Complement Component C1q Enhances Invasion of Human Mononuclear Phagocytes and Fibroblasts by Trypanosoma cruzi Trypomastigotes

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Abstract

Internalization and infectivity of Trypanosoma cruzi trypomastigotes by macrophages is enhanced by prior treatment of parasites with normal human serum. Heating serum or removing C1q from serum abrogates the enhancement, but augmentation of attachment and infectivity is restored by addition of purified C1q to either serum source. Although both noninfective epimastigotes (Epi) and vertebrate-stage tissue culture trypomastigotes (TCT) bind C1q in saturable fashion at 4°C, internalization by monocytes and macrophages of TCT but not Epi-bearing C1q is enhanced in comparison to untreated parasites. Adherence of human monocytes and macrophages to surfaces coated with C1q also induces a marked enhancement of the internalization of native TCT.

C1q enhances attachment of both Epi and TCT to human foreskin fibroblasts, but only when C1q is on the parasite and not when the fibroblasts are plated on C1q-coated surfaces. Only TCT coated with C1q show enhanced invasion into fibroblasts.

Although trypomastigotes produce an inhibitor of the complement cascade which limits C3 deposition during incubation in normal human serum, C1q binds to the parasite and enhances entry of trypomastigotes into target cells.

Introduction

Infection of monocytes and macrophages by Leishmania spp. (1–3), Legionella pneumophila (4), Mycobacterium tuberculosis (5), and Histoplasma capsulatum (6) is blocked with monoclonal antibodies to receptors for the third component of complement, CR1 and CR3. Furthermore, productive infection of macrophages by these organisms is markedly enhanced by incubation of the organisms in serum before allowing cell attachment and entry to proceed. These findings, which are most thoroughly developed with Leishmania major (2, 3) and Leishmania donovani (1), have led to the notion that C3 fragments deposited during incubation in serum or derived from local production by macrophages (7, 8) are necessary for cell attachment and entry. An alternative mechanism has now been proposed for serum-independent uptake of Leishmania mexicana via CR3 (9). Nonetheless, the central involvement of CR1 and CR3 has only been postulated for organisms with an obligatory residence in professional phagocytic cells.

Trypanosoma cruzi, the causative agent of Chagas disease, is an intracellular parasite closely related to Leishmania spp., but with a broad host cell range (reviewed in Zingales and Colli [10]). The type of disease caused by T. cruzi, in which cells of connective tissue origin such as fibroblasts and muscle cells are predominantly infected, differs markedly from that of clinical Leishmania infection involving macrophages of either the cutaneous, subcutaneous or visceral organs. Although vector-stage epimastigotes (Epi) and vertebrate-stage trypomastigotes of T. cruzi are internalized within mononuclear phagocytes, only the biologically relevant trypomastigote stage infects other types of cells (10).

The ligands and receptors involved in cell uptake of T. cruzi trypomastigotes following serum incubation are not defined. Nogueira and Cohn (11) suggested that C3 receptors were not involved in the uptake process, since trypsinization of macrophages did not affect trypomastigote attachment but did diminish binding of complement-coated erythrocytes. In more recent studies by our laboratory (Rimoldi, M. T., and K. A. Joiner, unpublished observations) and others (12), treatment of trypomastigotes with serum augmented uptake into human and mouse macrophages, but the augmentation was not blocked using monoclonal antibodies directed against CR1 and CR3. In contrast, uptake of non-infective epimastigotes after autologous serum incubation is significantly inhibited by anti-CR3 antibodies. These findings are reflective of the difference in deposition of C3 on the parasite surface during incubation in serum. Epi bear large numbers of C3 molecules after serum incubation (13–15), whereas deposition of C3 on the trypomastigote surface is limited by a C3 convertase inhibitor produced by this stage (16–18).

We therefore sought another explanation for the serum-mediated enhancement of internalization of trypomastigotes into phagocytic cells. We report here that complement component C1q enhances invasion of human mononuclear phagocytes and fibroblasts by trypomastigotes of T. cruzi.

