Enhanced Invasion of Mononuclear Phagocytes by Serum-treated Trypanosoma cruzi is Due to Clq.

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The ligands and receptors involved in cell uptake of T. cruzi trypomastigotes following serum incubation of the parasites are not defined. Studies by our laboratory and others suggested that C3 receptors were not involved in the uptake of trypomastigotes by macrophages. The notion that C3 fragments deposited during incubation in serum or derived from local production by macrophages are necessary for cell attachment and entry, has only been investigated for organisms with an obligatory residence in professional phagocytic cells. T.cruzi has a broad host cell range and although both epimastigotes (Epi) and tissue-culture trypomastigotes (TCT) are internalized within mononuclear phagocytes, only trypomastigotes infects other types of cells. We report here that enhancement of TCT internalization after serum treatment results from Clq deposition on the parasite surface. We showed that uptake of TCT by macrophages was augmented by incubation in normal human serum (NHS). Heating serum (HNHS) or depleting serum of Clq (ClqD) totally abrogated the serum mediated enhancement of attachment and entry. When HNHS or ClqD serum were reconstituted with purified Clq the enhancement was restored. The enhancement of attachment and entry of NHS-treated TCT into human macrophages was also reflected in more parasites/infected macrophage after 48 hours of culture. Treatment of TCT with ClqD serum resulted in fewer parasites/infected macrophage after 48 hrs. of culture. Adding Clq but not factor D to ClqD serum, which lacks both components, restored the level of infection to that observed

with NHS. We next sought to investigate, using a serum-free system, specific binding of human C1q to the parasite surface, as well as activation of human C1 and the role of C1q in the T.cruzi mononuclear cell interaction. We demonstrate that the binding of C1q to Epi and TCT at 4°C is concentration dependant and saturable. The calculated number of C1q binding sites at saturation was 5.2×10^3 sites per Epi and 5.8×10^3 sites per TCT, although Epi consistently showed higher apparent affinity for C1q. Although we could not demonstrate conventional activation of human C1, which had been reconstituted from purified components (C1q, $C1r_2$, ^{125}I-$C1s_2$), we found that partial degradation of C1s, which was more extensive with TCT (65% cleavage) than with Epi (43% cleavage) occurred in the presence and absence of C1 inhibitor. Partial proteolytic cleavage of purified ^{125}I-C1q, greater for Epi (91%) than for TCT (39%) was also detected during incubation at 37°C. Cleavage of native C1 and degradation of C1q by the parasites could result in exposure of C1q tails and does not preclude a role for free cleavage fragments in enhancing internalization, since purified collagenous-like tail region of C1q enhances ingestion of opsonized targets on phagocytic cells. Finally, the role of purified C1q on parasite internalization by phagocytic cells was tested. Pretreatment of TCT with C1q at 0°C produced an enhancement of internalization (% internalization: 2.2 fold; Index: 2.7 fold) over native TCT entry into human monocytes. The extent of enhancement was similar when serum-free culture derived human macrophages were used as target cells. No differences were found when Epi bearing C1q

were compared with native Epi entry into monocytes or macrophages. Plating monocytes or macrophages on Clq coated surfaces also produced an enhancement of parasites internalization (% internalization: 2.4 fold; Index: 3.7 fold for monocytes and when macrophages were used the enhancement was % internalization: 2.4 fold; Index: 2.5 fold) analogous to the effects of Clq on enhancing phagocytosis of other particles. When Epi were used, no increase in either parameters were found with monocytes or macrophages adhere to a Clq-coated surface. How the ClqR enhances the internalization of parasites or other particles is unknown. In our system it is likely that Clq can influence an unrelated receptor, since enhanced internalization of TCT was observed when either the parasites were opsoniced with Clq or the phagocytes were plated on Clq. Fibronectin can be the other receptor involved since TCT bind fibronectin and the interaction of Clq with fibronectin may also enhance parasite attachment and entry. If that is the case our results differ from those previously reported since Clq is acting in our system without an additional requirement for C3 fragments or IgG on the target particle. The fundamental differences in the mechanism of serum resistance in the infective forms of different parasites, depending on the amount of C3 fragments that bear on the membranes after serum incubation may thus dictate, at least in part, the ligand receptor interactions and the host-cell invasion. T.cruzi trypomastigotes produce a C3 convertasa inhibitor preventing deposition of C3 but not of Clq during serum incubation. These infective forms of T.cruzi are directed to cells bearing Clq receptors and fibronectin, of which connective tissue cells are the prototype