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M T Rimoldi, … , D A Bobak, K A Joiner

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Research Article

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

Maria T. Rimoldi,* Andrea J. Tenner,‡ David A. Bobak,§ and Keith A. Joiner*
**Laboratory of Parasitic Diseases and §Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; and ‡American Red Cross, Rockville, Maryland 20855*

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)[1] 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 macrophases, 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

Address reprint requests to Dr. Joiner, Division of Infectious Disease, P.O. Box 3333, Yale University School of Medicine, New Haven, CT 06510.

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1. *Abbreviations used in this paper:* BESM, bovine embryo skin muscle; Epi, epimastigote(s); HINHS, normal human serum heated to inactivate complement; HLB, hypotonic lysing buffer; NHS, normal human serum; NPGB, nitrophenyl guanidino benzoate; TCT, tissue culture trypomastigote(s).

serum-free defined media (Ventrex Lab, Portland, ME); phosphate-buffered saline (PBS); PBS containing 1% glutaraldehyde (PBS-glutaraldehyde); hypotonic lysing buffer (HLB) containing 10 mM Tris and 2 mM EDTA, pH 8.

Nitrophenyl guanidino benzoate (NPGB) was obtained from Sigma Chemical Co. (St. Louis, MO). Purified human plasma fibronectin was generously provided by Dr. Alex Kurosky, Galveston, TX. When analyzed by SDS-PAGE under reducing conditioning, > 95% of the fibronectin migrated as a band of 200–220 kD. The peptide arginine-glycine-aspartic acid-serine (RGDS) was purchased (Peninsula Laboratories, Inc., Belmont, CA). The peptide gave a single peak when analyzed by high-performance liquid chromatography (LKB Instruments, Inc., Gaithersburg, MD) using a reverse-phase C18 column (Altex, Berkeley, CA).

Parasites. The Y strain and the Miranda 88 (M88) clone of *T. cruzi* were obtained from Dr. J. A. Dvorak (Bethesda, MD). The Epi were maintained by serial passage in liver-infusion tryptose broth, (Oxoid, Basingstoke, Hanks, UK) containing 10% (vol/vol) fetal calf serum, 0.02 mg/ml hemin; 100 μ/ml penicillin, and 100 μg/ml streptomycin. Epi in log-phase growth (3 d) were harvested at a parasite density of 5 $\times$ 10^6/ml. M88 clone TCT were maintained by serial passages in bovine embryo skin muscle cell (BESM) cultures in RPMI-1640 containing 2% fetal calf serum, using culture conditions previously described (17). Y strain tissue culture trypomastigotes (TCT) were grown in $LLCMK_2$ cells as described (17, 18). The final suspensions from BESM or $LLCMK_2$ cultures consisted predominantly of motile trypomastigotes; amastigotes and intermediate developmental forms were always present but never exceeded 5% of the total suspension.

Serum. Normal human sera (NHS) 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 ^{125}I (Amersham International, Arlington Heights, IL) to an average specific radioactivity of 0.5 μC/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* neuraminidase (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 radioiodinated in the presence of 5 mM $CaCl_2$ (25) using Enzymobeads (Bio-Rad Laboratories) to a specific radioactivity of 0.5 μC/μg. Native C1 was reconstituted in the presence of 5 mM $CaCl_2$ by incubating equimolar amounts of purified C1q, $C1r_2$, and trace-labeled ^{125}I-$C1s_2$ 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% nonspecific esterase positive and had typical macrophage morphology. Cells expressed Fc, 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 ½-in. 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) (10^8/ml) were preincubated for 10 min at 37°C in medium (RPMI-1640 with 5% heated human AB serum) or 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 monocytes 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.

^{125}I-C1q binding to T. cruzi. Binding of monomeric ^{125}I-C1q to Epi and TCT (M88 clone) was performed by incubating triplicate tubes containing 10^7 parasites in HBSS-BSA with increasing amounts of a mixture of ^{125}I-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 MultiPrias 4, Packard Instrument Co., Inc., Downers Grove, IL). In certain samples, unlabeled C1q (up to 100-fold molar excess over ^{125}I-C1q) was also added to determine nonspecific binding of ^{125}I-C1q. Nonspecific uptake was usually 1% or less of total input and 15% or less of total bound radioactivity. Experimental values were corrected by subtraction of nonspecific uptake.