Methods

Buffers and reagents. The following buffers were used: Hanks' buffered salt solution (HBSS) containing 10 mg/ml bovine serum albumin (BSA) (Boehringer-Mannheim) (HBSS-BSA); HBSS containing 20 mg/ml sucrose (HBSS-S); RPMI-1640 (Gibco Laboratories, Grand Island, NY) containing 2 mM glutamine and 10 mg/ml BSA; HL1

1 Abbreviations used in this paper: BESM, bovine embryo skin muscle; Epi, epimastigote(s); HINHS, normal human serum heated to inactivate complement; HLb, hypotonic lysis buffer; NHS, normal human serum; NPGb, nitrophenyl guanidino benzoate; TCT, tissue culture trypomastigote(s).
buffered saline (Altex, Berkeley, Calif.) and fibronectin (Basingstoke, Hampshire, U.K.). The peptide was described in LLCMK2 cells at a concentration of 10^6/ml. 

Serum. Normal human sera (NS) were collected and frozen in aliquots at −70°C. Some samples of serum were heated at 56°C for 30 min to inactivate complement (HINHS). Human serum was also depleted of C1q and factor D (C1q D serum) using a BioRex 70 column (Bio-Rad Laboratories, Richmond, CA) at pH 7.3 as described (19). The absence of C1q was verified by hemolytic titer as described by Kolb et al. (20). This method can detect <0.4 ng C1q. Human AB serum was collected from a normal volunteer and heated at 56°C for 30 min. Neither sera contained detectable antibodies for T. cruzi as measured by indirect immunofluorescence at a dilution of 1:10.

Purification and radiolabeling of C1 subcomponents. C1q was isolated from human serum or plasma as described previously (19) and radiolabeled with Na^211I (Amersham International, Arlington Heights, IL) to an average specific radioactivity of 0.5 µCi/µg. Purified C1q gave one band on SDS-PAGE. When tested by double immunodiffusion, purified C1q gave no line when tested against antiserum to high density lipoprotein, to “Cruzin,” the serum inhibitor of T. cruzi neunnamidase (21) (antiserum kindly provided by M. E. A. Pereira, Tufts University, Boston, MA), or to fibronectin. C1r, C1s, and C1 inhibitor were isolated as previously described (22–24). C1s was radiodiodinated in the presence of 5 mM CaCl_2 (25) using Enzymobeads (Bio-Rad Laboratories) to a specific radioactivity of 0.5 µCi/µg. Native C1 was reconstituted in the presence of 5 mM CaCl_2 by incubating equimolar amounts of purified C1q, C1r, and trace-labeled ^125I-C1s for 20 min at 0°C (26).

Internalization of TCT by macrophages: effect of incubation in serum. Long-term culture monocyte-derived macrophages were prepared (Sechler, J. M., M. K. Warren, and J. I. Gallin, manuscript in preparation). Mononuclear cells were obtained from peripheral blood by separation on Histopaque cushions and were maintained in culture for at least 1 mo by replating those initially adherent cells which spontaneously lifted off of culture plates into new flasks. Cells were > 99% non-specific esterase positive and had typical macrophage morphology. Cells expressed Fe, CR1, and CR3 receptors, as determined by sheep cell rosetting, and expressed FMLP receptors, as determined by affinity labeling. Macrophages were avidly phagocytic for opsonized Candida albicans and responded to γ-interferon with enhanced production of superoxide on stimulation with phorbol myristate acetate (PMA). Macrophages prepared as described were plated in V24-well petri dishes in RPMI-1640 with 5% heat-inactivated human AB serum and allowed to adhere in the petri dishes for at least 24 h at 37°C in 5% CO_2. TCT (Y strain) (10^9/ml) were preincubated for 10 min at 37°C in medium (RPMI-1640 with 5% heat-inactivated AB serum) and then incubated with one of the following sources of serum: 20% NHS, 20% HINHS, 20% C1qD, 20% HINHS with addition of 200 µg/ml C1q, or 20% C1qD with addition of 200 µg/ml C1q. The TCT were then washed twice in RPMI-1640 containing 5% human AB serum, added to the petri dishes at a parasite/cell ratio of 10:1, and incubated for 2 h at 37°C in 5% CO_2. Noninternalized parasites were removed by hypotonic lysis with HLB. 

In all experiments reported in this manuscript using monococytes and macrophages, only internalized parasites were enumerated. Although this procedure will not identify ligands which enhance attachment only, it unambiguously identifies ligands which facilitate internalization. After fixation and staining with Leukostat (Fisher Scientific, Springfield, NJ), internalization was determined by light microscopy.

