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Toxoplasma gondii: Antibody-independent Binding of Human Complement Subcomponent C1q to the Parasite

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ABSTRACT

The binding capacity of C1q onto *Toxoplasma gondii (T.gondii)* parasites at each infectious stage was examined by using the immunofluorescence assay (IFA) with purified human C1q, rabbit anti-human C1q antibodies and fluorescein -isothiocynate conjugated goat anti-rabbit IgG antibodies in parallel with the IgM-binding to the parasite.

Any possibly contaminated natural antibodies to the parasite were completely removed from the anti-human C1q-preparations by repeated absorption with the parasite in advance. Parasites incubated with C1q before adding anti-C1q antibodies have shown the specific fluorescence on their outer surface membrane, whereas those without any addition of C1q have no specific fluorescence. The intensity of fluorescence differed in stages, and cystozoites and trophozoites seemed to have significantly higher C1q-binding activities than sporozoites. Furthermore, C1q-binding sites have been differently located from IgM-binding sites, or anterior poles, on the parasite.

These observations indicate that *T.gondii* parasite has the antibody- independent binding capacity to human C1q on its outer surface membrane.

INTRODUCTION

Intracellular parasitic organisms have evolved a wide variety of mechanisms that allow them to grow and multiply within cells and escape defense mechanisms of their hosts. *Toxoplasma gondii* (*T. gondii*) an obligate intracellular protozoa is well adapted to an intracellular growth and development during the extraintestinal phase of its life cycle. This apicomplexan parasite can penetrate phagocytic and non-phagocytic mammalian cells, and survive within parasitophorous vacuoles. Several organelles are believed to participate in its active penetration of the parasite into host cells. Recent studies have shown that substances released from rhoptries enhance the penetration into host cells during invasion process (Nichols et al. 1983; Schwartzman 1986; Kimata

and Tanabe 1987; Sadak et al 1988). Moreover, the existence of Fc receptor (Budzko et al. 1989) and C3 receptor (Fuhrman and Joiner 1989a) on *T. gondii* has been documented. Biological functions of these receptors may play an important role in internalization and infectivity of the parasite (Fuhman and Joiner 1989b). To date, however, the interaction between the parasite and host defense system after cell invasion, is still unclear.

C1q, a subcomponent of the first component of complement(C1) is an unusual plasma protein with the N-terminal half of the molecule composed of a triple helix with collagen-like amino acid sequence. The C-terminal end of the molecule lacks the collagen-like sequence, and is believed to contain the site that interacts with Fc regions of aggregated IgG and/or immune complexes. The collagen-like regions of C1q appear to be involved in the interaction with and activation of C1r and C1s proenzymes of C1 (Reid and Porter 1981). Substances such as polyinosinic acid, bacterial lipid A and antivenom polysaccharide (Loss 1982) and some constituents of the extracellular matrix such as fibronectin (Isliker et al. 1981) and laminin (Bohnsack et al 1985) have been shown to interact directly with C1q. Moreover, some of these substances activate subsequent components in the classical complement pathway. Thus, it has become apparent that C1q plays an important role in the triggering and activation of the classical complement pathway by immune complexes or other antibody-independent activators. Also C1q may carry out the essential function by clearing immune complexes and other antibody-independent activators via phagocytic mechanism.

To have a better understanding of the mechanism of intracellular parasitism, in this study, we examined the binding of C1q to each infectious stage of *T. gondii*.

MATERIALS AND METHODS

Adult BALB/c mice bred in the Department of Veterinary Physiology, Obihiro University, Hokkaido, Japan, were used. Mouse embryonal cells (MEC) were obtained from embryos on the 12th-14th day of gestation, and cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and antibiotics.

To examine the C1q binding capacity of high virulent and low virulent strains of *T. gondii*, RH and S-273 strains were used. Trophozoites of RH and S-273 strain were obtained from infected MEC cultures. S-273 strain cystozoites were collected from physically ruptured cysts from infected mouse brain. Trophozoites were isolated by Percoll (Pharmacia, Tokyo, Japan) sucrose density gradient centrifugation (Cornelissen et al. 1981). To remove host immunoglobulins from cysts surface, isolated cysts were incubated for 30 min in 50 mM acetate buffered saline (pH 2.9) at 4℃ and washed three times with phosphate buffered saline (PBS).