Cleavage of C1s by T. cruzi. An aliquot of 20 μl of reconstituted C1 containing ^{125}I-C1s in HBSS-BSA was added to a pellet containing 10^7 or 2 $\times$ 10^7 parasites (M88 clone). The samples containing C1 at or near serum concentration (1.8 $\times$ 10^{-7} M) were incubated at 30°C for 10 min. In some experiments, a physiologic concentration of C1 inhibitor (C1 Inh) (1.3 $\times$ 10^{-6} 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 $\times$ 10^7 parasites were incubated in a volume of HBSS-BSA identical to that of the ^{125}I-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 $\times$ 10^8 parasites/ml (M88 clone) was mixed with 5 μl (20 μg) of ^{125}I-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 autoradiogram.

Internalization of T. cruzi-bearing C1q by monocytes and macrophages. Human peripheral blood monocytes 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 monocytes that had been cultured in Teflon jars (Savillex Corp., Minnetonka, MN) at 1 $\times$ 10^6 cells/ml in

HL1 containing 2 mM L-glutamine and 10 μg/ml of gentamicin in 5% CO_2 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 MgCl, and 10 μg/ml gentamicin at 2.5×10^5/ml, and 250 μl (6.25×10^4 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% CO_2. TCT or Epi (M88 clone) at 1×10^8/ml were pretreated for 15 min at 0°C with C1q (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% CO_2. 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 C1q. Eight-well Lab-Tek chambers (Miles Laboratories, Naperville, IL) were incubated with C1q (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% CO_2. The experiments were performed by adding parasites (M88 clone) at a parasite/cell ratio of 10:1.

Attachment of T. cruzi-bearing C1q to human foreskin fibroblasts. Human foreskin fibroblasts were obtained from the American Type Culture Collection, Rockville, MD (ATCC No. CRL1635) 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 2×10^4 cells per coverslip. TCT or Epi in PBS at 2×10^7/ml were pretreated for 15 min at 0°C with buffer or (*a*) C1q at 100 μg/ml, (*b*) C1q (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 washed twice at 4°C in PBS. Aliquots of the sample from *a* 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-C1q 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% CO_2. Total cell-associated parasites were determined by washing coverslips six times in PBS followed 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 C1q. Human foreskin fibroblasts were plated on coverslips precoated with either buffer, C1q alone, or C1q followed by anti-C1q. Coating with C1q was exactly as described for macrophages and monocytes. Subsequent incubation with $F(ab')_2$ anti-C1q (200 μg/ml) was carried out for 30 min at 4°C after first washing away nonbound C1q; then unbound antibody was removed by washing. Parasites were added at a 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 C1q and factor D abrogated the serum mediated enhancement of cell entry (Fig. 1). When heated serum or C1q and fD deficient serum were reconstituted with purified C1q, the serum-dependent enhancement was restored. These results suggest that the C1q derived from normal human serum is necessary for the observed enhancement of internalization of serum-incubated TCT by macrophages.