In experiments to test the effect of C1q on TCT infectivity, parasites incubated in serum and washed as described above were added to long-term culture monocyte-derived macrophages. Incubation was carried out for 2 h at 37°C in 5% CO_2; non-cell-associated parasites were washed away, and incubation was continued for an additional 48 h. The number of internalized parasites, consisting almost exclusively of amastigotes, was assessed as described above.

125I-C1q binding to T. cruzi. Binding of monomeric ^125I-C1q to Epi and TCT (M88 clone) was performed by incubating triplicate tubes containing 10^7 parasites in HBSS-A with varying amounts of a mixture of ^125I-labeled and unlabeled C1q in a total volume of 100 µl. After incubation on ice for 20 min, the reaction mixture was layered over a 150-µl cushion of HBSS-S and centrifuged at 10,000 g for 30 s at 4°C in a microcentrifuge (Microfuge, Beckman Instruments, Inc., Fullerton, CA). The parasite pellet and the tube with the remaining supernatant were counted separately in a γ scintillation counter (Packard MultiPries 4, Packard Instrument Co., Inc., Downers Grove, IL). In certain samples, unlabeled C1q (up to 100-fold molar excess over ^125I-C1q) was also added to determine nonspecific binding of ^125I-C1q. Nonspecific uptake was usually 1% or less of total input and 15% or less of total bound radioactivity. Experimental values were corrected for subtraction of nonspecific uptake.

Cleavage of C1s by T. cruzi. An aliquot of 20 µl of reconstituted C1 containing ^125I-C1s in HBSS-BSA was added to a pellet containing 10^7 or 2 × 10^7 parasites (M88 clone). The samples containing C1 at or near serum concentration (1.8 × 10^{-7} M) were incubated at 30°C for 10 min. In some experiments, a physiologic concentration of C1 inhibitor (C1 Inh) (1.3 × 10^{-5} M) or 25 µM NPGB was added. Reactions were stopped by the addition of equal volumes of SDS-PAGE sample buffer containing 15 mM dithiothreitol and incubated for 45 min at 37°C. C1 activation was assayed by SDS-PAGE under reducing conditions as previously described (26). In some experiments, 10^7 or 2 × 10^7 parasites were incubated in a volume of HBSS-BSA identical to that of ^125I-C1 for 10 min at 30°C. Parasites were pelleted, and the supernatant was assayed for its ability to activate C1 in the presence and absence of C1 Inh. Control tubes contained aggregated IgG (1 mg/ml) in the presence or absence of C1 inhibitor.

Degradation of C1q by T. cruzi. A volume of 95 µl containing 2.5 × 10^7 parasites/ml (M88 clone) was mixed with 5 µl (20 µg) of ^125I-C1q at 0°C and incubated for 10 and 30 min at 37°C. The samples were centrifuged at 10,000 g for 30 s at 4°C in a microcentrifuge (Microfuge) and the pellet and the supernatant were separated. The pellet was divided into equal aliquots and treated with sample buffer with or without 2-mercaptoethanol. Samples were electrophoresed in 10% SDS-PAGE in the presence of urea, and the percentage of degradation of bound C1q was quantitated by densitometric scanning (Ultrascan XL, LKB Produkter, Bromma, Sweden) of the autoradiograms.

Internalization of T. cruzi-bearing C1q by monococytes and macrophages. Human peripheral blood monococytes were isolated by counterflow elutriation using a modification of the technique of Lionetti et al. (27) as described (28). Macrophages, as defined here for the serum-free experiments, were elutriated monocyes that had been cultured in Teflon jars (Savillex Corp., Minnetonka, MN) at 1 × 10^6 cells/ml in

