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Characterization of *Toxoplasma gondii* Antigens that React with Human Immunoglobulin M and Immunoglobulin G Antibodies

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Studies were performed to define the nature of the *Toxoplasma gondii* antigens that are recognized by human immunoglobulin M (IgM) and IgG antibodies. Both IgM and IgG antibodies were found to be directed mainly against *T. gondii* membrane antigens in sera obtained from patients with acute toxoplasmosis. Treatment of the membrane preparation with DNase, RNase, or lipase had no apparent effect on the reactivity of the membrane antigens with IgM and IgG antibodies. Lipids isolated from tachyzoites were not recognized by either IgM or IgG antibodies. Exposure of *T. gondii* membranes to heat, proteolysis, or oxidation with sodium periodate decreased the reactivity of the membrane preparations with both IgM and IgG antibodies. A preparation of *T. gondii* proteins and polysaccharides were recognized by both immunoglobulin classes. *T. gondii* polysaccharides reacted with human IgG antibodies produced during both the acute and chronic phases of the infection. We concluded that, after infection with *T. gondii*, IgM and IgG antibodies are elicited in response to both protein and carbohydrate constituents of the invading parasite.

Despite the large number of studies on the subject of the antibody response to infection with *Toxoplasma gondii* in humans and animals, relatively little is known about the antigens which elicit the antibody response to this intracellular parasite. Fractionated *T. gondii* antigens prepared from lysed organisms by low- or high-speed centrifugation have been demonstrated to react differently in the complement fixation test, hemagglutination test, and Sabin-Feldman dye test (DT) (6, 7, 25, 27, 29, 30, 34). Fleck (7) concluded that *T. gondii* contains at least two different antigens: a heavy one which reacts in the DT and the complement fixation test and a light soluble one which reacts in the complement fixation test but not in the DT.

Studies performed in our laboratory (10) have demonstrated that *T. gondii* tachyzoites have four major membrane surface proteins that are recognized by sera obtained from mice chronically infected with *T. gondii*. Analysis of precipitable immune complexes formed between these four membrane proteins and immunoglobulin G (IgG) antibodies from humans with acute *T. gondii* infection revealed that IgG antibodies of certain sera precipitated all four proteins, whereas those from other patients precipitated only two or three of the proteins (10). In addition to proteins, *T. gondii* polysaccharides have also been reported to be antigenic (22).

Mineo et al. (18) and Camargo et al. (2) observed a reaction between polysaccharides extracted from *T. gondii* and antibodies from infected individuals. These authors demonstrated that human IgM antibodies reacted with polysaccharides and proteinaceous supernatants obtained after centrifugation of sonicated tachyzoites. They found that IgG antibodies reacted preferentially with preparations containing proteins and less so with preparations containing polysaccharides. On the basis of these data, Mineo et al. (18) and Camargo et al. (2) suggested that IgM antibodies are elicited in response to both sugar and protein moieties of a glycoprotein complex and that IgG antibodies are directed mainly against protein components of the organism.

Although Mineo et al. did not actually isolate glycoproteins from *T. gondii*, recent data reported by Mauras et al. (17) reveal that, in contrast to previous findings by Handman et al. (10) and Sethi et al. (26), glycoconjugates do exist in *T. gondii* membranes. Subsequent studies by Johnson et al. have revealed that glycosylated polypeptides are present in the insoluble fraction

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obtained after ultracentrifugation of disrupted *T. gondii* but not in the soluble fraction (14). Hughes and Balfour (13) recently reported that 11 distinct antigens were recognized in a soluble antigen preparation of *T. gondii* by serum from an individual with toxoplasmosis. The authors state that of these antigens, nine were proteins, one was a lipopolysaccharide, and one was a glycoprotein.

Our study was performed in an attempt to reconcile some of the differences reported in the literature and to further characterize the biochemical nature of *T. gondii* antigens that are recognized by human IgM and IgG *T. gondii* antibodies.

MATERIALS AND METHODS

***T. gondii* antigens.** Antigens were prepared from tachyzoites of *T. gondii* RH harvested from peritoneal exudates of 22- to 24-g Swiss Webster female mice infected 2 days earlier. Debris and mouse cells were removed by filtration as previously described (37). The peritoneal fluids were centrifuged at 2,000 × *g* for 30 min at 4°C. The supernatants were separated and the organisms in pellets were washed three times in phosphate-buffered saline (PBS), pH 7.4, and disrupted by sonication (20). Intact cells were removed by centrifugation at 2,000 × *g* for 20 min. The cell-free supernatant was used as *T. gondii* sonic extract.