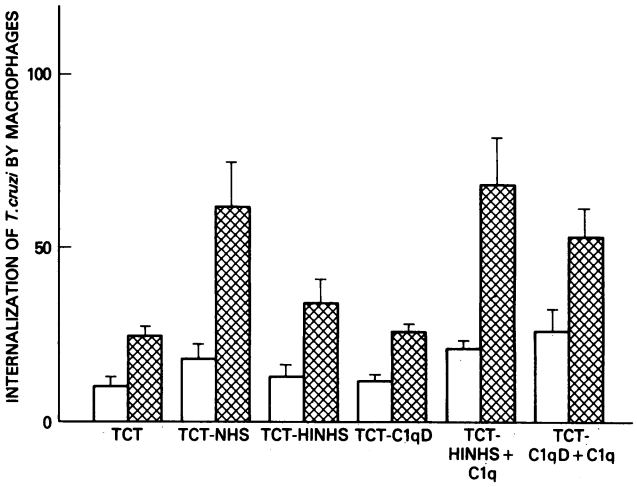

Figure 1. Uptake of TCT by macrophages: effect of incubation in serum. TCT (Y strain) were incubated for 10 min at 37°C in the serum sources indicated, then mixed with long-term culture monocyte-derived macrophages at a parasite cell ratio of 10:1. After incubation for 2 h at 37°C, internalized parasites were determined as described in Methods. The number of parasites inside 100 cells is defined as the internalization index (*cross-hatched bars*); the percentage of cells with at least one parasite is referred to as the "percent of internalization" (*open bars*). At least 200 cells were assessed per dish. Data shown are the mean±SD from three experiments, each done in duplicate dishes. Results with TCT-HINHS and TCT-C1qD were not different from TCT alone ($P > 0.05$ by *t* test).

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 C1q in serum also augmented parasite infectivity in macrophages. Compared with parasites incubated in NHS, treatment of TCT with C1qD and factor D–deficient serum resulted in fewer parasites per infected macrophage after 48 h of culture (Fig. 2). Adding C1q 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 C1q, 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 C1q augmented growth of internalized TCT.

Binding of C1q to T. cruzi. We next sought to determine if purified C1q bound to *T. cruzi*. Representative data are shown in Fig. 3 and demonstrate that, in a serum-free system, the binding of C1q to Epi and TCT at 4°C is concentration-dependent 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 (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 (C1q, $C1r_2$, ^{125}I-$C1s_2$). Fig. 4 shows that while C1 was activated by aggregated IgG, as detected by the shift of radioactivity from the 87-kD proenzyme

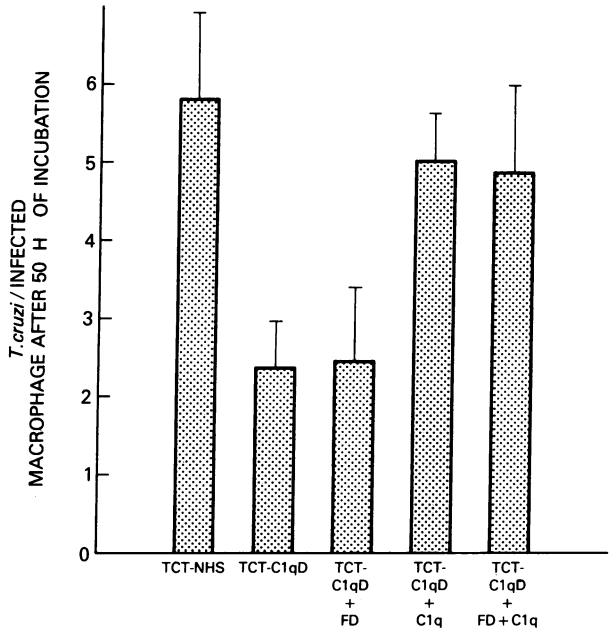

Figure 2. Infectivity of *T. cruzi* in macrophages: effect of incubation in serum. TCT (Y strain) were incubated with serum and added to macrophages as described in the legend to Fig. 1. Noninternalized parasites were removed by washing, and incubation was continued for 48 h. The number of parasites per infected cell at 50 h was determined as described in Methods. Data shown are the mean±SD from two experiments, each done in duplicate dishes.

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. ^{125}I-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

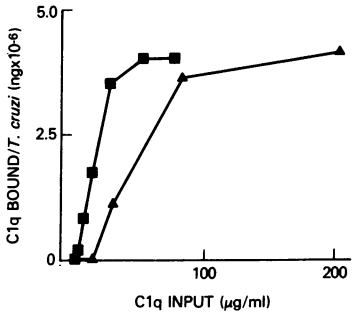

Figure 3. Binding of ^{125}I C1q to *T. cruzi.* ^{125}I-C1q binding to TCT (▲) and Epi (■) is plotted as a function of C1q concentration. Results shown are the mean of triplicate samples in a representative experiment.

