Fractionation of Membrane Components from Tachyzoite Forms of *Toxoplasma gondii*: Differential Recognition by Immunoglobulin M (IgM) and IgG Present in Sera from Patients with Acute or Chronic Toxoplasmosis

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Tachyzoite forms of *Toxoplasma gondii* were subjected to a sequential organic solvent extraction, which allows fractionation of membrane components according to their degrees of hydrophobicity, yielding three fractions named F1 (most hydrophobic) to F3 (least hydrophobic). Fractions F2 (80.85% specificity and 86.95% sensitivity) and F3 (89.36% specificity and 93.61% sensitivity) gave the best results, being preferentially recognized by immunoglobulin M (IgM) and IgG in sera from patients with acute and chronic toxoplasmosis, respectively. Improved scores of specificity (100%) and sensitivity (100%) were achieved when a secondary antibody against human IgG instead of total IgG was employed to measure the reactivity of IgG antibodies with the F3 fraction. To purify tachyzoite antigens recognized by human IgM or IgG antibodies, the F2 or F3 fraction was loaded onto an octyl-Sepharose column and eluted with a propan-1-ol gradient. The main antigen(s) recognized by IgM or IgG eluted in a single peak from the octyl-Sepharose resin loaded with either F2 (30 to 50% propan-1-ol) or F3 (15 to 35% propan-1-ol), respectively. These semipurified fractions gave improved scores when used to detect *T. gondii*-specific IgM (95.7% specificity and 81.8% sensitivity) or IgG (100% specificity and 93.75% sensitivity) in an enzyme-linked immunosorbent assay. Further biochemical and immunological analyses of antigens partially purified from F2 and F3 indicate that glycosylphosphatidylinositol (GPI)-anchored proteins are preferentially recognized by IgM, whereas proteins of approximately 30 to 40 kDa are recognized by IgG, elicited during *T. gondii* infection in humans.

*Toxoplasma gondii* is widespread throughout the world, with no geographic or zoological boundaries, so that human populations are constantly exposed to and infected with this parasite (7). It is estimated that toxoplasmosis exists in a chronic, asymptomatic form in 500 million to 1 billion of the world’s human population (17). Whereas infection with *T. gondii* is usually innocuous or asymptomatic in most individuals, it causes serious morbidity and mortality in fetuses of primarily infected pregnant women (19) and in immunocompromised individuals (4). The simultaneous infection with *T. gondii* and human immunodeficiency virus type 1 is of increasing concern, since it is reported that this parasite is the major infectious cause of encephalitis in AIDS patients, being among the top 10 opportunistic infections which are more often encountered as AIDS-defining illness (22).

Therefore, there are at least two major situations in which the diagnosis of *T. gondii* infection, leading to therapeutic intervention, is of medical importance. The first one is the detection of *T. gondii*-specific immunoglobulin M (IgM) in sera from pregnant women, who, if not treated with specific chemotherapy, may have serious fetal problems, including malformation or abortion (19). Second, different studies indicate that up to 15% of AIDS patients who have positive serological tests for *T. gondii* may develop toxoplasmic encephalitis. Toxoplasmic encephalitis is often difficult to diagnose and has to be treated immediately after the initial symptoms to avoid fatality (2, 16).

Different studies have defined the major targets for *T. gondii*-specific IgM or IgG antibodies found in sera from acutely or chronically infected individuals (6, 19). However, most serological tests used in the laboratory employ parasite extracts rather than purified or recombinant antigens. This is especially true in the case of tests to detect *T. gondii*-specific IgM that target complex glycolipids that are difficult to synthesize in the laboratory. In addition, false-positive and false-negative results, using commercial kits for parasite-specific IgM detection, are often reported (15). Even in tests for detection of tachyzoite-specific IgG, the vast majority of which recognize parasite proteins, the use of recombinant protein or synthetic peptides has been problematic (23), also yielding dubious results.