***T. gondii* membranes and cytoplasmic antigens.** To separate membranes from cytoplasmic antigens, *T. gondii* sonic extracts were centrifuged at 34,000 × *g* for 2 h at 4°C. The supernatant was separated and used as the source of cytoplasmic antigens (11, 21), and the pellets were washed twice in PBS and used as membrane antigens (11, 12, 20, 21, 24, 31).

Protein and carbohydrate determinations. For preparations containing protein concentrations of >10 μg/ml, protein concentrations were determined by the method of Lowry et al. (15). To measure protein concentrations of <10 μg/ml, we used a protein assay kit from Bio-Rad Laboratories, Richmond, Calif. Bovine serum albumin was used as a standard for both of these assays. Carbohydrates were determined by the anthrone reaction (16), with D-galactose used as standard.

Heat treatment. Samples (1 ml) of *T. gondii* sonic extracts or membrane preparations at a concentration of 500 μg of protein per ml were heated for 1 h at 100°C in a water bath.

Treatment of membrane antigens with enzymes. Samples (500 μg of protein) of *T. gondii* membrane preparations suspended in 1 ml of 0.1 M phosphate buffer (pH 7.5) supplemented with 0.02 M $MgCl_2$ were subjected to 250 μg of protease (P-5130; Sigma Chemical Co., St. Louis, Mo.), 250 μg of both DNase (D-0876; Sigma) and RNase (R-5875; Sigma), or 250 μg of lipase (L-2253; Sigma). After incubation for 2 h at 37°C, the enzymatic reaction was stopped by the addition of 20 ml of cold PBS, and the preparation was centrifuged at 34,000 × *g* for 2 h at 4°C. The resulting pellets were washed in PBS and resuspended in 1 ml of PBS. Antigens incubated in parallel without enzymes were used as controls. Under these experimental conditions, 50 to 58% of the proteins were digested by protease, as revealed by protein determinations in the digested preparations. Lipase or DNase and RNase had no apparent effect on the protein concentrations in the preparations.

Treatment of *T. gondii* membrane antigens with sodium periodate. Membrane preparations at concentrations of 500 μg of protein in 1 ml of PBS were exposed to 0.01 M (final concentration) of sodium *m*-periodate (S-1878; Sigma) for 18 h at 4°C (5). Control antigens with buffer instead of sodium periodate were run in parallel. After incubation, the antigen preparations were extensively dialyzed against PBS for 2 days at 4°C. Under these conditions, protein concentrations of *T. gondii* antigens were not significantly affected.

Lipid extraction. Lipids were prepared from *T. gondii* sonic extracts by two successive extractions with chloroform-methanol by the method of Folch et al. (8). Organic solvents were removed from the lipid fractions and from the lipid-depleted residues by evaporation under a stream of N_2, followed by extensive dialysis against PBS. After dialysis, both the lipid fractions and the delipidated residues were concentrated to the original volumes of the *T. gondii* sonic extracts. Proteins could not be detected in the lipid fractions.

Protein-enriched preparations. Proteins were precipitated from lipid-depleted *T. gondii* sonic extracts or directly from *T. gondii* sonic extracts after solubilization with detergents. Solubilization was performed with a mixture of sodium dodecyl sulfate and sodium deoxycholate at concentrations of 0.5 and 2 mg of detergent, respectively, per mg of sonicated proteins. After incubation for 15 min at 37°C, ammonium sulfate was added to 12% saturation (3, 23) at 4°C. The precipitate was subsequently separated by centrifugation at 10,000 × *g* for 20 min at 4°C, redissolved in sodium dodecyl sulfate-sodium deoxycholate at the same concentrations, and precipitated again. The pellet was washed in PBS and finally suspended in PBS at the original volume of *T. gondii* sonic extracts.