Complement C1q and Trypanosoma cruzi
HL1 containing 2 mM l-glutamine and 10 μg/ml of gentamicin in 5% CO₂ at 37°C. On day 7 of culture, macrophages were harvested from the Teflon jars by vigorous pipetting and washed twice in PBS before use. Effector cells (monocytes or macrophages) were suspended in RPMI-1640 medium containing 2 mM l-glutamine, 5 mM MgCl₂, and 10 μg/ml gentamicin at 2.5 × 10⁷/ml, and 250 μl (6.25 × 10⁶ cells) was added to each well of a Lab-Tek chamber previously coated with BSA (40 μg/ml). The cells were allowed to adhere for 1 h at 37°C in 5% CO₂. TCT or Epi (M88 clone) at 1 × 10⁶/ml were pretreated for 15 min at 0°C with Clq (200 μg/ml), washed twice at 4°C, and added in a parasite/mononuclear cell ratio of 10:1. Chambers were centrifuged at room temperature for 3 min at 100 g and were incubated for 30 min at 37°C in 5% CO₂. Noninternalized parasites were removed by hypotonic lysis with HLB. After fixation with PBS-glutaraldehyde and staining with Giemsa, internalization was determined by light microscopy.

**Internalization of T. cruzi by monocytes or macrophages adhered in the presence or absence of Clq.** Eight-well Lab-Tek chambers (Miles Laboratories, Naperville, IL) were incubated with Clq (30 μg/ml) or BSA (40 μg/ml) in 0.1 M carbonate buffer, pH 9.5, for 2 h at room temperature. Chambers were washed twice with PBS immediately before use. Monocytes and macrophages, prepared as described above, were added and allowed to adhere for 1 h at 37°C in 5% CO₂. The experiments were performed by adding parasites (M88 clone) at a parasite/ cell ratio of 10:1.

**Attachment of T. cruzi-bearing Clq to human foreskin fibroblasts.** Human foreskin fibroblasts were obtained from the American Type Culture Collection, Rockville, MD (ATCC No. CRL-1635) and maintained at low passage number in Eagle’s minimal essential medium containing 10% fetal calf serum, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. For use in experiments, cells were released with trypsin/EDTA and plated overnight on glass coverslips (12 mm, No. 1, Fisher Scientific Co., Pittsburgh, PA) at 2 × 10⁵ cells per coverslip. TCT or Epi in PBS at 2 × 10⁷/ml were pretreated for 15 min at 0°C with buffer or (a) Clq at 100 μg/ml, (b) Clq (100 μg/ml) and RGDS (50 μg/ml), (c) human plasma fibronectin at 100 μg/ml, or (d) human plasma fibronectin (100 μg/ml) and RGDS (50 μg/ml). Parasites were allowed to adhere at 37°C in PBS. Aliquots of the sample from c were suspended in PBS and incubated for an additional 20 min in buffer alone or buffer containing 200 μg/ml of the F(ab')₂ fragment of anti–Clq prepared as described earlier (29). All parasite preparations were added to fibroblasts on coverslips in 24-well plates at a 20:1 parasite/cell ratio. Plates were centrifuged at room temperature for 3 min at 100 g and incubated for 60 min at 37°C in 5% CO₂. Total cell-associated parasites were determined by washing coverslips six times in PBS following by fixation with PBS-glutaraldehyde and staining with Leukostat. Internalized parasites were assessed by first removing noninternalized parasites with HLB.

**Attachment of T. cruzi to human foreskin fibroblasts plated on Clq.** Human foreskin fibroblasts were plated on coverslips precoated with either buffer, Clq alone, or Clq followed by anti–Clq. Coating with Clq was exactly as described for macrophages and monocytes. Subsequent incubation with F(ab')₂ anti–Clq (200 μg/ml) was carried out for 30 min at 4°C after first washing away nonbound Clq; then unbound antibody was removed by washing. Parasites were added at 20:1 parasite/cell ratio, and further incubations carried out as described above.

**Results**

**Uptake of TCT by macrophages: effect of incubation in serum.** Initial experiments showed that uptake of TCT by macrophages was augmented by incubation in normal human serum (Fig. 1). Heating serum or depleting serum of Clq and factor D abrogated the serum mediated enhancement of cell entry (Fig. 1). When heated serum or Clq and D deficit serum were reconstituted with purified Clq, the serum-dependent enhancement was restored. These results suggest that the Clq derived from normal human serum is necessary for the observed enhancement of internalization of serum-incubated TCT by macrophages.