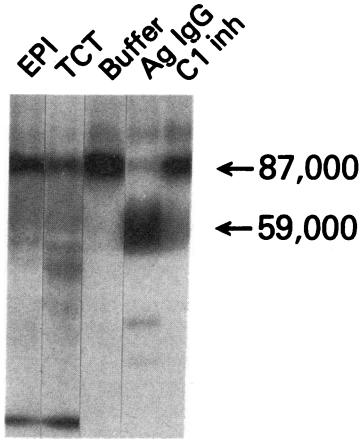

Figure 4. Activation of C1 by *T. cruzi.* SDS-PAGE analysis of ^{125}I-C1s within reconstituted C1 after incubation for 10 min at 30°C with Epi, TCT, or buffer. Control tubes were incubated with aggregated IgG in the presence or absence of C1 inhibitor.

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 trypomastigotes 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 pretreated 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

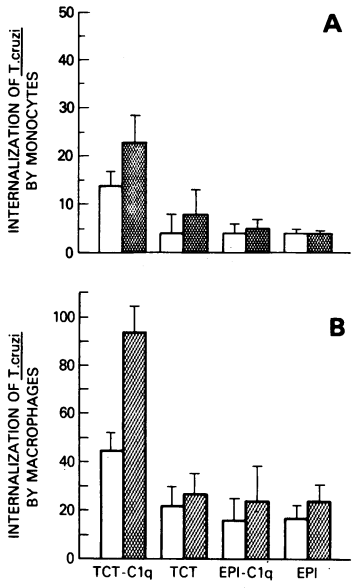

Figure 5. Internalization of *T. cruzi* by monocytes and macrophages: effect of incubating parasites with C1q. TCT and Epi were preincubated for 15 min at 0°C with C1q, then washed and incubated with either (*A*) monocytes or (*B*) macrophages at a 10:1 parasite/cell ratio. The percentage of mononuclear 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. Each experiment with duplicate wells was performed three times. The results were compared by using the Student's *t* test. When TCT-C1q were used to infect the monocytes, the mean values differ significantly from the mean value of the control at the $P < 0.01$. No differences were found when Epi treated with C1q were compared with native Epi for internalization by monocytes. When TCT pretreated with C1q were used to infect macrophages, internalization was enhanced significantly in comparison to non-pretreated TCT. Internalization of Epi by macrophages was not significantly enhanced by pretreatment with C1q ($0.1 < P < 0.2$). Results were similar for all conditions when parasite/cell ratios of 5:1 and 20:1 were compared with the data presented here (not shown).

