, usually asymptomatic phase, followed by the chronic stage of the disease. 60–70% of infected individuals remain asymptomatic throughout their lifetime. Patients who progress to chronic symptomatic Chagas disease usually present with cardiomyopathy, but can also present with megacolon and/or megaesophagus, with or without cardiomyopathy [1]. Sleeping sickness manifests initially with fever and headache, and progresses to sleep disturbances and neurological disorders. It is typically fatal. Disease can be acute when caused by *Trypanosoma brucei rhodesiense* or chronic in the case of *Trypanosoma brucei gambiense* infection [2]. Leishmaniasis is found in three main forms: (i) self-healing but scarring cutaneous lesions; (ii) mucocutaneous disease with parasite dissemination to the nose, mouth and throat; and (iii) potentially fatal visceral

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Keywords: trypanosomes; leishmaniasis; Chagas disease; sleeping sickness; virulence factors; omics techniques.

1471-4922/

 http://dx.doi.org/10.1016/j.pt.2014.05.001

Glossary

Amastin: small surface glycoproteins in trypanosomatids, implicated in disease progression.
Cardiomyopathy: deterioration of heart muscle function, usually leading to heart failure.
Chagas disease: American trypanosomiasis; disease caused by *T. cruzi* parasites. Chagas disease progresses from an acute phase immediately following parasite infection to a chronic, lifelong phase. Both phases can be asymptomatic; only 30–40% of infected individuals develop clinically manifest chronic Chagas disease with cardiac, digestive, or combined cardiodigestive symptoms.
Cutaneous leishmaniasis (CL): skin disease caused by *Leishmania* parasites, characterized by parasite replication and lesion formation at the site of vector bite. Lesions are usually self-limiting and can include papules, nodules, or ulcers.
Discrete typing units (DTUs): subdivision used to classify *T. cruzi* strains. Strains within a DTU are genetically more similar to each other than to strains from other DTUs (Box 1).
gp90 and gp35/50 mucins: surface glycoproteins in *T. cruzi.* Their levels relative to other *T. cruzi* surface glycoproteins modulate the ability of the parasite to invade host cells.
Hemolymphatic stage: initial stage of African trypanosomiasis (sleeping sickness) in which parasites are found in the blood and lymph. Symptoms include fever and headaches.
Isolate: refers here to clinical isolates. Parasites isolated from a patient.
Megacolon: enlargement of the colon.
Megaesophagus: enlargement of the esophagus.
Meningoencephalitic stage: second stage of African trypanosomiasis in which parasites cross the blood–brain barrier and are found in the central nervous system. Symptoms include sleep disturbances and neurological manifestations.
Mucocutaneous leishmaniasis: disease caused by metastasis of some *Leishmania* spp to facial mucosal tissues, leading to ulceration and destruction of mucosal tissues in the nose, mouth, and throat.
Serum resistance-associated (SRA) gene: gene responsible for *T. brucei rhodesiense* resistance to human serum. The encoded protein promotes TLF1 degradation.
Strain: genetically distinct variant of a given species.
Surface antigens: markers found on the parasite surface that elicit a host immune response.
Tropism: preferential colonization of specific tissues by the parasite.
Trypanosome lytic factors (TLF): human serum high-density lipoproteins that cause lysis and killing of *T. brucei* parasites that infect animals. The toxic components of TLF1 are apolipoprotein L-1 and haptoglobin-related protein. Human-pathogenic *T. brucei* subspecies have evolved mechanisms to resist killing by these factors.
Trypomastigote small surface antigen (TSSA): a surface glycoprotein expressed in the trypomastigote stage of the *T. cruzi* lifecycle and which is involved in host cell invasion.
Visceral leishmaniasis (VL): disease caused by some *Leishmania* spp in which parasites disseminate to the liver, spleen, and bone marrow. Symptoms include high fever and enlargement of the liver and spleen.

leishmaniasis with liver and spleen parasitemia [3]. Although host and environmental factors clearly influence disease, a common link between all three trypanosomatids is that parasite factors are a key determinant of disease phenotype. Underlying genetic differences between parasite strains and species lead to changes in the structure, activity, or levels of these factors. They are usually proteins, but can also include carbohydrates and nucleic acids.