**Infectivity of TCT for macrophages: effect of incubation in serum.** Enhanced entry of parasites into cells is not synonymous with enhanced survival and replication within cells. We therefore investigated whether Clq in serum also augmented parasite infectivity in macrophages. Compared with parasites incubated in NHS, treatment of TCT with ClqD and factor D–deficient serum resulted in fewer parasites per infected macrophage after 48 h of culture (Fig. 2). Adding Clq but not factor D restored the level of infection to that observed with NHS. A portion of the infected cells, greater in the presence of Clq, was released into the supernatant during 48 h of culture. It was not possible, therefore, to accurately compare the total number of cell associated parasites at 2 and 50 h under different serum incubation conditions to determine whether Clq augmented growth of internalized TCT.

**Binding of Clq to T. cruzi.** We next sought to determine if purified Clq bound to T. cruzi. Representative data are shown in Fig. 3 and demonstrate that, in a serum-free system, the binding of Clq to Epi and TCT at 4°C is concentration-dependent and saturable. The calculated number of Clq binding sites at saturation was 5.2 × 10⁷ sites per Epi and 5.8 × 10⁸ sites per TCT, although Epi consistently showed higher apparent affinity for Clq (data not shown).

**Activation of C1 by T. cruzi.** We examined the ability of Epi and TCT to activate human C1 which had been reconstituted from purified components (Clq, Cl₁₂₁₂₃ -Cl₁₂). Fig. 4 shows that while C1 was activated by aggregated IgG, as detected by the shift of radioactivity from the 87-kD proenzyme
C1s polypeptide chain to the 59-kD chain resulting from cleavage of C1s, no C1 activation was found during incubation with either Epi or TCT. Instead, a decrease in intensity of the 87-kD band was seen, with the appearance of multiple lower molecular weight degradation fragments. This degradation of C1s, which was more extensive with TCT (65% cleavage by densitometric scanning) than with Epi (43% cleavage), occurred in the presence and absence of C1 inhibitor and in the presence of the serine esterase inhibitor NPGB (data not shown). No activation or proteolysis of C1 was detected when the supernatant of the parasites was used in the C1 activation assay (data not shown).

Cleavage of C1q by T. cruzi. Since C1s within intact C1 was degraded by Epi and TCT, we questioned whether isolated C1q was also cleaved by the parasites. 125I-C1q was incubated with TCT and Epi for 10 and 30 min at 37°C. After 10 min of incubation, 36% of C1q bound to Epi was cleaved, whereas < 10% of C1q on TCT was degraded. By 30 min of incubation, 91% of C1q on Epi was proteolytically cleaved, in comparison with 39% on TCT. No cleavage was observed when C1q was incubated with buffer or with the supernatant of parasites (not shown).

Internalization of T. cruzi by monocytes and macrophages: effect of incubating parasites with C1q. The role of purified C1q on parasite internalization by monocytes and macrophages was tested. Preliminary experiments indicated that there was a dose-related increase in internalization of trypanosomites by monocytes when parasites were pretreated with concentrations of C1q ranging from 25 to 200 μg/ml (not shown). Since C1q at 200 μg/ml gave saturable binding on TCT (Fig. 3), this concentration was used to provide maximum reproducibility between experiments. The percentage of internalization and the internalization index for TCT pre-treated with 200 μg/ml of C1q for 15 min at 0°C were enhanced 2.2- and 2.7-fold, respectively, over native TCT entry into monocytes (Fig. 5 A). The extent of enhancement was similar when macrophages were used as the target cell (Fig. 5 B). No differences were found when Epi bearing C1q were compared with Epi alone for entry into monocytes (Fig. 5 A) or macrophages (Fig. 5 B).

Internalization of T. cruzi by monocytes and macrophages: effect of plating cells on C1q. We tested whether plating monocytes or macrophages on C1q-coated surfaces enhanced parasite internalization, analogous to the effects of C1q on enhancing phagocytosis of other particles (28). A marked enhancement of internalization of TCT but not Epi resulted when monocytes were adhered to C1q-coated surfaces (Fig. 6 A). The percentage of internalization and the internalization index were increased 2.4- and 3.7-fold, respectively, when TCT entry into monocytes adhered to C1q-coated surfaces was compared to entry into cells adhered onto BSA-coated surfaces (Fig. 6 A). In contrast, when Epi were used as target cells, no significant increase in either percentage of internalization or internalization index was found with monocytes adhered to a C1q-coated surface (Fig. 6 A). Results for TCT were similar when macrophages were adhered to C1q-coated wells (Fig. 6 B). Internalization of Epi into macrophages plated on C1q was not enhanced in comparison to cells adhered to a BSA-coated surface.