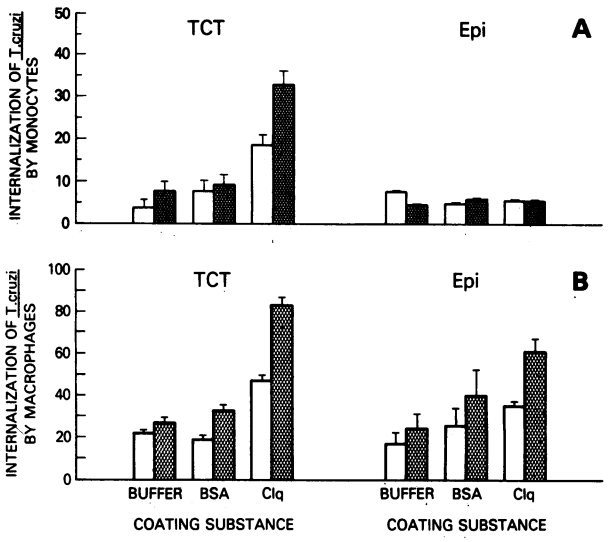

Figure 6. Internalization of *T. cruzi* by monocytes and macrophages: effect of plating cells on C1q. TCT were added at a 10:1 parasite/cell ratio to (*A*) monocytes or (*B*) macrophages adhered to buffer-coated, BSA-coated, or C1q-coated surfaces. *Open* and *stippled bars* are as defined in the legend to Fig. 5. With monocytes, only when TCT were added to cells adhered to a C1q-coated surface was the mean value significantly different from the mean value of the control at the $P < 0.01$ level. No significant differences were found in the case of Epi. When TCT were used to infect macrophages plated on C1q, the mean values differ significantly from the mean value of the control at the $P < 0.005$. Internalization of Epi by macrophages plated on C1q 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 C1q. Incubation of C1q-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 C1q 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 C1q. Attachment of Epi and TCT to fibroblasts was not altered when cells were plated on C1q-coated surfaces or C1q 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 C1q (Fig. 5) or the human phagocytes were plated on C1q (Fig. 6). With fibroblasts, C1q functions only as a ligand for attachment (Fig. 7), since plating cells on C1q does not augment TCT internalization (Table I).

Interaction of C1q with fibronectin (32–37) may also enhance parasite entry. *Treponema pallidum* presensitized with C1q showed increased adherence to fibronectin 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., unpub-