In the present study, we used a methodology that employs a sequential organic solvent extraction, which allows the fractionation of membrane components according to their degrees of hydrophobicity (1, 10). This methodology yielded two distinct fractions, named F2 and F3, which were preferentially recognized by IgM and IgG present in sera from patients with acute and chronic toxoplasmosis, respectively. Because the major targets for either IgM or IgG have been defined as a specific subset of glycosylphosphatidylinositol (GPI)-anchored proteins (14, 25), respectively, we used hydrophobic interaction chromatography to further purify the parasite molecules which are major targets for human antibodies. The antigens recognized by IgM or IgG

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were eluted as a single peak from octyl-Sepharose resin loaded with either F2 (30 to 50% propan-1-ol) or F3 (15 to 35% propan-1-ol) and highly enriched. The fractions obtained from octyl-Sepharose loaded with F2 and F3, when used in an enzyme-linked immunosorbent assay (ELISA), resulted in an assay of much higher specificity and approximately the same sensitivity to detect T. gondii-specific IgM and IgG, respectively.

**MATERIALS AND METHODS**

**Population studied.** Coded serum samples were obtained from 23 patients with acute T. gondii infection (IgG positive and IgM positive) and 47 patients with chronic T. gondii infection (IgG positive and IgM negative). Sera from 47 uninfected individuals (IgG and IgM negative) were used as controls. The patients with acute infection were further classified as high (n = 5; average serum titer, 1:960) and low (n = 18; average serum titer, 1:120) IgM producers, as indicative of early and late acute toxoplasmosis, respectively. Toxoplasma-specific IgM serological testing was performed with a commercially available immuno-fluorescence assay (IFA) kit with fixed tachyzoites (Immunotox; bioMérieux, Marcy l’Étoile, France), using the anti-human IgM (whole-molecule) fluorescein isothiocyanate conjugate (Fuoline H; bioMérieux) (3). Toxoplasma-specific IgG serological testing was performed using a ELISA kit employing a tachyzoite extract (Toxonostika IgG; Organon, Bottel, The Netherlands) (9). All of the patients with chronic toxoplasmosis were asymptomatic. In contrast, patients with acute infection presented variable clinical symptoms, ranging from no symptoms to fever, headache, lymphoadenopathy, and/or pneumonia.

**Parasites.** Tachyzoites of RH strains of T. gondii were maintained by in vitro passage in human foreskin fibroblasts at 37°C (12). Tachyzoites were harvested at 4 to 5 days postinfection, centrifuged at 70 × g for 10 min in order to remove cell debris, and then pelleted at 590 × g for 10 min. The parasite pellet was washed twice by resuspension in cold phosphate-buffered saline (PBS) and centrifugation at 590 × g for 10 min. The final pellet was stored at −70°C until used for sequential organic solvent extraction.

**Sequential organic solvent extraction of tachyzoite membrane components.** The tachyzoite pellet frozen at −70°C was homogenized and subjected to extraction with chloroform-methanol-water (5/1/4, vol/vol/vol) (Fig. 1A) (1, 10). Ten volumes of chloroform-methanol-water was added to the parasite pellet and sonicated for 15 min, followed by centrifugation at 5,000 × g for 15 min at 10°C. The resulting pellet was subjected to same protocol twice more, and the supernatants were pooled, dried in a speed vacuum (Savant Instruments Inc., Farmingdale, N.Y.), and subjected to a butan-1-ol–water (1/1, vol/vol) partition. The butanolic and aqueous phases generated by the butan-1-ol–water partition were named F1 and F2, respectively. The pellet obtained after the chloroform-methanol-water extractions was dried in a speed vacuum and extracted three times with 10 volumes of 95% butan-1-ol with shaking at room temperature, followed by centrifugation at 5,000 × g for 15 min at 10°C. The 95% butan-1-ol supernatants were pooled and named F3. The resulting pellet (cell debris) and fractions F1 to F3 were all dried and resuspended in water, and their protein concentrations were determined by the Bradford method (Bio-Rad Laboratories, Richmond, Calif.) using bovine serum albumin as a standard. Cell debris and F1 to F3 samples were then stored at −70°C until used in the ELISA and Western blotting assay.

**Octyl-Sepharose chromatography.** Frozen F2 and F3 fractions were resuspended in 100 mM ammonium acetate containing 5% propan-1-ol and subjected to hydrophobic interaction chromatography using octyl-Sepharose resin (Pharmacia Biotech, Uppsala, Sweden) elution with a propan-1-ol (5 to 60%) gradient. Two-milliliter fractions were collected and assayed for myo-inositol content, protein concentration, and the ability to bind to IgM and IgG present in sera from patients with acute and chronic toxoplasmosis, respectively.

