Trypanosoma cruzi trypomastigotes, but not epimastigotes, are normally resistant to the lytic effects of complement from vertebrate hosts susceptible to infection. This resistance facilitates parasite survival and infectivity. During the course of chronic infections, however, the vertebrate hosts produce antibodies that render the trypomastigotes sensitive to lysis, primarily via the alternative complement cascade and amplified by the classical pathway. Here, Greice Krautz, Jessica Kissinger and Antoniana Krettli summarize research on lytic antibodies, and on their respective target(s) on the T. cruzi surface. These targets are useful in tests aimed at the diagnosis of chronic Chagas disease for control of care after specific treatment and for vaccine development.

Trypanosoma cruzi, the parasite responsible for Chagas disease in humans, has evolved a mechanism whereby its infective trypomastigote forms can evade the vertebrate host’s natural immune responses. Specifically, T. cruzi trypomastigotes are resistant to direct lysis by the complement (C) system, thus enabling them to survive in the vertebrate host’s bloodstream. This resistance is a developmentally regulated phenomenon because the parasite is susceptible to C lysis when it is in its multiplicative insect form, the epimastigote. It is known that active, lysis-promoting C3 is deposited on the C-sensitive epimastigote forms, whereas it is primarily the inactive C3b fragment (C3b) that is deposited on the resistant trypomastigote forms. Resistance to direct C lysis is ablated by pretreating trypomastigotes with proteases or by heating them to 45°C. The C3 and factor B components of the C cascade have been detected on the surface of trypsin-treated trypomastigotes. These studies suggest that trypomastigotes contain C acceptor(s) on their surfaces to protect them from serum lysis. Thus, these C acceptors represent important targets for vaccine development, because of their key role in immune system evasion.

What are ‘lytic antibodies’?

The term ‘lytic antibodies’ acknowledges that sera from chronic T. cruzi infections (in mice and humans), which could agglutinate parasites and cause them to lose infectivity (reviewed in Ref. 4), converted trypomastigotes into a target for C lysis. Incubation of trypomastigotes with human C does not lead to lysis when the trypomastigotes do not have immunoglobulins on their surfaces (eg, in the case of trypomastigotes isolated from cell culture or from the bloodstream of mice that have been lethally irradiated or cyclophosphamide treated). However, if such trypomastigotes are pre-incubated with sera obtained from chronically infected hosts, IgG immunoglobulins bind to their surface and the parasites become sensitive to lysis by fresh human sera as a source of C. This antibody-dependent C-mediated lysis (CoML) is primarily via the alternative pathway. This was initially suggested by the finding that parasites precoated with specific antibodies, in vivo or in vitro, were not lysed by diluted fresh normal sera.

Three fundamental observations showed that CoML of trypomastigotes in vitro requires an intact alternative pathway: (1) serum depletion of factor B and properdin (factor P) completely abrogates lysis of trypomastigotes precoated with IgG; however, parasite lysis is restored when the purified factors are added to the system; (2) only high levels of C in fresh normal sera trigger antibody-mediated trypomastigote lysis; and (3) the classical pathway enhances lysis by the alternative pathway: sera from individuals genetically deficient in the classical C protein C2 can still lyse 50% of IgG-coated trypomastigotes compared with controls treated with normal sera; the addition of increasing amounts of purified C2 to the deficient sera restores the lytic activity to levels comparable to that of controls.

More insight into the lytic antibody-dependent activation of the alternative pathway by trypomastigotes came from experiments demonstrating that Fab and Fab fragments derived from the IgG of chagasic patients could also promote CoML of trypomastigotes. Therefore, the lysis-promoting activity of the antibodies was independent of Fc and was instead related to the binding of such specific IgG molecules on the parasite surface. In subsequent studies, 87–93 kDa glycoproteins that could accelerate the decay of C3 convertases were isolated from trypomastigotes using sera from T. cruzi chronically infected patients. Hence, it was clear that decay-accelerating factor (DAF)-like molecules were present on trypomastigotes, enabling them to escape the lytic effects of C, and it became essential to establish whether or not the DAF-like molecules on the parasite surface were also the targets of the lytic antibodies. Lytic antibodies are not easy to elicit by immunization with dead parasite or with purified T. cruzi antigens, although they are readily detected in mice, rats, rabbits and humans infected with T. cruzi. Inoculation of mice with glutaraldehyde-fixed or freeze-thawed parasites leads to the production of antibodies detected by conventional serological tests using air-dried trypomastigote antigens, but not lytic antibodies. Lytic antibodies were later elicited by inoculation with attenuated trypomastigotes and with trypomastigote antigens shed into the culture media, suggesting that the target is labile and the shed protein fragment is necessary, and sufficient, to confer C resistance.
**Focus**

