Austin Clinical Microbiology

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Mini Review

Trypanosoma cruzi: The Genius of Escape from the Complement System

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Received: May 02, 2020; **Accepted:** May 23, 2020; **Published:** May 30, 2020

Introduction

Chagas Disease (CD) is a highly influential parasitic infection in 21 endemic countries of Latin America. Its impact has transcended the borders of this region and has reached regions such as North America, Japan, Europe and Australia. The large spread of CD is largely due to the migration of infected people from endemic areas to non-endemic areas [1]. The number of infected individuals worldwide reaches 6 million, of whom around 7000 die during the course of the year. These statistics indicate that CD is the parasitic infection that causes the greatest number of deaths in Latin America. It is also a major contributor to the global burden of cardiovascular disease, as this parasitosis is the primary source of infectious cardiomyopathy around the planet [2]. In addition to all these problems, most of the people affected by CD are of low socioeconomic resources and live in remote areas with difficult access. In addition, it must also be considered that less than 1% of chagasic individuals can be diagnosed and have chemotherapeutic treatment.

The parasite-host relationships are determining factors in the appearance of CD, as well as in the severity level of chronic symptomatic forms. Complement is the first defense barrier to prevent *Trypanosoma cruzi* infection from establishing, the functions of this protein complex are participation in the detection of these microorganisms and opsonization, as well as, they are involved in their elimination in order to avoid the entrance to the host cells. Activation of complement during the acute phase of CD allows controlling parasitaemia, while its proinflammatory effects favor the chronic phase. For all these reasons, this first line of defense for mammals constitutes a very strong and difficult system to violate, however, *T. cruzi* is a very ingenious parasite and always provided with counter-attack tactics, since it has developed different evasion mechanisms, to avoid the action of the complement cascade, which enables it to overcome the barriers imposed by the immune system and continue with the progress of CD.

T. cruzi Interactions with the Innate Immune System

The human invasion process by *T. cruzi* is affected by the activation of the complement system during the acute phase where it plays a key role in the control of parasitaemia, while in the chronic phase it participates in the appearance of symptomatic manifestations because it induces the production of proinflammatory molecules [3]. The complement system is made up of around 40 circulating proteins present in the plasma/blood, which work together with regulators or receptors of the cell surface, whose activation can be carried out in three ways: Classical Pathway (CP), Alternative Pathway (AP) and Lectin Pathway (LP). These routes have some points in common: 1 the degradation of C3 in C3a and C3b and 2 the convergence in a common point, the assembly C3 convertase complex.

Classical Pathway (CP)

CP begins mainly due to C1 binding with immune complexes (antigen/antibody); as well as, it depends to a lesser degree on PAMPs (pathogen-associated molecular patterns) such as porins and lipopolysaccharides from Gram-negative bacteria; pentraxins (pentraxin 3 and C-reactive protein) are some examples [4]. The C1 complex is structured by a C1q molecule plus two molecules of C1r and C1s, which results in a molecule of the C1q C1r2 C1s2 type. After C1q formation, its activation is mediated by its interaction with the CH3 Fc domain of IgM or CH2 Fc domain of IgG, which produces modifications in the conformation of C1q. Then C1r and C1s are activated, in addition to the serine proteases cut C4 and C2 that give rise to C3 and C5 convertases from CP [5].

Lectin Pathway (LP)

LP is activated in the presence of mannose containing polysaccharides present on the surface of the parasite, which interact with Mannan Binding Lectin (MBL), as well as other receptors such as L-ficolin, H-ficolin or M-ficolin. This pathway contributes around 70% of parasite complement-mediated lysis during the *T. cruzi* invasion process [6,7].

Alternative Pathway (AP)

AP is the simplest route, because it only needs to be activated due to spontaneous hydrolysis of the thiol-ester bond of C3 α-chain that produces C3 (H_2O), which interacts with plasma protein FB and produces C3 (H_2O) B. FD degrades FB into Ba and Bb, this last fragment binds to C3 (H_2O) and forms the first C3 convertase (C3 (H_2O) Bb), which has serine protease activity by acting on C3 and converting it to C3a and C3b. This latter fragment binds to FB, which allows FD to break it down and forms the second C3 convert, C3b Bb that binds to C3 to produce C3b Bb C3b, a complex structure possessing C5 convert activity [8, 9].

The complement activation system is the first defense barrier against pathogenic microorganisms, whose mechanism of action depends on a proteolytic cascade that triggers a very powerful lytic effect. For these reasons, this chain of events is an ideal target for microorganisms to implement their immune evasion strategies that allow them to survive and advance the infection process until reaching the chronic phase of the disease [10]. During the first steps

Citation: Maria Elizabeth MC. *Trypanosoma cruzi*: The Genius of Escape from the Complement System. Austin Clin Microbiol. 2020; 4(1): 1013.

of infection, *T. cruzi* is attacked by the AP and LP pathways of the complement, since the CP pathway is activated at later times when the production of antibodies begins.

T. cruzi is a very robust parasite, with multiple tools to escape the action of the mammalian host complement system, by inhibiting this proteolytic cascade, which allows it to survive its lytic mechanisms, as well as opsonization and chemotaxis. Epimastigotes, the non-infective form of the parasite, that live in triatomine vectors, are very susceptible to complement-mediated lysis while trypomastigotes, the infective form, present in host bloodstream, are resistant. The susceptibility of *T. cruzi* to complement is stage (amastigote, epimastigote, trypomastigote) and dependent strain. The morphological stages of this microorganism have different sensitivities to complement-mediated lysis. In the case of epimastigotes, their presence has been determined to allow the activation of the 3 complement pathways and they are sensitive to this mechanism [6]. In contrast, metacyclic trypomastigotes are resistant to the action of complement [10].