**myo-Inositol measurements.** Briefly, samples were preincubated with 40 pmol of deuterated myo-inositol, dried in a SpeedVac centrifuge (Savant Instruments), resuspended in 50 mM sodium bicarbonate, sonicated for 10 min in order to denature proteins, and transferred to glass capillary tubes. Samples were dried again, and 50 μl of 6 N HCl was added. The capillary tubes were then sealed under vacuum and subjected to hydrolysis at 110°C for 16 to 18 h. Samples were dried under vacuum, and the residual HCl was removed by evaporation after addition of 50 μl of water. For dehydrogenation, 50 μl of methanol was added to each sample and dried under vacuum. The samples were then incubated with fresh trimethylsilyl (TMS) reagent for 15 to 30 min at room temperature. TMS derivatives were analyzed (1 μl per sample) in an SE-54 (0.25 mm by 5 m) (Alltech) capillary column using a linear temperature gradient of 140°C for 1 min, 140 to 250°C for 7.3 min (15°C/min), and 250°C for 5 min. Selective ion monitoring was carried out for TMS derivatives of d6-myoinositol at 307 and 321 m/z and of myo-inositol at 305 and 318 m/z (5).

**ELISA.** Immunol-2 plates (Dynatech Laboratories, McLean, Va.) were coated with 100 μl of either F1, F2, or F3 at a protein concentration of 10 μg/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6. Alternatively, 0.5 pmol of F2-derived eluate E1 or 1.0 pmol of F3-derived eluates E1 and E2 per well in 50 μl of 0.05 M carbonate-bicarbonate buffer (pH 9.6) was used to coat the Immunol-2 plates. Plates were incubated overnight at 4°C, blocked with 2% casein (Calbiochem, La Jolla, Calif.) for 2 h at 37°C, and then washed four times with 0.15 M PBS (pH 7.2)–0.05% Tween 20 (Sigma, St. Louis, Mo.) (PBS-T). One-hundred-microliter portions of sera at dilutions of 1:50 to 1:2,000 in PBS-T–1% bovine serum albumin (Biobras, Montes Claros, Brazil) were added and incubated for 1 h at 37°C. Plates were then incubated with biotinylated conjugates of anti-human IgG, IgM, IgG2, and IgG3 at a concentration of 1:20,000 in PBS-T for additional 1 h at 37°C and washed with PBS-T. Streptavidin-peroxidase conjugate (Sigma) at a 1:1,000 dilution was added and incubated for 30 min at 37°C. The plates were then washed with PBS-T and developed using ABTS (2,2′-azino-bis(3-ethylbenz-thiazoline-sulfonyl acid)) as a substrate. The reaction was terminated by the addition of 50 μl of 1% sodium dodecyl sulfate (SDS) solution, and results were read at 405 nm.

**SDS-PAGE.** Different tachyzoite antigen preparations were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 10 or 15% gels under reducing conditions as previously described (8). Gels were silver stained after fixation for 30 min in 50% methanol–10% acetic acid and for 30 min in 5% methanol–7% acetic acid, followed by 30 min of incubation with 20 mM dithiothreitol and 0.1% AgNO3. The gels were developed in 3% Na2CO3–0.05% formaldehyde.

**Immunoblotting.** Proteins separated by SDS-PAGE were transferred to nitrocellulose paper (27), using the Mini-Protein system (Bio-Rad). Alternatively, 5 μl of parasite antigen suspension in PBS was added to a nitrocellulose paper, which was left drying for 10 min. Blots or dot blots were first soaked in 2% casein–PBS-T for 1 h at room temperature to block free binding sites. The blots were then incubated overnight at 4°C with a pool of sera, at a 1:200 dilution, from patients with either acute or chronic toxoplasmosis or from individuals who did not have any evidence of T. gondii infection. The nitrocellulose sheets were then incubated with biotin-conjugated goat anti-human IgM or IgG antibody (Sigma) for 1 h at room temperature and then reincubated for 30 min at room temperature with streptavidin-peroxidase conjugate (Sigma) at a 1:1,000 dilution. After each incubation, the membranes were washed three times with PBS-T. Finally, after being rinsed with 0.05 M carbonate-bicarbonate buffer (pH 9.6), blots were
Identification of tachyzoite antigens that are preferentially recognized by IgG antibodies from sera of patients chronically infected with T. gondii. Our experiments also indicate that F3 gave the best results for IgG detection, with higher and lower averages for infected and uninfected individuals as well as the best sensitivity (93.61%) and specificity (89.36%) scores (Table 2).