Isolation of *T. gondii* polysaccharides. The procedures described by Westphal et al. (36) were used to isolate polysaccharides. Whole or lipid-depleted sonic extracts of the organism were extracted with 45% phenol in the presence of 8-hydroxyquinoline (10) for 30 min at 68°C. After centrifugation at 10,000 × *g* for 10 min, the aqueous phase was separated. The phenol layer was reextracted with an equal volume of water and separated again into two layers by centrifugation. Combined aqueous phases were dialyzed for 2 to 3 days against PBS and concentrated to the original volume of the sonic extracts. To further purify the polysaccharide fraction in certain experiments, the preparations were exposed to 50 μg each of DNase and RNase per ml in the presence of 5 mM (final concentration) $MgCl_2$. Digestion was carried out for 2 h at 37°C. Thereafter, protease was added at 100 μg/ml for an additional incubation period of 24 h at 37°C.

IgM- and IgG-ELISAs. The conventional IgM and IgG–enzyme-linked immunosorbent assay (ELISA) procedures were carried out essentially as described previously (2, 18, 32, 33), with wells coated with 100 μl of the different *T. gondii* antigen preparations. Antigen preparations were used at 10 μg of protein per ml to compare the activities of different preparations in which proteins were not affected during treatment or during isolation procedures. Since in each procedure

we concentrated the different preparations to the original volumes of either *T. gondii* sonic extracts or *T. gondii* membranes, we coated wells with equal volumes at equal dilutions of the antigens to be compared.

Antigens were diluted in 0.1 M carbonate buffer (pH 9.8) and incubated in wells of microtiter plates overnight at 4°C. Plates were then washed with PBS containing 0.05% Tween 20 three times for 5 min each. Wells were postcoated with 250 μl of 4% bovine serum albumin in PBS–0.05% Tween 20 for 1 h at 37°C. Plates were washed as described above. Sera to be tested were added at fourfold dilutions (in PBS), beginning with a dilution of 1:16, and incubated for 1 h at 37°C. After washing, 100 μl of alkaline phosphatase-conjugated rabbit IgG antibodies to human IgM (μ-specific) or alkaline phosphatase-conjugated rabbit IgG antibodies to human IgG (γ-specific), prepared as previously described (20, 21) and diluted in 4% bovine serum albumin–PBS–0.05% Tween 20 were added to each well, and the plates were incubated for 1 h at 37°C. After washing, 100 μl of enzyme substrate was added and the plates were reincubated for 1 h at 37°C. Absorbance at 405 nm was determined directly in wells with a micro-ELISA reader (PR-580; Dynatech Laboratories, Inc., Alexandria, Va.).

IgM and IgG antibody titers were determined as the highest serum dilution that exhibited an absorbance of at least twice that of the same dilution of the negative control serum.

Other serological tests. The DT (11), the IgM–indirect fluorescent-antibody test (35), and the double sandwich (DS)-IgM-ELISA (19, 20) were performed as previously described.

Human sera. Six sera obtained from patients diagnosed as having acute acquired toxoplasmosis were either used individually or pooled. Diagnosis was established by previously described criteria (1, 4, 20, 35). These sera were all positive in the DT, the IgM–indirect fluorescent-antibody test, and the DS-IgM-ELISA.

Six sera obtained from seronegative healthy individuals were pooled to serve as a negative control. These sera were negative in the DT, the IgM–indirect fluorescent-antibody test, and the DS-IgM-ELISA. All sera were negative for rheumatoid factor and antinuclear antibodies, determined as previously described (19).

A serum sample, obtained from a patient 1 month after the clinical onset of acute toxoplasmosis, which had titers of 1:16,384 in the DT, 1:1,280 in the IgM–indirect fluorescent-antibody test, and 1:16,384 in the DS-IgM-ELISA served as the positive control for IgM *T. gondii* antibodies and was included in each IgM-ELISA. A serum sample, obtained from a patient 14 months after the clinical onset of acute toxoplasmosis, which had titers of 1:8,192 in the DT and was negative in the IgM–indirect fluorescent-antibody test (<1:16) and in the DS-IgM-ELISA (<1:4) served as the positive control for IgG *T. gondii* antibodies. This serum was included in each IgG-ELISA.

Separation of IgM and IgG serum fractions. IgG was separated on Sepharose-4B columns conjugated to staphylococcal protein A as described by Goding (9).

RESULTS

To study the antigens that are recognized by human IgM and IgG antibodies produced in response to infection with *T. gondii*, we studied a series of IgM- and IgG-ELISAs, using *T. gondii* sonic extract and membranes and the fractions enriched with cytoplasmic components.