Parasite determinants of disease manifestation in Chagas disease

Differences in tropism between discrete typing units (DTUs)

T. cruzi is now classified into six DTUs, *T. cruzi* I (TcI), TcII, TcIII, TcIV, TcV, and TcVI, with multiple strains within each DTU (Box 1, Table 1). DTUs vary with regard to main geographic location and ecological niche, host, and vector preference [5]. Digestive syndromes are more prevalent in areas where TcII, TcV, and TcVI parasites predominate, including Argentina, Brazil, Bolivia, and Chile, but are rarer in areas where TcI and TcIV are found [1,5], suggesting that there may be an association between infecting DTU and disease manifestation, although this has yet to be confirmed.

The strongest link between DTUs and disease phenotype was observed in Colombia: infection with TcI was associated with increased prevalence of cardiac alterations compared to infection with TcII, even after controlling for the lower overall prevalence of TcII [6]. Different strains were also observed between heart and esophagus biopsy samples in Brazil. However, the heart and esophagus samples came from separate patients in all but two cases, making it difficult to distinguish between variations in patient exposure to parasite strains and variations in parasite tropism [7]. Similarly, a possible link was suggested between TcII, TcV, and TcVI and megacolon [8]. By contrast, a later Colombian study using cardiac tissue samples rather than peripheral blood was unable to find a correlation between strain and disease manifestation [9]. A similar lack of association was observed in Brazil [10] and Bolivia [11,12].

Overall, a major limitation is that many of these studies were performed with samples only from peripheral blood [6,8,10,11]. There are reports of variations between circulating and organ-associated parasites [13], suggesting that peripheral blood samples may not be representative of the situation within affected organs. This is further complicated by the fact that coinfection with multiple parasite strains may be common [6]. Finally, these studies are limited to associations between local parasites and disease, making it difficult to determine whether the absence of a specific DTU in patients with a given disease phenotype is due to parasite factors or to lack of patient exposure to this DTU.

Variations in pathology between *T. cruzi* strains from TcI and TcII were maintained in separate mouse strains, demonstrating that parasite factors are involved in determining disease severity in mice [14]. This forms the basis for the comparison of disease tropism between *T. cruzi* strains in mice. With regard to studies of cardiac pathology, some TcII strains caused more severe lesions than TcI or TcVI strains, whereas other TcII strains were less virulent [14–16]. Inflammation during chronic infection caused by TcVI was higher than during TcI infections, whereas TcV did not cause any inflammation [17]. In organs other than the heart, higher parasitism of spleen, liver, smooth muscle, and bone marrow was observed for TcII than for TcVI [18]. A large-scale study of 25 *T. cruzi* strains from TcI or TcIV found a broader organ tropism for TcI, whereas TcIV inflammation was restricted to skeletal muscle [19]. By contrast, CA-I strain (TcI) infected only cardiac and skeletal muscle of immunocompetent mice, leading to cardiac lesions and cardiac failure that recapitulate the disease seen in human infections [20].

Generalized conclusions are difficult to form from these studies. There is significant intra-DTU variation [21]; most of these studies used a single isolate from each DTU, and none compared all six DTUs. In addition, many of these studies used single reference strains, and often detailed patient information is lacking. This makes it difficult to determine whether disease severity and phenotype in mouse models accurately reflect disease phenotype in humans.

Parasite factors influencing disease phenotype

The variations in tropism and virulence described above in murine models are the basis for investigations into factors responsible for this variation. The diversity of surface

Table 1. Representative *T. cruzi* strains by DTU

DTU	Strain[a]	Ref
TcI	CA-I Colombian Dm28c[b] G[b] Sylvio X10	[87]
TcII	12SF 21SF Peruvian Y	[87]
TcIII	3869	[87]
TcIV	Can III	[87]
TcV	NRcl3	[87]
TcVI	CL-Brener[c] Tulahuen	[87]

[a]Unless otherwise specified, isolates are from a human source.

[b]Isolated from reservoir.

[c]Isolated from vector.

Box 1. *T. cruzi* classification into DTUs

There is considerable genetic diversity and significant variations in disease manifestations and disease severity within the *T. cruzi* species. To address this, *T. cruzi* has recently been reclassified into six discrete typing units (DTUs), TcI to TcVI, in which TcV and TcVI are hybrids derived from TcII and TcIII. DTUs differ in their geographical distribution, ecological niche, and vector and reservoir hosts. Their associations with specific disease phenotypes are an important topic currently under investigation.

Each DTU can include multiple parasite strains which are more closely related to each other than to strains from other DTUs. This is manifested by a greater genetic similarity within a DTU than between DTUs, such that members of one DTU can be distinguished from other DTUs by the presence of common molecular markers. However, strains within a DTU are not necessarily identical and can also be distinguished from each other within the DTU using other molecular markers. The concept of DTUs has recently been reviewed in detail by Zingales *et al.* [5].