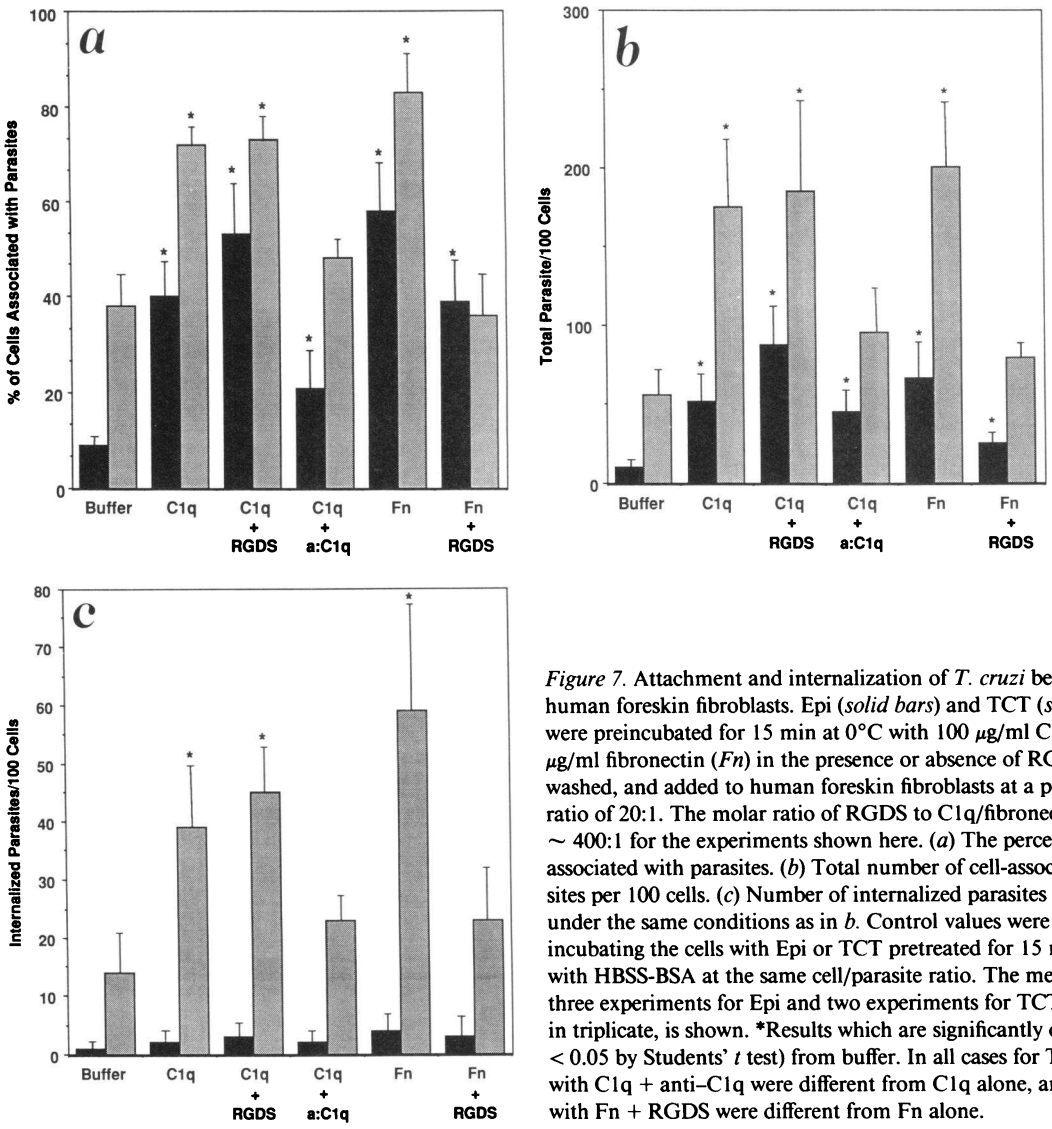

Figure 7. Attachment and internalization of *T. cruzi* bearing C1q by human foreskin fibroblasts. Epi (*solid bars*) and TCT (*stippled bars*) were preincubated for 15 min at 0°C with 100 μg/ml C1q or 100 μg/ml fibronectin (*Fn*) in the presence or absence of RGDS, then washed, and added to human foreskin fibroblasts at a parasite/cell ratio of 20:1. The molar ratio of RGDS to C1q/fibronectin was ~ 400:1 for the experiments shown here. (*a*) The percentage of cells associated with parasites. (*b*) Total number of cell-associated parasites per 100 cells. (*c*) Number of internalized parasites per 100 cells under the same conditions as in *b*. Control values were obtained by incubating the cells with Epi or TCT pretreated for 15 min at 0°C with HBSS-BSA at the same cell/parasite ratio. The mean±SD from three experiments for Epi and two experiments for TCT, each done in triplicate, is shown. *Results which are significantly different ($P < 0.05$ by Students' *t* test) from buffer. In all cases for TCT, results with C1q + anti-C1q were different from C1q alone, and results with Fn + RGDS were different from Fn alone.

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 C1q alone potentiates internalization of TCT without an additional requirement for C3 fragments or IgG on the target particle.

Table I. Attachment and Internalization of T. cruzi by Human Foreskin Fibroblasts Plated on C1q

	Cells associated with parasites		Total parasites per 100 cells		Internalized parasites per 100 cells	
Ligand on plate	Epi	TCT	Epi	TCT	Epi	TCT
	%		*n*		*n*	
BSA	44±11*	43±14	54±10	276±96	2±2	26±13
C1q	38±17	62±21	62±29	361±134	3±2	42±10
C1q + anti-C1q	50±14	54±17	64±27	294±40	5±4	37±17

* Mean±SD from two experiments, each performed in triplicate.

The collagen-like tail domain of C1q mediates the enhancement of phagocytosis of particles by monocytes and macrophages (28). In normal human serum, however, C1 exists as a loosely associated macromolecular complex composed of C1q, $C1r_2$, $C1s_2$ (reviewed in Cooper [44]) in which the cell-binding region of the collagen-like tail region of C1q is not exposed. Activation of C1, which is initiated by interaction of the globular head regions of C1q with the activating surface, renders the $C1r_2s_2$ enzyme susceptible to inactivation by the serum regulatory glycoprotein, C1 inhibitor. In this process, C1 inhibitor dissociates $C1r_2$, $C1s_2$ from the C1q–activator complex, thereby exposing the collagen-like tail domain of C1q to the microenvironment (44–47). Although conventional activation was not observed in this in vitro system, proteolytic degradation of native C1 by TCT and Epi (Fig. 4) could result in exposure of C1q tails to receptors on phagocytic cells. Although degradation of C1q by both Epi and TCT occurs, > 60% of C1q remains intact on the infectious TCT stage, whereas < 10% of the C1q remains intact on Epi. Furthermore, it is possible that free cleavage fragments enhance internalization, since the purified collagenous-like tail region of C1q enhances ingestion of opsonized targets by monocytes

and macrophages (27). Finally, it remains to be determined if both C1s and C1q degradation occur in normal serum or plasma.

Serum components other than C1q and fibronectin enhance trypomastigote 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 $LLCMK_2$ 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 trypomastigote 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. Trypomastigotes 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).

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