Attachment and internalization of T. cruzi bearing C1q by human foreskin fibroblasts. The capacity of C1q to augment parasite attachment and internalization by fibroblasts was tested. We examined attachment as well as internalization with fibroblasts, since Epi are not internalized by these cells (30, 31). Attachment of both Epi and TCT was augmented by
Figure 5. Internalization of T. cruzi by monocytes and macrophages: effect of incubating parasites with Clq. TCT and Epi were preincubated for 15 min at 0°C with Clq, then washed and incubated with either (A) monocytes or (B) macrophages at a 10:1 parasite/cell ratio. The percentage of mononuclear cells with at least one parasite inside (open bars) and the number of parasites per 100 mononuclear cells (stippled bars) are displayed. Control values were obtained by exposing the cells to TCT or Epi pretreated for 15 min at 0°C with HBSS-BSA at the same parasite/cell ratio.

Figure 6. Internalization of T. cruzi by monocytes and macrophages: effect of plating cells on Clq. TCT were added at a 10:1 parasite/cell ratio to (A) monocytes or (B) macrophages adhered to buffer-coated, BSA-coated, or Clq-coated surfaces. Open and stippled bars are as defined in the legend to Fig. 5. With monocytes, only when TCT were added to cells adhered to a Clq-coated surface was the mean value significantly different from the mean value of the control at the P < 0.001 level. No significant differences were found in the case of Epi. When TCT were used to infect macrophages plated on Clq, the mean values differ significantly from the mean value of the control at the P < 0.005. Internalization of Epi by macrophages plated on Clq was not enhanced in comparison to macrophages adhered to BSA, but was augmented in comparison to buffer (P < 0.02).

C1q. Both the percentage of cells associated with parasites (Fig. 7 a) and the total number of parasites/100 cells (Fig. 7 b) were enhanced. Results were not altered by inclusion of the peptide RGDS with the parasites during incubation with Clq. Incubation of Clq-coated parasites with F(ab')2 anti-C1q significantly decreased the percentage of cells associated with parasites and the total number of parasites per 100 cells. Fibronectin also enhanced attachment of both Epi and TCT to fibroblasts, an effect blocked by RGDS.

In contrast to results with attachment, internalization of TCT but not Epi was augmented by Clq and fibronectin (Fig. 7 c). These findings are analogous to the results with monocytes and macrophages (Fig. 5).

Attachment and internalization of T. cruzi by human foreskin fibroblasts plated on Clq. Attachment of Epi and TCT to fibroblasts was not altered when cells were plated on Clq-coated surfaces or Clq and anti-C1q-coated surfaces (Table I). Furthermore, there was no significant effect of either ligand on parasite internalization when compared to cells plated on buffer alone.

Discussion

We have shown that internalization of trypomastigotes of T. cruzi by both phagocytic cells and fibroblasts is enhanced by the complement subcomponent C1q. The molecular mechanism by which C1q enhances the internalization of T. cruzi or of other particles is unknown. Conceptually, in any interaction between ligand and a cell surface receptor, the ligand can directly mediate internalization via interaction with a cell surface receptor, or the interaction of ligand with its cell receptor can influence an unrelated opsonic receptor to enhance internalization. This latter mechanism appears to operate for the C1q-mediated enhancement of phagocytosis of erythrocytes bearing IgG (28). For T. cruzi, it is likely that C1q serves a similar function with phagocytic cells, since enhanced internalization of TCT by monocytes or macrophages was observed when either the parasites were opsonized with Clq (Fig. 5) or the human phagocytes were plated on Clq (Fig. 6). With fibroblasts, C1q functions only as a ligand for attachment (Fig. 7), since plating cells on Clq does not augment TCT internalization (Table I).