Representative results obtained in the IgM-ELISA when performed in wells coated with equal protein concentrations of the different antigen preparations are shown in Fig. 1. IgM antibodies appeared to react preferentially with the membrane preparation, as compared with their activity with both unfractionated sonic extracts and cytoplasmic antigens. The same pattern of results was obtained with six sera containing both IgM and IgG antibodies from individuals with recent onset of infection.

Effects of heat, enzymatic, and chemical treatments on antigenicity of *T. gondii* membranes. Having established that antigens with *T. gondii* membranes are recognized by both human IgM and IgG antibodies, we initiated a series of studies to identify the biochemical nature of these membrane antigens.

The IgM- and IgG-ELISA titers obtained for each serum when tested with each of the different antigen preparations are shown in Tables 1 and 2. Treatment of the membrane preparation with DNase, RNase, or lipase had no effect on the activity of the preparations with IgM or IgG antibodies. In contrast, treatment of the membrane preparations with heat, protease, or sodium periodate did affect the antigenic properties, as demonstrated by reduction of the IgM- and IgG-ELISA titers.

Heat denaturation of proteins or protease digestion decreased the serum IgM-ELISA titers

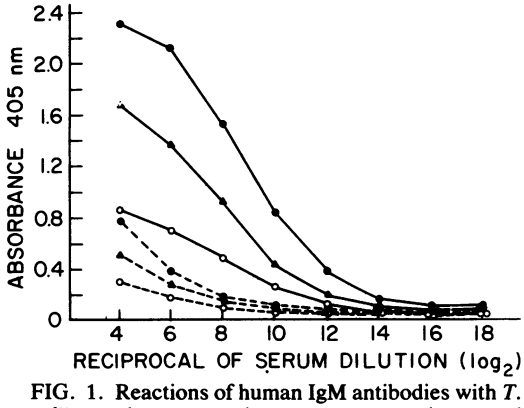

FIG. 1. Reactions of human IgM antibodies with *T. gondii* membranes, sonic-extract preparations, and fractions enriched with cytoplasmic antigens. The IgM-ELISA was performed in plates coated with 10 μg of protein per ml of membranes (●), sonic extract (▲), or fractions enriched with cytoplasmic antigens (○). Reactions of a positive serum (———) are compared with those of a pool of negative sera (– – – –).

TABLE 1. Effect of heat, enzymes, and sodium periodate on the reactivity of *T. gondii* membranes with human IgM antibodies[a]

Serum no.	IgM-ELISA titers^{-1} obtained with *T. gondii* membranes exposed to:					
	No treatment	Heat	Protease	DNase and RNase	Lipase	Sodium periodate
1	4,096	1,024	1,024	4,096	4,096	64
2	16,384	4,096	1,024	16,384	16,384	1,024
3	4,096	256	256	4,096	4,096	256
4	1,024	256	256	1,024	1,024	64
5	1,024	64	64	1,024	1,024	16
6	256	64	16	256	256	16

[a] Sera were applied to wells coated with treated or untreated membranes. Wells were coated with 100 μl of each of the antigen preparations at a concentration of 10 μg of protein per ml, except for protease-digested membranes, which were used at 4.2 μg of protein per ml.

by 4- to 16-fold. A greater effect (16- to 64-fold decrease) on the activity of IgM antibodies was noted when the membrane preparations were oxidized with sodium periodate.

The activity of IgG antibodies with the treated membrane preparation was affected in a different manner. Sodium periodate treatment decreased the activity of IgG antibodies with the membrane preparation by 4- to 16-fold. Heat treatment or digestion reduced the activity of these antigen preparations with human IgG antibodies by 16- to 256-fold.

Reaction of human IgM and IgG antibodies with lipids isolated from *T. gondii*. Treatment of the membrane preparation with lipase had no apparent effect on the reaction of this preparation with either IgM or IgG antibodies, indicating that membrane lipids might not be involved in these antigen-antibody reactions. Isolated lipids concentrated to the original volume of *T. gondii* sonic extract and the original sonic extract were used at various dilutions to coat wells of ELISA plates. Six sera containing both IgM and IgG antibodies were applied and their titers were determined in IgM- and IgG-ELISAs. The results revealed that the isolated lipid preparation did not react with either IgM or IgG antibodies (data not shown). To confirm these latter observations, we used the lipid preparation in the IgM- and IgG-DS-ELISA systems. These DS-ELISA systems are based on a different methodology than that of the conventional ELISA systems and do not involve adsorption of the antigens to polystyrene or polyvinyl surfaces (20, 21). The results obtained were essentially the same as those obtained with conventional ELISA tests; lipids isolated from the sonic extract were not recognized by either IgM or IgG antibodies.