To examine the quantitative difference of C1q binding capacity at each infectious stage of *T.gondii*, only S-273 strain sporozoites were used, inasmuch as cats inoculated with RH strain did not shed any oocysts. Oocysts of the S-273 strain were isolated from cats' feces using sucrose density gradient centrifugation and excysted at 37℃ through incubation in PBS containing 0.5% taurocholic acid and 0.1% trypsin for 30 min. Sporozoites were isolated from oocyst suspension

using Percoll-sucrose density gradient centrifugation and washed extensively with cold PBS (Arrowood and Sterling 1987). Isolated parasites were fixed in PBS with 1% paraformaldehyde at 4℃ for 15 min.

Purification of C1q from healthy human serum was done following the method of Yonemasu and Stroud (1971), then stored at -105℃, until use. Absence of immunoglobulins in the material was confirmed by immunoblotting analysis using peroxidase-conjugated anti-human Immunoglobulins.

Rabbit anti-human C1q antiserum was prepared as described by Yonemasu and Stroud (1971) and stored at -105℃, until use. All antiserum preparations were adsorbed repeatedly with fixed parasites to remove any possibly contaminating natural antibodies to *T. gondii*. F(ab')2 fragments were prepared and purified from the IgG fraction of antiserum by pepsin digestion and subsequently by Sephadex G-150 chromatography (Nisonoff 1964).

The binding of C1q to fixed smears of the RH and the S-273 strains and a live suspension of the RH strain were examined by indirect IFA (IIFA). Fixed or live parasites were incubated with different concentrations of C1q or without C1q at 37 ℃ for 30 min in PBS containing 5% skim milk. The parasites were subsequently incubated at 37℃ for 30 min with an appropriate dilution of F(ab')2 fragments prepared from anti-human C1q antiserum. The parasites were extensively washed to remove nonspecifically bound F(ab')2 and incubated at 37℃ for 30 min with an appropriate dilution of FITC-conjugated goat anti-rabbit IgG.

The capacity of *T. gondii* to bind IgM was examined with a direct IFA (DIFA) technique with various dilutions of normal human serum and with goat FITC-conjugated anti-human IgM antibodies. All dilutions were prepared in PBS. The healthy adult human serum was inactivated at 56℃ for 30 min to destroy all C1q antigenicity (Yonemasu and Stroud 1971). Observation was carried out using a fluorescence microscope.

Trophozoites of the RH strain and human erythrocytes obtained from healthy individuals were incubated at 37℃ for 30 min in PBS containing C1q(50 ug/ml). Control preparations were incubated without C1q. After extensive washing, the parasites were solubilized at 100℃ for 3 min with 125 mM Tris buffer, pH 6.8, containing 10% 2-mercaptoethanol and 4.6% sodium dodecyl sulfate(SDS). The preparations were centrifuged to remove insoluble aggregates and the supernatant was applied to a 10% polyacrylamide gel containing SDS(SDS-PAGE) (Fairbanks et al. 1971). The peptide chains of C1q (A: 27,500; B: 25,200; C: 23,800) were used as protein standards for estimation of molecular weights. Proteins were transferred from SDS-PAGE gels to nitrocellulose filters with a Trans-Blot apparatus (Bio-Rad, Richmond, Calf., U.S.A.) as described by Towbin et al. (1979). The membrane was blocked with 1% bovine serum albumin (BSA) in PBS and incubated for 60 min at room temperature with appropriate dilutions of rabbit anti-human C1q and/or rabbit anti-human IgG (H and L chains) antiserum. After washing with PBS containing 0.05% Tween-20, the membrane was incubated for another 60 min with horseradish peroxidase-conjugated F(ab')2 fragments prepared from the IgG fraction of goat anti-rabbit IgG antibodies. After extensive washing, the nitrocellulose membrane was stained with

4-chloro-1-naphthol containing H_2O_2.

RESULTS AND DISUCUSSION

Incubation with C1q revealed a rim-like specific fluorescence on the membrane of both RH and S-273 strains of *T. gondii* (Figure 1). Also, membrane associated fluorescence was observed with live parasites of RH and/or S-273 strains incubated with C1q; while the parasites incubated without C1q did not show any specific fluorescence. Trophozoites of RH (Figure 1a) and S-273 (Figure 1b) strains had diffuse and slightly patchy fluorescence. Sporozoites from fractured oocysts (Figure 1c) also exhibited fluorescence on their membrane, but were stained less intensely than trophozoites.