T. cruzi Proteins Involved in the Inactivation of the Complement System

T. cruzi is a very successful microorganism because it efficiently invades and infects mammals, which allows it to multiply and perpetuate itself to guarantee its existence. Metacyclic trypomastigotes (infective forms of the host mammal) can escape complement-mediated destruction, because these parasites have the surprising ability to express on their surface a variety of Complement Regulatory Proteins (CRPs), which act at different levels in the plugin path, as described in detail in the following sections:

Trypomastigote Decay-Accelerating Factor (T-DAF)

T-DAF is a glycoprotein with a MW of 87–93 kDa [11], which remains attached to the plasma membrane by means of a GPI anchor [12]. It has been determined that the blood, metacyclic and cultured trypomastigotes [13] express T-DAF, which manages to establish interactions with C3b and C4b, which induces the inhibition of the assembly and favors the accelerated decay of C3 and C5 convertase from AP and CP of the complement cascade [14,15]. These events inhibit parasitic lysis and allow their escape from the complement cascade of different mammalian species. From a cDNA expression library corresponding to T-DAF, it is possible to obtain a clone using monoclonal anti-DAF antibodies to carry out the immunosearch. It is possible to determine the nucleotide sequence that codes for a fragment of the protein and the high homology of T-DAF with human DAF can be evidenced [16,17].

Trypanosoma cruzi Calreticulin (TcCRT)

TcCRT is a Ca^{++} binding protein present in *T. cruzi* trypomastigotes, where it is located mainly in the Endoplasmic Reticulum (ER) and then transported to the surface of the parasite, mainly through the region where the flagellum arises. According to histochemical evaluations, it has other cellular locations, such as in the Golgi apparatus, flagellar pocket, reservosomes, kinetoplast, nucleus and cytosol [18], but the reasons for its ubiquity are unknown [19]. TcCRT has functions on several steps involved in the parasite/host relationship, such as the inhibition of the formation of C3 and C5 convertase of CP due to its union by S-domain with the C1q collagen stems, which prevents the activation of C1s , as well as the degradation of C4 dependent on C1s [20]. However, C1s inactivation has been observed to occur only when she is forming the C1 complex [C1q, (C1r, C1s) 2] [21]. As well as, it interacts with ficolines [22] and MBL [20] and therefore, inhibits the initial steps of complement LP. The deletion of the TcCRT gene makes *T. cruzi* sensitive to CoML, on the other hand, when parasites are modified to induce TcCRT over expression, their resistance to complement by CP and LP increases considerably [23]. Likewise, TcCRT has an important role as a virulence factor, whose infective potential is enhanced by binding to C1q [24,25].

Trypanosoma cruzi Complement Regulatory Protein (TcCRP/gp160)

TcCRP has been initially characterized by Norris et al (1989) [26], it is a 160 kDa glycoprotein (gp160) expressed by trypomastigotes, absent in epimastigotes [27]. It constitutes an integral protein of the plasma membrane of metacyclic and tissue-culture trypomastigotes of *T. cruzi*, whose insertion occurs through a GPI anchor. According to studies of sub cellular location, it has been detected in the flagellar pocket and the flagellum [28].

Transfection assays of epimastigotes with TcCRP indicate that these forms become more resistant to CoML, which strongly indicates that this protein is the protagonist in complement evasion by *T. cruzi* [29,30]. TcCRP is an inactive trans-sialidase that forms covalent bonds with C3b and C4b, which prevents the formation of functional C3 convertase from AP and CP, thus blocking the lysis of parasites mediated by the complement cascade [27,31].

Trypanosoma cruzi Complement C2 Receptor Inhibitor Trispanning Protein (TcCRIT)

TcCRIT is a protein of 32 kDa with an N-terminal extracellular domain of 27 aa called ed1, with much similarity to the C4β chain that participates in the interaction with C2 [32,33]. Trypomastigotes express it on its surface [34], where the binding of the ed1 domain with C2 occurs and prevents its degradation caused by MASP2 and C1s, which blocks the formation of C3 convertase [33,6,32] and ultimately inhibits the activation of LP and CP. TcCRIT is a C2 receptor present on *T. cruzi* (competes for C4 for binding to C2) and inhibits C2 cleavage by C1s, that is, it participates in the modulation of CP [34,35,19]. Cestari Idos *et al* (2009) [33], found that transgenic parasites that overexpress TcCRIT are highly resistant to Complement-Mediated Lysis (CoML). MBL, Ficolins and the MASP-2 enzyme have been shown to bind to the surface of metacyclic trypomastigotes and LP activates, but the mechanism of these processes is still unknown [33]. But this morphological stage is resistant to CoML because it inhibits LP, due to the expression of TcCRIT [34]. This protein have homology with ShCRIT of Schistosoma haematobium and hCRIT of humans.

Glycoprotein 58/68 (gp58/68)

This protein has a MW of 58 kDa in its unreduced form and 68 kDa in a reduced condition [36]. Gp58/68 and an 80-85 kDa protein form a collagen/fibronectin receptor in trypomastigotes [37], which plays a fundamental role in the binding of trypomastigotes to mammalian cells [38,39,36]. This glycoprotein has complement regulatory functions, because it prevents the formation of C3 convertase from AP on the surface of *T. cruzi* and in the fluid medium. Its mechanism of action depends on blocking the binding of complement Factor B

(FB) with C3b.

Conclusion

A detailed review of the mechanisms of complement evasion by *T. cruzi* shows its high efficiency and great ability to escape the action of this protein cascade. This microorganism produces a diversity of complement regulatory proteins, this function is to exert its action at various levels of the complement cascade and thus ensure the inhibition of the different routes of this prodigious mechanism belonging to the innate immune system.

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