Figure 2B shows that the pattern of recognition of different fractions by IgG was more complex than the pattern generated by IgM antibodies. A band of approximately 30 kDa was the major antigen recognized by T. gondii-specific IgG from sera of patients with chronic toxoplasmosis. This antigen was present in both F2 and F3. In addition to the 30-kDa antigen, many other antigens with molecular masses of above 30 kDa were also recognized by IgG antibodies in sera from chronically infected individuals. The same 30-kDa antigen appears to be recognized by IgM antibodies, but with much lower intensity (Fig. 2A and B) than IgG antibodies, from chronically infected patients. Accordingly, in the ELISA the total tachyzoite sonicate and F3 fraction were poorly recognized by T. gondii-specific IgM antibodies compared to the F2 fraction.

Identification of tachyzoite antigens that are preferentially recognized by IgM antibodies from sera of patients acutely infected with T. gondii. The results presented in Table 1 show the ability of the F2 fraction (80.85% specificity and 86.95% sensitivity) to detect specific IgM antibodies present in sera of patients with acute toxoplasmosis. However, a high number of false-positive results were observed in the experiments using the F2 fraction, as indicated by the relatively low (80.85%) specificity of the assay.

The pattern of antigen complexity of this fraction was further analyzed by immunoblotting analysis, using sera from patients with acute toxoplasmosis. Figure 2A shows that the main antigen recognized in the F2 fraction by the IgM antibodies present in sera from acutely infected patients was an antigen with a diffuse pattern of migration and an apparent molecular mass of below 14 kDa. This antigen was also recognized by a monoclonal antibody (MAb), T33F12, against GIPLs from T. gondii tachyzoites (Fig. 2C) (23). A less diffuse band of approximately 30 kDa and a more defined band at 70 kDa present in the F2 fraction were also recognized by IgM antibodies present in sera from acutely infected patients.
We also determined the main IgG isotype present in sera of patients with chronic toxoplasmosis that recognized the tachyzoite antigens present in F3. The results presented in Table 2 show a clear dominance of the IgG1 isotype among IgG antibodies specific for T. gondii antigens. Importantly, the use of anti-IgG1 instead of anti-total IgG secondary antibody also resulted in an increased specificity (100%) and sensitivity (100%) to discriminate infected from uninfected individuals.

Purification and partial characterization of tachyzoite molecules recognized by human IgM and IgG from sera of patients with acute or chronic toxoplasmosis.

In order to improve the scores of our ELISA test, we decided to furtherpurify components of the tachyzoite membrane by hydrophobic interaction chromatography using octyl-Sepharose. In fact, different studies suggest that GPIPLs and GPI-linked proteins are the main targets of IgM (19, 20, 22) and IgG (13, 23) antibodies present in sera from humans infected with T. gondii.

Figure 3A shows protein concentrations (absorbance at 280 nm) and myo-inositol concentrations of fractions A to H released during the propan-1-ol gradient treatment used to release the tachyzoite molecules from octyl-Sepharose columns loaded with F2 and F3. Major protein and myo-inositol peaks were observed in eluate B (5% propan-1-ol) for the column loaded with either F2 or F3, and these correspond to unbound material. Two additional major myo-inositol peaks were detected in eluates E (30% propan-1-ol) and F (40% propan-1-ol) from the octyl-Sepharose column loaded with F2. Minor myo-inositol peaks were also observed in eluates E (15% propan-1-ol), F (25% propan-1-ol), and G (35% propan-1-ol) from the octyl-Sepharose column loaded with F3.

