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Latex Agglutination Assay of Human Immunoglobulin M Antitoxoplasma Antibodies Which Uses Enzymatically Treated Antigen-Coated Particles

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An assay of immunoglobulin M (IgM) antitoxoplasma antibodies which is rapid (<30 min), homogeneous, and reliable (interassay coefficient of variation, <11%) is proposed. Its principle is based on the observation that a suspension of latex particles coated with toxoplasma antigens, after treatment with proteinase K, becomes less agglutinable by IgG antibodies but more agglutinable by IgM antibodies. The difference between the activities of the two classes of antibodies is increased by the addition of a monoclonal antibody directed against the Fc region of IgM. Agglutination is measured with a special instrument which optically counts the particles that remain free after the reaction. Turbidimetric reading, although less sensitive, is also suitable. No significant interferences either by IgG antitoxoplasma antibodies or by rheumatoid factor or antinuclear antibodies were observed. The sensitivity was similar to that of the immunosorbent agglutination assay.

For the diagnosis of recent symptomatic or asymptomatic infection by *Toxoplasma gondii*, immunoglobulin M (IgM) antibodies can be assayed by various techniques, including immunofluorescence (IgM-IFA) (10), direct agglutination (3, 4), enzyme-linked immunosorbent assay (ELISA) that uses antibody capture (9), immunosorbent agglutination assay (IgM-ISAGA) (2), and passive latex agglutination (11).

Our group has developed various immunoassays that are based on latex agglutination, using a special instrument which optically counts the particles that remain free after the reaction (6). Hence, with this instrument we tried to set up an assay for IgM antitoxoplasma antibodies. The work was greatly facilitated by our observation that when the toxoplasma antigen bound to latex was digested by proteinase K, the level of agglutinability of the particles by IgM antibodies increased, whereas that by IgG antibodies decreased drastically.

MATERIALS AND METHODS

Buffers. Glycine-buffered saline (GBS) contained 0.1 M glycine, 0.17 M NaCl, and 6.15 mM NaN_3, the pH being adjusted to 9.2 with NaOH. GBS-BSA was GBS containing 10 g of bovine serum albumin per liter (Fraction V; Biomedical Corporation). GBS-EDTA was GBS supplemented with 50 mM EDTA, giving a pH of 6.5 or of 9.2 when adjusted with NaOH.

Antigens. Antigens were prepared from *T. gondii* tachyzoites, which were harvested from peritoneal exudates of mice that had been infected 2 days previously with the RH strain. The peritoneal cavity was washed with 3 to 5 ml of sterile physiological saline. To 1 ml of this suspension in conical tubes were added 0.015 ml of the antibiotic Pentrexyl and 10 U of heparin, and the preparation was allowed to stand for 30 min at room temperature. The tubes were then centrifuged for 4 min at 40 × *g*, and the pellets were discarded. The suspension was again centrifuged for 30 min at 2,000 × *g* at 4°C, and the supernatant was discarded. Erythrocytes were hemolyzed by the addition to the pellet of 2 ml of 1 M NH_4Cl, and after centrifugation at 2,000 × *g* at 4°C, the pellet was washed three times with saline. The pellet was resuspended in 2 ml of distilled water and then (three times) successively frozen, thawed, and submitted to ultrasounds (B12 Sonifier; Branson, Danbury, Conn.) at an output of 20 W for 10 min. Finally, after centrifugation at 2,000 × *g*, the supernatant was stored at −20°C. The protein concentration was estimated to be 15 mg/ml by Lowry's method, with BSA as standard.

Preparation of latex. Polystyrene particles of 0.8 μm (Estapor K 109; Rhône-Poulenc, Courbevoie, France) were coated with antigen as follows. A 50-μl volume of the antigen solution, a 400-μl volume of fivefold-diluted GBS, and a 50-μl volume of the 10% (wt/vol) latex suspension were mixed and incubated for 30 min at room temperature. The latex was centrifuged and washed two times with diluted GBS and once with GBS-BSA. The coated particles were resuspended in 1 ml of GBS-BSA and stored at −20°C. Before use, the antigen-coated latex was submitted to ultrasounds for 10 s and then diluted 10-fold with GBS-BSA to reach a 200-fold final dilution.

Enzymatic and periodate treatments. One volume of antigen-coated latex was washed three times with 0.1 M Tris-HCl buffer (pH 7.5) containing 20 mM $CaCl_2$ (Tris-Ca) and then suspended in 1 volume of lipase (Merck, Darmstadt, Germany) trypsin, pronase, or proteinase K (Boehringer Mannheim) at an enzyme concentration of 1 mg/ml in Tris-Ca, and the resulting suspension was incubated for 2 h at 37°C. Pepsin (Sigma) was used at a final concentration of 5 mg/ml in 0.15 M HCl with a 10-min incubation time. Finally, the suspension was washed two times with GBS-BSA, adjusted to the original volume with GBS-BSA, and stored at −20°C. For the digestion of the soluble antigen by proteinase K, equal volumes of substrate and enzyme (0.2 mg/ml in Tris-Ca) were mixed and incubated for 2 h at 37°C, and the reaction was stopped by addition of phenylmethylsulfonyl fluoride.