Reaction of human IgM and IgG antibodies with *T. gondii* proteins. Reduction of the activity of the membrane preparation with human IgM and IgG antibodies after exposure of the preparation of heat or proteolysis indicated that proteins of the organism participate in these antigen-antibody reactions. To further explore this observation, proteins were isolated either directly from the sonic extracts or from sonic extracts

TABLE 2. Effect of heat, enzymes, and sodium periodate on the reactivity of *T. gondii* membranes with human IgG antibodies[a]

Serum no.	IgG-ELISA titers^{-1} obtained with *T. gondii* membranes exposed to:					
	No treatment	Heat	Protease	DNase and RNase	Lipase	Sodium periodate
1	16,384	256	256	16,384	16,384	1,024
2	65,536	4,096	1,024	65,536	65,536	16,384
3	16,384	1,024	1,024	16,384	16,384	1,024
4	16,384	64	64	16,384	16,384	4,096
5	65,536	1,024	1,024	65,536	65,536	4,096
6	4,096	64	64	4,096	4,096	256

[a] Sera were applied to wells coated with treated or untreated membranes. Wells were coated with 100 μl of each of the antigen preparations at a concentration of 10 μg of protein per ml, except for protease-digested membranes, which were used at 4.2 μg of protein per ml.

from which lipids had previously been extracted. Subsequently, IgM- and IgG-ELISAs were performed. Plates were coated with equal protein concentrations of either isolated proteins or with the original sonic extract. The reactions of the pool of the six positive sera containing IgM and IgG antibodies were compared with those of a pool of negative sera. The isolated protein preparation reacted with both IgM and IgG antibodies (Fig. 2). However, the reactivity was less than that obtained with equal protein concentrations of the unfractionated sonic extract. Because partial denaturation of proteins may have occurred during the protein isolation procedure, we performed additional experiments in which the isolated proteins were used at concentrations threefold higher than those of the control unfractionated sonic extract. The results were essentially the same as those obtained with the lower protein concentrations (Fig. 2). Regardless of the concentrations, the activity of IgM and IgG antibodies with the protein preparations was lower than with the sonic extracts.

Reactions of human IgM and IgG antibodies with polysaccharides isolated from *T. gondii*. In initial experiments, we observed that both IgM and IgG antibodies reacted with the isolated polysaccharides. To confirm these observations, we fractionated the pooled positive serum on Sepharose-4B conjugated to staphylococcal protein A. The IgG fraction and the IgM-containing fraction concentrated to original serum volume were then used in the IgM- and IgG-ELISAs. Both the IgM and IgG fractions reacted with the polysaccharides (Fig. 3).

Treatment of the polysaccharide preparations with DNase, RNase, and protease did not affect the reactions of human IgM and IgG antibodies with the polysaccharide preparations.