Each of the eluates obtained from octyl-Sepharose columns loaded with F2 and F3 were also characterized for their ability to be recognized by IgM and IgG from sera of patients with acute or chronic toxoplasmosis (Fig. 3B). These studies were performed using the dot immunoblotting analysis. Our results show that the eluate F and, to a lesser extent, eluate E eluted from octyl-Sepharose loaded with F2 were preferentially recognized by IgM antibodies present in sera from patients acutely infected with T. gondii. This same eluate F was recognized specifically by MAb T33F12. In contrast, eluate F obtained from the octyl-Sepharose column loaded with F3 reacted preferentially with IgG antibodies from sera of patients chronically infected with T. gondii. No reactivity with any of the eluates was observed when we used sera from uninfected individuals.

Each of the fractions that showed reactivity with either human IgM or IgG was further analyzed by SDS-PAGE and immunoblotting analysis. Our results demonstrate that eluates E and F did not present a single protein band when silver stained. Only a major diffuse band with molecular mass of below 14 kDa was prominent in eluate F obtained from the column loaded with F2 (Fig. 4A, left panel). This low-molecular-mass diffuse band was recognized by IgM antibodies from sera of patients with acute toxoplasmosis (Fig. 4A, middle panel). After a further butan-1-ol–water partition, the butanolic phase of F2-derived eluate F showed by ES-MS (negative mode) a group of doubly charged [(M+2H)+2] pseudomolecular ions at m/z 900 to 1150. At least four major species at m/z 905, 959, 1015, and 1028 were observed. Interestingly, two of the less abundant species, at m/z 905 and 986, have estimated molecular masses (1,812 and 1,974 Da, respectively) consistent with two major GPIPL structures previously reported (Fig. 4A, right panel) (23). These structures correspond to (i) (ethanolamine-PO₄)₂-Mano₁-2Mano₁-6(GalNAcβ1-4)
Man\(_1\)4Glc\(\alpha\)-inositol-PO\(_4\)-diacyl(C\(_{16:0}\)/C\(_{18:0}\))-glycerol (molecular mass, 1,812 Da) and (ii) (ethanolamine-PO\(_4\))-Man\(_1\)2Man\(_1\)-6(Glc\(_1\)-4GalNAc\(_\beta\)-1-4)Man\(_1\)-4Glc\(\alpha\)-inositol-PO\(_4\)-diacyl(C\(_{16:0}\)/C\(_{18:0}\))-glycerol (molecular mass, 1,974 Da). In fact, most of the major doubly charged species observed (m/z 959, 1015, 1028, 1040, 1096, 1109, and 1121) could be derived from the species at m/z 905 and 986, as indicated in Fig. 4A (right panel) and Table 3.

**TABLE 3. Proposed assignments for *Toxoplasma* GIPL species observed by ES-MS**

<table>
<thead>
<tr>
<th>GIPL series</th>
<th>([\text{M} - 2\text{H}]^{2+}) (m/z)</th>
<th>Mass (Da)</th>
<th>Proposed assignment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>905</td>
<td>1,812</td>
<td>(Hex(<em>\alpha),HexNAc)(EtNP)-Hex-InsP-(C(</em>{16:0})/C(_{18:0}))DAG(^a)</td>
</tr>
<tr>
<td></td>
<td>986</td>
<td>1,974</td>
<td>(Hex(<em>\alpha),HexNAc)(EtNP)-Hex-InsP-(C(</em>{16:0})/C(_{18:0}))DAG(^a)</td>
</tr>
<tr>
<td>B</td>
<td>959</td>
<td>1,920</td>
<td>(Hex(<em>\alpha),HexNAc)(EtNP)(AEP)-Hex-InsP-(C(</em>{16:0})/C(_{18:0}))DAG</td>
</tr>
<tr>
<td></td>
<td>1,040</td>
<td>2,082</td>
<td>(Hex(<em>\alpha),HexNAc)(EtNP)(AEP)-Hex-InsP-(C(</em>{16:0})/C(_{18:0}))DAG</td>
</tr>
<tr>
<td></td>
<td>1,121</td>
<td>2,244</td>
<td>(Hex(<em>\alpha),HexNAc)(EtNP)(AEP)-Hex-InsP-(C(</em>{16:0})/C(_{18:0}))DAG</td>
</tr>
<tr>
<td>C</td>
<td>1,015</td>
<td>2,032</td>
<td>ND(^c)</td>
</tr>
<tr>
<td></td>
<td>1,096</td>
<td>2,194</td>
<td>ND</td>
</tr>
<tr>
<td>D</td>
<td>1,028</td>
<td>2,058</td>
<td>(Hex(<em>\alpha),HexNAc)(EtNP)-Hex-InsP-(C(</em>{16:0}/22:0))DAG</td>
</tr>
<tr>
<td></td>
<td>1,109</td>
<td>2,220</td>
<td>(Hex(<em>\alpha),HexNAc)(EtNP)-Hex-InsP-(C(</em>{16:0}/22:0))DAG</td>
</tr>
</tbody>
</table>