**Table 1. Known possible targets of anti-*Trypanosoma cruzi* lytic antibodies**

<table>
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<th>Possible lytic antibody targets</th>
<th>Developmental stage of protein synthesis</th>
<th>Possible function and known characteristics</th>
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<tr>
<td>160 kDa protein (alias, gp160, CRP-160, CRP-10)</td>
<td>Trypomastigote specific</td>
<td>Induces lytic antibodies; detects cure of chronic infection; GPI-anchored glycoprotein, binds C3b and C4b; interferes with C3 convertase formation; confers complement resistance to gp160-transfected epimastigotes</td>
<td>13–15,29–31</td>
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<tr>
<td>87–93 kDa protein (alias T-DAF)</td>
<td>Trypomastigote specific</td>
<td>Accelerates decay of C3 convertase and factor B binding; cDNA clone shares similarity with human DAF; recombinant protein induces lytic antibodies and inhibits convertase activity</td>
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<td>58/68 kDa protein</td>
<td>Trypomastigote specific</td>
<td>Glycoprotein, interferes with function of the alternative pathway of complement activation by interacting with factor B</td>
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<td>Mucin-like GPI-anchored glycoproteins</td>
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<td>Target of lysis-supporting anti-sialic acid antibodies; detects cure of chronic infection</td>
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<tr>
<td>77, 82 and 88 kDa proteins</td>
<td>Trypomastigote</td>
<td>Unknown</td>
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<tr>
<td>Variety of proteins in the 72–160 kDa range</td>
<td>Trypomastigote</td>
<td>Unknown; authors suggest that these different proteins share a common epitope recognized by lytic antibodies</td>
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<td>85 and 90 kDa proteins</td>
<td>Epimastigote, trypomastigote</td>
<td>Passive transfer of monoclonal antibodies against these proteins to mice confers protection</td>
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<td>15 and 45 kDa proteins</td>
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<td>24 kDa flagellar protein</td>
<td>Epimastigote, epimastigote, amastigote</td>
<td>Binds Ca2+; might be involved in motility; protects against infection; detects cure of chronic infection</td>
<td>26,32–34</td>
</tr>
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*Abbreviations: CRP, complement regulatory protein; GPI, glycosphosphatidylinositol; T-DAF, trypomastigote decay-accelerating factor.*

These early observations set the stage for two main lines of research: (1) the search to identity the target, or targets, of the lytic antibodies on the parasite surface and the determination of how antibody binding to these targets activates CoML via the alternative pathway; and (2) the use of the CoML test as a diagnostic tool to assess parasitological cure after specific therapies, based on data in treated mice and patients (negative by CoML and parasitological tests). CoML is consistently positive in non-treated patients and animals with ongoing chronic infections detected by repeated hemocultures\(^1\). Because most treated patients remain serologically positive to other *T. cruzi* crude antigens in conventional serology, the hypothesis of cure based on seroconversion of the CoML is still disputed among clinicians (for review, see Ref. 12). We will focus here on the potential antigen targets of lytic antibodies, emphasizing the 160 kDa C regulatory protein (CRP) and trypomastigote-DAF (T-DAF), for which the encoding genes have been identified.

**Identification of lytic antibody targets**

Researchers studying *T. cruzi* surface proteins have uncovered a number of potential lytic antibody targets (summarized in Table 1). The first potential target for lytic antibodies, a glycoprotein of 160 kDa (gp160), was identified on trypomastigotes, but not epimastigote forms, on western blots (WBs) probed by sera from chronic infections in humans and mice. Sera from non-infected individuals (human or mice), from immunized non-infected mice and from cured subjects (mice and humans) unable to transform trypomastigotes into cells sensitive to CoML cannot bind to gp160 on WB\(^12\). Gp160 is trypomastigote specific, contains a glycosphosphatidylinositol (GPI) anchor and is actively released by the parasite into culture media; it elicits anti-gp160 antibodies in vivo that convert the C-resistant trypomastigotes into cells susceptible to CoML\(^13,15\). These proteins to mice confers protection against infection induced by metacyclic trypomastigotes in mice\(^14,15\). gp160 has C3b- and C4b-binding affinity and can inhibit the formation of the C3 convertases of both the alternative and classical pathways\(^16\); thus, gp160 prevents C-mediated lysis of trypomastigotes. These findings, combined with earlier proposed models for other C regulatory proteins, allowed Norris et al.\(^17\) to propose that gp160 prevents C lysis of the trypomastigotes in the absence of antibodies. This model takes advantage of the functional and genetic similarities of gp160 CRP to the human DAF. Accordingly, when 160 kDa CRP binds to C3b and prevents interaction of C3a with factor B, C3 convertase formation is inhibited and hence no parasite lysis occurs. In the presence of anti-gp160 antibodies, the binding of 160 kDa CRP to C3b is blocked, C3 convertase is formed and lysis of the parasite occurs as a result of the C cascade. Therefore, antibody to 160 kDa CRP are necessary to activate the C alternative pathway on the surface membrane of the trypomastigote for lysis to occur (Fig. 1).