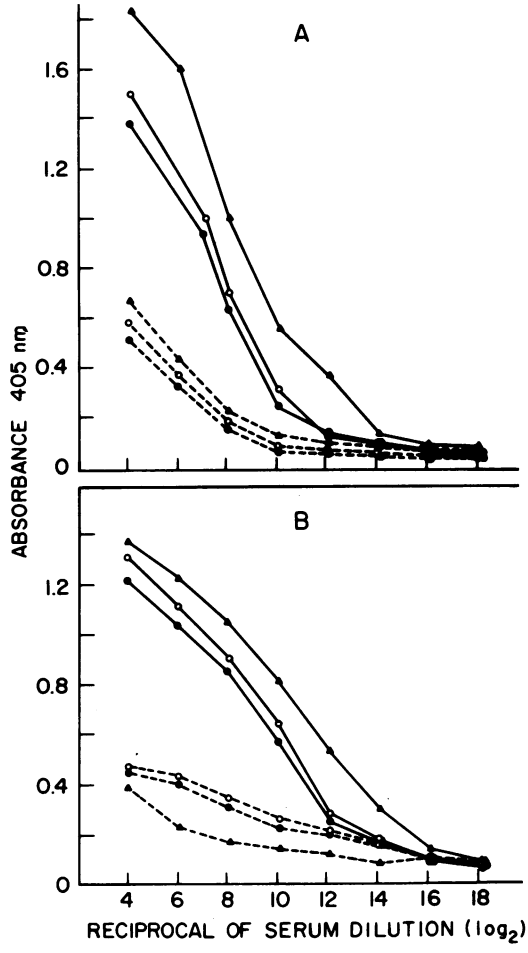

FIG. 2. Reactions of human IgM and IgG antibodies with proteins isolated from *T. gondii*. IgM- (A) and IgG- (B) ELISAs were performed in plates coated with 10 μg of protein per ml of sonic extract (▲), 10 μg of protein per ml of proteins isolated from *T. gondii* (●), or 30 μg of protein per ml of proteins isolated from *T. gondii* (○). With each antigen, reaction of a pool of six positive sera (———) were compared with those of a pool of six negative sera (– – – –).

DISCUSSION

The results presented above demonstrate that both IgM and IgG antibodies produced during the acute stage of infection with *T. gondii* react preferentially with *T. gondii* membranes and less with cytoplasmic antigens of the organism. In 1963, Fleck (7) reported that human antibodies to *T. gondii* which react in the DT are directed against a heavy antigen obtained by high-speed centrifugation. Since the DT detects mainly IgG antibodies, the results of Fleck correlate with our data on the preferential reactions of IgG antibodies with membranes of the organism. More recently, Handman et al. (10) demonstrated that human IgG antibodies produced during acute infection with *T. gondii* formed precipitable immune complexes with radioiodinated membrane proteins.

Our result with human IgM antibodies and membranes isolated from *T. gondii* revealed that IgM antibodies from acutely infected patients are also directed mainly against membrane antigens.

To further characterize the biochemical nature of the antigens recognized by human IgM and IgG antibodies, we subjected *T. gondii* membranes to heat, oxidation with sodium periodate, and enzymatic digestion. Treatment with lipase, DNase, or RNase did not affect recognition with either IgM or IgG antibodies. Treatment of the membranes with protease or heat at 100°C or with sodium periodate decreased the titers obtained with the membrane antigen prep-

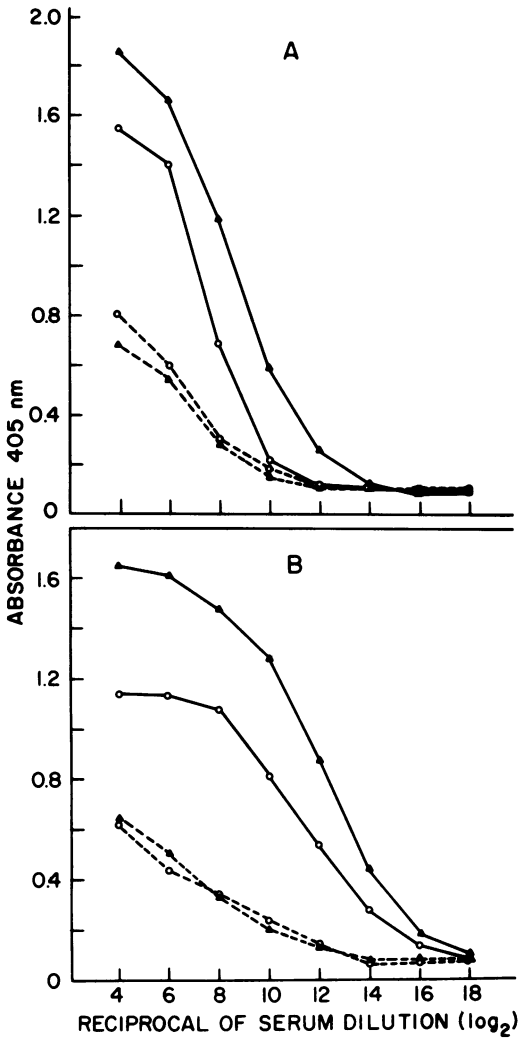

FIG. 3. Reactions of human IgM and IgG antibodies with polysaccharides isolated from *T. gondii*. IgM- (A) and IgG- (B) ELISAs were performed in plates coated with 10 μg of protein per ml of sonic extract (▲) or equal volumes of polysaccharides isolated from *T. gondii* containing 4 μg of carbohydrates per ml (○). The reactions of the IgM-rich fractions and IgG fractions obtained from a pool of positive human sera by fractionation on protein A columns were compared with those of a pool of negative sera.

aration and both human IgM and IgG antibodies. These results indicate that the antigenic reactivity of the membrane antigens with IgM and IgG antibodies depends upon the integrity of both proteins and carbohydrates (or glycoproteins) within *T. gondii* membranes. Moreover, our data suggest that the reactions of IgM antibodies with *T. gondii* membranes may be more dependent on the integrity of membrane carbohydrates than on that of membrane proteins and that IgM may recognize antigenic determinants located preferentially on the carbohydrate moiety of membranes.