* Hex, hexose; HexNAc, N-acetylhexosamine; EtNP, ethanolaminephosphate; HexN, hexosamine; InsP, myo-inositol-phosphate; DAG, diacylglycerol; AEP, 2-aminoethylphosphonate.

\(^a\) Based on structures described by Striepen et al. (21).

\(^b\) ND, not determined.
eluates with higher performance in discriminating sera from patients at different
obtained from individual sera from patients of the same group. Asterisks indicate
The ELISA was developed as described in Materials and Methods. The means
to be bound by human IgG present in sera (dilution, 1:100) from patients with
loaded onto an octyl-Sepharose column and eluted in a gradient of propan-1-ol
and IgM present in sera (dilution, 1:200) from patients with
onto an octyl-Sepharose column and eluted in a gradient of propan-1-ol, as
described for Fig. 3A. The octyl-Sepharose eluates were tested for their ability to
be bound by human IgM present in sera (dilution, 1:200) from patients with
acute toxoplasmosis. The results represent the means and standard deviations for
for discriminating acutely infected from chronically
infected and uninfected individuals, as seen by their high spec-
ificity (100%) and sensitivity (93.75%) scores. Treatment with
proteinase K destroyed most reactivity of F3 eluates E and F
with IgG from sera of patients with chronic toxoplasmosis
(data not shown), indicating the proteinaceous nature of these
epitopes.

The results presented in Fig. 6 show the individual values of
parasite-specific IgM (Fig. 6A) or IgG (Fig. 6B) in ELISA
using different antigen preparations as well as sera from pa-
patients with acute toxoplasmosis, patients with chronic toxopla-
mosis, and uninfected controls. These results show an already
substantial improvement after the sequential organic extrac-
tion when comparing the serology results using F2 (IgM) and
F3 (IgG) with total tachyzoite extracts. The data were further
improved when eluate F from F2 and eluates E and F from F3
were used to measure IgM and IgG specific for tachyzoite
antigens, respectively. The latter improvement was mainly due
to an increase in the specificity of the assay, i.e., a decrease in
the number of false-positive results with sera from chronically
infected patients in the ELISA to measure T. gondii-specific
IgM as well as a reduction in the number of false-positive
results with sera from uninfected controls in the assay used to
measure parasite-specific IgG.

**DISCUSSION**

Despite major advances in the field of DNA technology,
most serological tests used for diagnosis of T. gondii infection
still employ paraformaldehyde-fixed parasites or crude extracts
from tachyzoites instead of parasite recombinant antigens. Thus,
in addition to the Sabin-Feldmen test (17), which is considered the standard serological test for toxoplasmosis, im-
munofluorescence of fixed parasites is used for detection of
IgM present in sera of acutely infected patients. For detection
of IgG present in sera of chronically infected patients, an
ELISA using total tachyzoite extracts is the most usual method
employed. The failure of recombinant antigens to provide a
test with high specificity and sensitivity scores may be in part
attributed to the facts that (i) carbohydrates instead of pep-
tides are the major targets for IgM antibodies elicited during
the acute infection with T. gondii and (ii) improper folding of
recombinant antigens may result in a dramatic reduction in the
binding of a considerable amount of anti-Toxoplasma IgG anti-
obodies, which may recognize tertiary rather than primary
peptide structures.