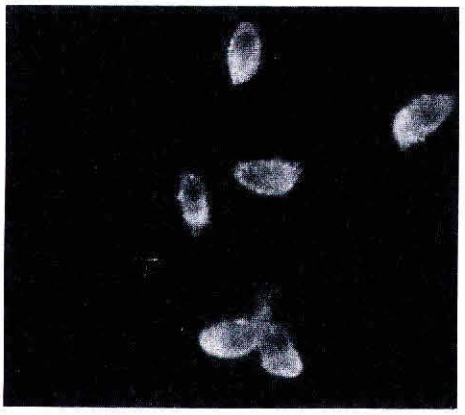

Fig. 1a

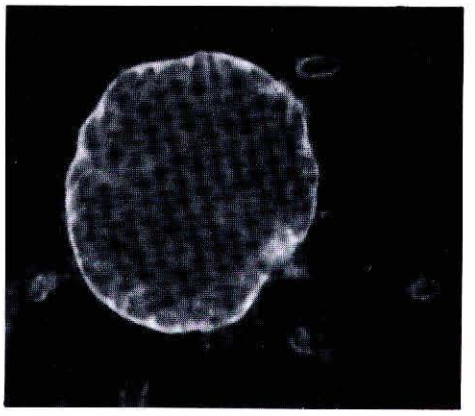

Fig. 1b

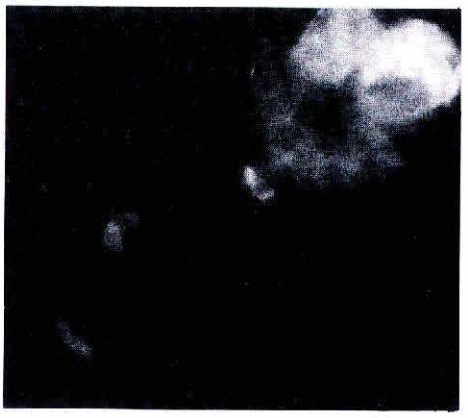

Fig. 1c

Fig. 1d

Fig.1a-d. Immunofluorescence localization of human C1q binding sites on fixed parasites. Parasites were incubated for 30 min at 37℃ with purified human C1q and then incubated with rabbit anti-human C1q F(ab')2 fragments, and reacted with FITC-conjugated anti rabbit IgG.
Extracellular trophozoites of RH strain (1a). trophozoites were harvested from cultured MEC cells. Magnification:× 1,000. Trophozoites of S-273 strain (1b). Parasites were collected from infected mice brains. Magnification:×1,000. Sporozoites of S-273 strain (1c). Magnification: ×400.
Human IgM binding sites on fixed trophozoites of RH strain. Parasites were incubated with heat-inactivated human serum from healthy adults and reacted with FITC-conjugated anti-human IgM (1d). Magnification: × 1,000.

Table 1.

Localization of human C1q on *Toxoplasma gondii* by immunofluorescence assay

parasite or host cell		Localization and intensity of staining host cell		
		living	fixed	concentration of C1q*
MEC		NT	-	
Trophoozoite	(RH)	rim +	rim +	60 ng/ml
	(S-273)	rim +	rim +	60 ng/ml
Sporozoite	(S-273)	NT	rim ±	240 ng/ml

Intensity of fluorescent staining: negative, - ; slightly positive, ± ; positive, +; NT, not tested.
*Values are expressed as minimum concentrations of C1q giving a distinct positive staining.

Specific and distinct immuno-staining was detected on the membrane of trophozoites incubated with C1q at concentrations higher than 60 ng/ml. Weak, but significant immuno-staining was detected on the membrane of sporozoites incubated with C1q at concentrations higher than 240 ng/ml (Table 1). Treatment of sporozoites with trypsin and taurocholic acid during excystation had no effect on the binding of C1q to the parasites (data not shown).

Trophozoites incubated with heat-inactivated human serum and stained by the DIFA procedure exhibited dot-like specific fluoresence on their anterior end (Figure 1d).