Reactivity of the membrane antigen preparation with IgG antibodies was more susceptible to proteolytic digestion and heat denaturation than to oxidation of carbohydrates.

The preparation of lipids isolated from *T. gondii*, in contrast to *T. gondii* sonic extracts, were not recognized by IgM or IgG antibodies. Since IgM and IgG antibody titers were also not affected when the membrane preparations were treated with lipase, it seems plausible that lipids are not involved, at least in the antigen-antibody reactions observed in the ELISA systems we employed. In a preliminary report, Hughes and Balfour (13) suggested that a glycolipid antigen isolated from *T. gondii* appears to be important in the DT reaction. However, in their report, the source of antibodies used in the DT was not given. Therefore, it is unclear whether a glycolipid antigen from *T. gondii* is recognized by human antibodies and whether such an antigen was not recognized in our studies owing to its low concentration within the bulk of the lipid preparation. Moreover, in a more recent extensive study reported by these same authors, neither lipids nor glycolipids were identified among 11 different antigens of *T. gondii* which were recognized by a human serum containing *T. gondii* antibodies (13). Since glycolipids isolated from other infectious organisms are present in the lipid fraction isolated by the procedure we used (28), it appears that, in the case of *T. gondii*, if lipids or glycosylated lipid antigens are present, they are not recognized as antigens by human IgM and IgG *T. gondii* antibodies in our ELISA systems.

Proteins and polysaccharides isolated from the organism reacted with IgM and IgG antibodies. We observed that the reactions of both immunoglobulin classes with isolated components was always lower than that with the unfractionated sonic extracts. This conclusion differs somewhat from that of Mineo et al. (18) and Camargo et al. (2), who used an ELISA to compare the reactions of IgM and IgG antibodies with *T. gondii* polysaccharides to the reactions of these antibodies with a soluble fraction obtained after centrifugation of sonicated organisms at 10,000 × *g* for 15 min. On the basis of their results, these authors suggested that the initially strong IgM antibody response in human toxoplasmosis frequently occurs to determinants in both the sugar and protein moieties of a glycoprotein complex (2, 18). In addition, they stated that the IgG antibody response to polysaccharides is low or even absent but is marked against parasite protein components (18). In our

studies, we treated the polysaccharide fraction of *T. gondii* with DNase, RNase, and protease in an attempt to remove possible contaminants. We demonstrated that both IgM and IgG fractions separated from a positive serum reacted with *T. gondii* polysaccharides. These results led us to conclude that not only IgM but also IgG antibodies recognize *T. gondii* polysaccharides. The differences between our results and those of Mineo et al. can probably be explained by differences in the methods used for preparation of the polysaccharides. For maximal extraction of polysaccharides from *T. gondii*, we found that a longer incubation period and a higher incubation temperature than those used by Mineo et al. are required (18). It is, therefore, possible that our preparations contained carbohydrate components which were absent in their preparations.

Our data revealed that, in addition to polysaccharides, *T. gondii* proteins are also recognized by both IgG and IgM antibodies. These data are in agreement with previous reports by Hughes and Balfour (13) on reactions between proteins isolated from *T. gondii* and sera of patients infected with this organism. Our study extends these observations and demonstrates that both IgM and IgG antibodies recognize polysaccharides and proteins isolated from *T. gondii*. Hughes and Balfour reported that 1 of the 11 different antigens present in their soluble antigen preparation was a glycoprotein (13) and that it reacted with human serum containing IgM and IgG *T. gondii* antibodies. However, it remains to be determined whether it is a membrane or cytoplasmic antigen.

Studies with lectins by Johnson et al. showed that glycosylated peptides exist in *T. gondii* but only at very low concentrations (15). Such glycosylated polypeptides were present in the insoluble fraction obtained from *T. gondii* sonic extracts by ultracentrifugation (15). With membrane preparations obtained by centrifugation at similar centrifugal forces, our data revealed that the integrity of both carbohydrate and protein structures may be necessary for optimal reactivity of IgM and IgG antibodies with *T. gondii* membranes.

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