After proteinase K treatment or no treatment, antigen-coated latex was oxidized with sodium periodate as follows:

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after two washings with 1 ml of phosphate-buffered saline, the particles (100 μl of a 1/20 suspension) were resuspended in 100 μl of 0.01 M sodium periodate (Merck) and incubated overnight at 4°C. After washing, the pellet was suspended in 100 μl of GBS-BSA. In the control, the sodium periodate solution was replaced by buffer.

Antitoxoplasma antibodies and F(ab')$_2$-latex conjugate. Antibodies were raised in a rabbit by subcutaneous inoculations of a brain homogenate from mice chronically infected with the Beverley strain of *T. gondii* (gift from J. P. Tomasi, Université Catholique de Louvain, Brussels, Belgium) (14). The IgG fraction was isolated by successive precipitations with acridine lactate (Rivanol) and with half-saturated ammonium sulfate. The F(ab')$_2$ fragments, which were prepared by Ultrogel AcA 4-4 chromatography of the IgG peptic digest, were coupled to 0.8-μm carboxylated polystyrene particles (Estapor K 150; Rhône-Poulenc) by carbodimide (5) in a proportion of 100 μg of F(ab')$_2$ to 50 μl of a 10% (wt/vol) latex suspension. The coated particles were stored at −20°C and, before use, were submitted to ultrasounds for 10 s and diluted 10-fold with GBS-BSA to reach a 200-fold final dilution.

DTT treatment. To 50 μl of serum were added 170 μl of GBS-EDTA (pH 9.2) and 15 μl of a 10-mg/ml dithiothreitol (DTT) solution (Boehringer Mannheim). The mixture was incubated at 37°C for 15 min, and the excess of DTT was inactivated by the addition of 15 μl of 0.2% hydrogen peroxide.

Additives. Either serum from a rheumatoid patient or a monoclonal antibody directed against the Fc region of IgM (anti-Fcμ MAb) was added to the reagents to reinforce the agglutinating activity of the IgG or IgM antibodies, respectively. The rheumatoid serum, which had an agglutination titer of 1/1,250 in the Behringwerke latex test, was used at a 1/500 dilution in GBS-BSA. The mouse anti-Fcμ MAb was prepared as described elsewhere (15).

Latex assay. Equal volumes (30 μl) of sample, latex, and additive were dispensed with micropipettes into 1-ml polystyrene tubes. The mixture was placed in a specially designed incubator-agitator that created a vortex at a fixed temperature of 37°C. After the reaction was stopped by the addition of 750 μl of GBS-EDTA (pH 6.5), the unagglutinated particles were counted with an optical counter which discriminates the unagglutinated from the agglutinated particles (6). The number of unagglutinated particles was inversely proportional to the concentration of agglutinator. Agglutination was also measured by turbidimetry (decrease in absorbance) at 360 nm in 1-cm light path cuvettes of a Spectronic 1001 spectrophotometer (Milton Roy Company, Rochester, N.Y.). For inhibition experiments, the samples were incubated for 10 min with the agglutinator before addition of the additive and latex.

ELISA. Microtiter plates were incubated overnight at 4°C with a solution (100 μl per well) of 100 μg of antigen per ml in fivefold-diluted GBS and then washed five times with physiological saline containing 0.05% Tween 20. To one half of the wells was added Tris-Ca (100 μl per well) containing proteinase K at a concentration of 1 mg/ml, and the plates were incubated for 2 h at 37°C. The remaining wells were treated with Tris-Ca alone and incubated for the same amount of time. After incubation, all plates were washed five times with physiological saline containing 0.05% Tween 20. Patients' sera were serially diluted from 1/50 to 1/350,000 in GBS-EDTA (pH 9.2) containing 0.1% Tween 80. To each well was added 50 μl of the diluted serum, and the plate was allowed to incubate for 1 h at room temperature and then washed five times with saline-Tween. After addition to each well of 50 μl of peroxidase-labelled anti-IgM (4 μg/ml) or anti-IgG antibodies (2.5 μg/ml) in GBS-EDTA containing Tween 80 and 1 h incubation at room temperature, the plates were washed five times and 100 μl of the enzyme substrate (10 mg of *o*-phenylenediamine per ml plus H_2O_2) per well was introduced. The reaction was stopped with 25 μl of 0.1 N H_2SO_4 after 20 min of incubation in the dark, and the optical density at 405 nm was read. The IgM and IgG antibody titers were expressed as the highest serum dilutions giving absorbances at least twice those of the same dilutions of the negative control serum.