Immunoblotting analysis of bound C1q is shown in Figure 2. Bands corresponding to the A-, B-, and C-chains of purified C1q (shown with arrows) were only detected in lane of trophozoites of RH strain incubated with C1q (lane 2); while, no other bands associated with A-, B-, C-chains of C1q were detected in the other lanes of trophozoites without C1q (lane 4). Human erythrocytes

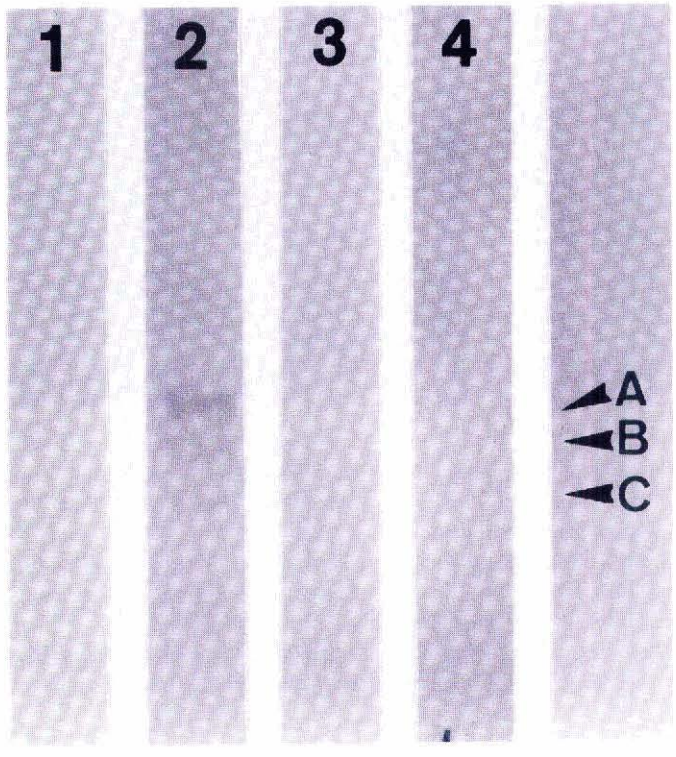

Figure 2. Immunoblotting of trophozoites of RH strain or human erythrocytes incubated either with or without C1q. Parasites incubated with C1q (lane2) or without C1q (lane4),human erythrocytes incubated with C1q (lane 3) or without C1q (lane 1), and purified C1q were electrophoresed in 10% SDS-PAGE gel. Transblotted membrane sheet was reacted with rabbit anti-C1q antiserum, followed with peroxidase-conjugated F(ab')2 anti-rabbit IgG.

incubated with (lane 3) or without C1q (lane 1) also showed no bands similar with those of C1q components.

Results of the immunofluorescence and immunoblotting analysis demonstrate that the binding sites of human C1q on the parasite are morphologically and immunochemically different from those of the IgM molecules. The use of purified C1q and anti-human C1q F(ab)'2 fragments adsorbed with parasites, effected the elimination of immunoglobulins that exhibited non-specific binding with *T. gondii*. Budzko et al. (1989) described the presence of receptors for the Fc portion of human immunoglobulins on the surface of *T. gondii* by IIFA technique. These receptors, however, exhibited a polar pattern of immunofluorescence which is different from the C1q binding sites noted in the present study. Our findings suggest the binding of C1q to *T. gondii* seems not to be mediated by Fc receptors.

It is of interest that the intracellular proliferative forms of the parasites, such as trophozoites of both low virulence (S-273) and high virulence (RH) strains showed higher C1q-binding activity than sporozoites (Figure 1 and Table 1). This observation seems to suggest that constituents or receptor(s) on the membrane of *T. gondii* responsible for C1q-binding has function(s) associated with intracellular survival, rather than with pathogenicity or virulence. Our finding is consistent with a related study on C1q receptors on fibroblastoid cells by Bordin et al. (1983). For now, we may hypothesize that binding of native C1q to the membrane of trophozoites must be important in bringing parasites in contact with host cells during the initial phase of their penetration. Moreover, the binding of C1q may also assist the parasite in terms of its adaptation to an intracellular survival by way of physico-chemical and metabolic modification on its cell membrane. To clarify these speculations, topographical and quantitative studies concerned with C1q-binding site on the parasite are inevitably necessary.

The present study was not able to determine whether C1q-binding substances are derived from the parasites themselves, from host cell products, or from extracellular matrix components, such as fibronectin or laminin. Furtado et al (1992) demonstrated that tachyzoites of *T.gndii* recognized multiple laminin receptors in attaching to different target cells. Further experiments to clarify these points, and the determination of the biological significance of C1q binding are in progress.

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