As previously established, patients in the early stages of
acute toxoplasmosis produce high levels of parasite-specific
IgM (3). Therefore, our acutely infected patients were divided
into those producing high and low levels of T. gondii-specific
IgM, independent of the levels of parasite-specific IgG. The
sera from uninfected controls were all negative for T. gondii-
specific IgM and IgG, whereas sera from patients with chronic
 toxoplasmosis were all IgM negative and IgG positive as de-
termined by parasite-specific IFA and ELISA, respectively. In
the present study we compared different extracts prepared from
tachyzoite antigens in regard to their ability to discrimi-
nate sera from patients acutely or chronically infected with
T. gondii from those from uninfected individuals.

Several studies suggest that the main targets for antibody
production during the acute and chronic phases of infection
are the surface antigens present in the tachyzoite membrane.
More precisely, in humans most of the IgM responses against
T. gondii are directed against the carbohydrates (11), which
were recently shown to be a branch derived from the glycan
core of a unique GIPL structure (21). In addition, the surface
antigens of approximately 20 (SAG-2), 30 (SAG-1), and 40

![Image](https://example.com/image.png)
SAG-3) kDa have also been shown to be major targets for IgG responses during chronic infection with T. gondii in humans; several studies suggest a dominant response to SAG-1 (6). It is noteworthy that most of the surface molecules are linked to the tachyzoite surface through GPI anchors (13, 18, 25, 26). The strategy used to prepare tachyzoite extracts was the adaptation of a protocol first used for fractionation of Leishmania donovani (10) and Trypanosoma cruzi (1) membrane components based on their hydrophobicities. As described in Materials and Methods, this protocol generates three fractions, F1 to F3, which consist of highly hydrophobic molecules (F1) (e.g., phospholipids), amphipathic components (F2) (e.g., GPIPs), and hydrophilic molecules (F3) (e.g., GPI-linked glycoproteins).

This study shows that by using sequential organic solvent extraction, we were able to produce a tachyzoite extract, named F2, which was highly enriched for GPIPs and displayed a pronounced ability to identify sera from patients with high Toxoplasma-specific IgM titers. However, this fraction still gave a high number of false-positive results and therefore low score for specificity (80.85%). In contrast, the F3 extract gave excellent results in discriminating sera from T. gondii-infected individuals from those from uninfected individuals, with specificity and sensitivity scores in the ranges of 95.7 and 81.8%, respectively. The biochemical and immunochemical data are consistent with the fact that eluate F, obtained from the octyl-Sepharose column loaded with F2, consisted mainly of GPIPs derived from tachyzoite membranes. Furthermore, this is in agreement with previous studies showing that the IgM antibodies from acutely infected patients recognize mainly carbohydrate epitopes (11).

We also observed a small increase in the specificity score when F3-derived eluates E (100%) and F (100%) were used instead of the F3 extract to discriminate sera of chronically infected individuals from those of uninfected individuals. These eluates E and F consisted mainly of protein of approximately 30 and 40 kDa, respectively. In contrast to the antibodies of the IgM isotype, the IgG antibodies were directed mainly against proteinaceous epitopes, as previously suggested by Hadman et al. (6) and Noat et al. (14).

Thus, our study shows that by using a simple biochemical procedure we can fractionate the major membrane components of the tachyzoite membrane. The use of these proteins of 30 and 40 kDa (eluates E and F from F3) leads to an improvement of the specificity and sensitivity scores of the ELISA for detecting sera from patients with chronic toxoplasmosis.

![Graph](https://example.com/graph.png)
addition, false-negative results are common finding in IFA used to detect tachyzoite-specific IgMs. The main reason for the false-negative results is the saturation of IgM binding sites by IgG antibodies. In order to avoid this problem, the use of IgM capture assays to measure T. gondii-specific antibodies has been recommended. Our data indicate that the direct recognition of fraction F2 eluate F by IgM is minimally affected by tachyzoite-specific IgG. Therefore, the chemical isolation of a fraction highly enriched for tachyzoite-derived GPIs that are preferentially recognized by IgM, but not IgG, antibodies may help in the development of a simpler direct ELISA for detecting T. gondii-specific IgM antibodies, with high specificity and fewer problems with false-negative results.

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