IDENTIFICATION OF MEMBRANE COMPONENTS OF *Trypanosoma cruzi* MODULATORS OF COMPLEMENT SYSTEM

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Resistance to complement (C)- mediated killing is an important acquisition for the infective forms of *Trypanosoma cruzi*. While epimastigotes (Epi) are C susceptible, the metacyclic trypomastigote (MT) which develops within the vector, the bloodstream trypomastigotes (BTF), and the tissue culture derived trypomastigotes (TCT) are C resistant (1-4).

We have shown recently that MT, TCT and BTF produce molecules, which interfere with the C3 convertases of the classical and alternative pathway. These substances are shed when parasites are incubated for 10 minutes at 45°C (5) or for 4 hours at 37°C. These molecules, which are therefore analogous in their function to human decay accelerating factor (DAF), a substance which prevents lysis of cells by autologous C (7, 8) may explain the developmental resistance of trypomastigote stages of T. cruzi to C- mediated lysis.

We now describe the purification and identification of T. cruzi-DAF molecule from CMT and TCT from the Y strain and Miranda 88- clone.

RESULTS

³⁵S Labelled parasites were allowed to shed spontaneously by incubating them either at 37°C for 4 hours or at 45°C for 10 minutes. Parasite pellets and supernatants (sup) were obtained after centrifugation and thereafter protease inhibitors (NPGB and leupeptin) were added.

Supernatants were assessed for T-DAF activity, by measuring decay/ inhibition of EAC14b2a (C3 convertase of the classical pathway) (5).

By PFLC chromatofocusing we were able to obtain a fraction with functional DAF activity which co-elutes with a 87-93 Kd ³⁵S containing band. By using solid phase lectins the purified molecule was completely retained by ConA-Sepharose but not by WGA, PNA or Soybean-Sepharose columns.
Whole sup or purified fraction were treated with solid phase proteases: trypsin, chymotrypsin and papain. Papain completely eliminated DAF activity, whereas trypsin decreased it by 30% and chymotrypsin had no effect.

We attempted to remove DAF activity from sup by absorption with antibodies (Ab) directed to T. cruzi molecules: 1) Mab 2F1 that recognizes a 90 Kd surface molecule specific for trypomastigotes, 2) polyclonal Ab against 2F1, 3) polyclonal Ab against Tc 45, 4) polyclonal 'anti Epi, 5) human chronic chagasic serum (HCS), or 6) anti serum to the cross reacting determinants (CRD) found within molecules bearing phospholipid anchors. In all these circumstances only HCS immunoprecipitated the 35S band as well as removing the DAF activity from TCT sup.

Immunoprecipitation was then done with Abs to human C proteins which bind to C3b and C4b and are part of a super gene family and share sequence homology (reviewed in 10, 11). Those are factor B, H, DAF, C2, C4Bp and CR1. No specific immunoprecipitation was observed with these Abs, as assessed by absence of 35S bands (DAF, C2, C4Bp, CR1) or by the lack of change in intensity of faint bands recognized by Ab (H, B) if the solid phase resins were saturated with the complement ligand prior to adding TCT sup.

**DISCUSSION**

We have shown that functional DAF activity co-elutes from an FPLC chromatofocusing column with a 87-93 Kd 35S- containing band. The DAF activity is destroyed by protease treatment and remove on lectin affinity column, procedures which are associated respectively with complete cleavage or complete removal of the 87-93 Kd band from the supernatant. Therefore, the evidence suggests that the molecule with functional decay accelerating activity is a glycoprotein.

We do not know the relationship between the 87-93 Kd shed molecule containing decay accelerating activity, and other T. cruzi (12-15) molecules of similar molecular weight described previously. The 90 Kd band in M88 CMT and TCT recognized by Mab 2F1 is also recognized by the broadly reactive monoclonal antibody 1G7 but not by other monoclonal antibodies to 90 Kd with more restricted specificity (15). However none of these antibodies immunoprecipitate the 87-93 Kd molecule with decay accelerating activity. It would also appear unlikely that the 87-93 Kd molecule is the same as the 85 Kd - 86 Kd molecular weight glycoprotein already described by Katzin and Colli and further characterized by Andrews; Zingales and Colli (16), apparently involved in cell attachment since this molecule was agglutinated by WGA (17, 18).

Finally, we were unable to immunoprecipitate the 87-93 Kd band with a rabbit antiserum raised against the nonapeptide repeat of the 86 Kd cell surface molecule of T. cruzi recently cloned by Peterson, et al. (19).

The recent demonstration that human DAF bears a phospholipid membrane anchor (20, 21) and the observation that T. cruzi decay accelerating antigens are shed into the supernatant, raise the possibility that the trypomastigote antigens were released by an
endogenous phospholipase which cleaves a phosphatidylinositol (PI) membrane anchor (reviewed in 22). Although preliminary evidence indicating that the membrane form of the 87-93 Kd antigen is soluble exclusively in detergent whereas the supernatant form is soluble in aqueous buffers, no endogenous phospholipase activity in T. cruzi CMT, was detected. Furthermore, no immunoprecipitation of the released 87-93 Kd band was observed with antiserum to the cross reacting determinant (CRD) common to PI- linked proteins, nor were bands of this molecular weight intrinsically labelled with myristate or palmitate in intact parasites. Therefore, we have no direct evidence that 87-93 Kd band has a phospholipid membrane anchor which is cleaved when the molecule is released into the supernatant.

The DAF activity of the molecule or molecules obtained at 45°C previously shown to inhibit the C3 convertase of the classical pathway, as herewith shown using intrinsically labelled forms of the same parasite, co-eluted with an 35S band of 87-93 Kd. In fact no major bands of 80 Kd or lower were detected in Y strain TCT supernatants. Since we have also demonstrated that TCT and CMT of the M88 clone shed molecules of 87-93 Kd which co-elute with DAF activity, the possibility that the molecules were antigenically related, is substantial. However, the relationship between the decay accelerating molecules produced by MCT, TCT and BTB trypomastigotes requires further investigation.

The isolation of a complement decay accelerating molecule from bacteria, parasites or fungi has not previously been accomplished. It is foreseen that production of DAF-like molecules will be a strategy for complement evasion employed by many different microorganisms. Certainly, the most interesting issue will be an investigation of the relationship at the gene and amino acid levels between the human and T. cruzi decay accelerating factors and their role in protective immunity.
REFERENCES


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