Interaction of C1q with fibronectin (32–37) may also enhance parasite entry. Treponema pallidum prepsensitized with C1q showed increased adherence to fibroconnectin coated surfaces, although phagocytosis by neutrophils was not enhanced (38). Sorvillo and Pearlstein (39) reported similar findings with E. coli and S. aureus. TCT and Epi bind fibronectin (40–43); therefore, the interaction of C1q with fibronectin on the parasite surface may be responsible for some of the effects of C1q reported here. Inclusion of RGDS during incubation of C1q and the parasites did not block the C1q-mediated augmentation of attachment and internalization, whereas the peptide did block enhancement of these functions seen with fibronectin. The possibility that C1q on the parasite binds to fibronectin on the macrophage (monocytes do not synthesize or express fibronectin) or fibroblast surface has not been excluded, although inclusion of RGDS during incubation of C1q-coated parasites with fibroblasts did not decrease the C1q-mediated enhancement of attachment and entry (Joiner, K. A., unpublished data).
lished observations). Whether or not fibronectin or other extracellular matrix proteins are involved, our results differ from those previously reported (28, 32, 38, 39), since purified Clq alone potentiates internalization of TCT without an additional requirement for C3 fragments or IgG on the target particle.

The collagen-like tail domain of Clq mediates the enhancement of phagocytosis of particles by monocytes and macrophages (28). In normal human serum, however, Cl exists as a loosely associated macromolecular complex composed of Clq, C1r2, C1s2 (reviewed in Cooper [44]) in which the cell-binding region of the collagen-like tail region of Clq is not exposed. Activation of Cl, which is initiated by interaction of the globular head regions of Clq with the activating surface, renders the C1r2s2 enzyme susceptible to inactivation by the serum regulatory glycoprotein, Cl inhibitor. In this process, Cl inhibitor dissociates C1r2, C1s2 from the Clq-activator complex, thereby exposing the collagen-like tail domain of Clq to the microenvironment (44–47). Although conventional activation was not observed in this in vitro system, proteolytic degradation of native Cl by TCT and Epi (Fig. 4) could result in exposure of Clq tails to receptors on phagocytic cells. Although degradation of Clq by both Epi and TCT occurs, > 60% of Clq remains intact on the infectious TCT stage, whereas < 10% of the Clq remains intact on Epi. Furthermore, it is possible that free cleavage fragments enhance internalization, since the purified collagenous-like tail region of Clq enhances ingestion of opsonized targets by monocytes.

### Table 1. Attachment and Internalization of T. cruzi by Human Foreskin Fibroblasts Plated on Clq

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* Mean±SD from two experiments, each performed in triplicate.
and macrophages (27). Finally, it remains to be determined if both C1s and C1q degradation occur in normal serum or plasma.

Sera components other than C1q and fibronectin enhance trypanastigote invasion of cultured cells. Protease (48), phospholipase D (49), the serum lipoprotein cruzin (21), and specific antibodies (50), the first three of which are present in normal serum, also enhance infection. In at least one report, inclusion of fetal calf serum in the assay decreased attachment and invasion of LLCMK2 cells by TCT (31), whereas other workers report an increase in internalization with both calf and human serum (51). Given the complex interplay among C1q, extracellular matrix proteins, and TCT, it is unlikely that only one serum component will mediate the enhanced trypanastigote invasion after serum incubation. Nonetheless, our results indicate that a substantial portion of the enhanced invasion following serum treatment is due to C1q.

These results with T. cruzi contrast dramatically with those for infection of cells by related Leishmania spp. Attachment and infectivity of metacyclic promastigotes of L. major is enhanced by serum treatment (2, 52). In this instance, however, enhancement is due to high-level deposition of C3 on the promastigote surface during serum incubation (53) and the subsequent interaction of bound C3 fragments with CR1 (52) or CR3 on macrophages. The fundamental difference in the mechanism of serum resistance between infective forms of T. cruzi and L. major may thus dictate, at least in part, the ligand receptor interactions and the host-cell range which lead to cell invasion. Leishmania spp., which resist serum killing at the terminal portion of the complement cascade, bear large numbers of C3 fragments after serum incubation (53) and are thus suited to enter their obligatory host cell, the macrophage, via receptors for C3. Trypanastigotes of T. cruzi, which produce a C3 convertase inhibitor that prevents deposition of C3 (15-18) but not of C1q during serum treatment, are directed to cells bearing C1q receptors and fibronectin, of which connective tissue cells such as fibroblasts are the prototype (54).

References