In addition to 160 kDa CRP, there are two other trypomastigote-specific glycoproteins with C regulatory
activity able to induce lytic antibodies—the 85–93 kDa T-DAF and a 58–68 kDa glycoprotein (gp 58/68) T-DAF, similar to 160 kDa CRP, is actively released by the parasite and is functionally analogous to human DAF, interfering with the efficient assembly of the C3 convertase of both the alternative and classical pathways, by binding to C3b and C4b. By contrast, gp 58/68 inhibits only the C3 convertase of the alternative pathway, by interacting with factor B rather than C3b. In a recent review, Tomlinson and Raper described the known mechanisms by which T. cruzi trypomastigotes evade C activation in the normal mammalian susceptible host.

Some of the targets for lytic antibodies have been used to immunize mice and have been found to protect against subsequent T. cruzi infection. Other targets for lytic antibodies have also been used in serological assays, potential replacements for the CoML test for monitoring cure of chagasic patients treated with trypanocidal drugs (Table 1). Among antigens capable of detecting cure there is a 74 kDa antigen identified as one of the mucin-like GPI-anchored glycoproteins. These glycoconjugates are extremely abundant on the trypomastigote surface, and they contain α-galactosyl epitopes present in a series of O-linked oligosaccharide chains recognized by lytic anti-α-galactosyl antibodies from chronic chagasic patients. Mucin-like glycoproteins are also acceptors by sequencing the N-terminal region of the native, purified 160 kDa CRP. Therefore, it was proposed that the trypomastigotes escape from C-mediated lysis by masking their target epitopes with sialic acid residues, thus preventing recognition by anti-α-galactosyl antibodies.

Cloning candidate target genes

Cloning the T. cruzi genes encoding the C inhibitory proteins T-DAF and 160 kDa CRP has been difficult because of their similarity to members of large heterogenous T. cruzi families, especially genes related to the trans-sialidase superfamily. However, molecular cloning of these proteins is essential to verify their genetic relationship and confirm their function in T. cruzi trypomastigotes.

A partial cDNA clone expressing the gene encoding T-DAF was isolated from a trypomastigote cDNA library using antibodies to the native, purified T-DAF. This gene showed sequence homology to human DAF and, unexpectedly, to the 85 kDa major surface protein of T. cruzi, characterized as a member of the trans-sialidase superfamily. The recombinant protein had T-DAF activity and antibodies raised against the fusion protein are lytic for trypomastigotes in the presence of C2.

The cloning of the gene encoding 160 kDa CRP began by sequencing the N-terminal region of the native, purified glycoprotein. The sequence obtained revealed that it is most closely related to the flagellar protein (FL-160) family of genes, which are trypomastigote-specific and of unknown function, and is distinct from the active trans-sialidase gene. Based on the C-terminal sequence of known gene members of the FL-160 family, a full-length cDNA, called CRP-10 (putatively encoding 160 kDa CRP), was cloned by reverse transcription–PCR and sequenced. As expected, CRP-10 had striking similarity to the FL-160 family of genes (80% identity), but the inferred N-terminal protein sequence of CRP-10 was different from that of the 160 kDa CRP, sharing only four of 14 clearly identified residues. Thus, it became necessary to verify that CRP-10 was indeed a CRP, by expressing the gene and analyzing the protein function.

The recombinant CRP-10 was able to bind C3b and antibodies raised to CRP-10; it recognized the native, purified 160 kDa CRP. Therefore, it was proposed that the CRP-10 gene encodes a variant of 160 kDa CRP. CRP-10 does not have sequence homology with mammalian CRPs.

After these results, the ability of CRP to inhibit lysis was assessed. The cloned CRP-10 gene was stably transfected into the normally C-sensitive epimastigote stage of T. cruzi. Epimastigotes transfected with CRP-10 produced a functional C3b-binding protein and became resistant to lysis when exposed to C. As is seen for T-DAF and 160 kDa CRP, antibodies to recombinant Tc24 can induce lytic antibodies. Umezawa et al. suggested the
interesting hypothesis that the large number of apparent targets for the lytic antibodies might in fact be the result of a shared epitope, an idea that deserves further investigation.

**References**