Gel filtration chromatography. A serum sample (3 ml) containing IgG and IgM antitoxoplasma antibodies was fractionated by gel filtration on a column of Ultrogel AcA 3-4 (2.5 by 90 cm) in 0.01 M phosphate buffer (pH 7.5) containing 1 M NaCl.

Commercial kits. ISAGA (Toxo-ISAGA; API-Biomerieux, Charbonières-les-Bains, France), Toxonostika IgM (Organon Teknika N. V., Turnhout, Belgium), and Toxo-G EIA (Abbott Laboratories Diagnostics Division, Chicago, Ill.) were applied according to the manufacturers' instructions. IFA was carried out according to the method of Remington et al. (10) with Biomerieux reagents.

Standards and serum samples. As a standard, we used a serum sample with a high titer of IgM antitoxoplasma antibodies. It was obtained from a patient 1 month after the clinical onset of acute toxoplasmosis. A pool of sera from individuals without serological evidence of toxoplasmosis was used as normal serum. The following three collections of sera were studied: collection 1, serum samples (137 patients) sent to the laboratory for the diagnosis of toxoplasmosis; collection 2, serial samples from 6 patients with acute toxoplasmosis; and collection 3, randomly collected serum samples (n = 1,047) that were used in the comparison of the results of the determination of levels of IgM antibodies by latex agglutination and by ISAGA.

In each serum sample of collection 1, IgG and IgM antibodies were titrated at 3-week intervals by enzyme immunoassay and IgM-IFA, respectively. The patients were classified into four groups according to the results. Group A contained the samples without IgM or IgG antibodies (nonimmune patients). In group B, IgM antibodies were undetectable, whereas significant titers of IgG antibodies which remained constant were found, indicating past infections (protected patients). Group C was similar to group B except that the titers of IgG antibodies varied with time, suggesting that the infections were rather recent. In group D, the fact that the infections were recent was suggested by the presence of IgM antibodies concomitant with either the absence of or an increase of IgG antibody titer. The first serum sample of each patient was tested for IgM antibodies by ISAGA, ELISA Toxonostika, and latex agglutination.

RESULTS

Strengthening of agglutination. To reach an optimal sensitivity, we reinforced the agglutinating activity of IgM antibodies by adding to the reaction mixture a monoclonal antibody specific for the μ chain. At a concentration of 60 μg/ml, it enhanced by a factor of 30 the level of agglutination initiated by IgM antibodies, the effect clearly depending on the concentration of the anti-μ antibody. Ascites containing the same monoclonal antibody could be used at a dilution of 1/100.

Treatment of toxoplasma antigen-coated latex. Proteinase

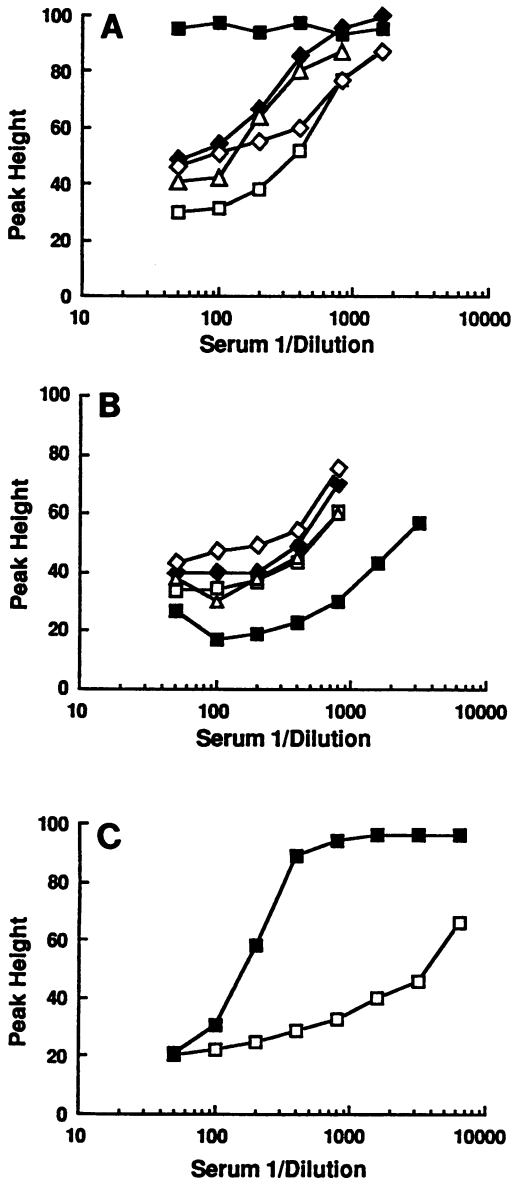

FIG. 1. Agglutination curves of latex coated with toxoplasma antigen. The particles were treated as indicated by various enzymes. The agglutinators were human serum containing IgG (A) or IgM (B) antitoxoplasma antibodies and serum from a rabbit immunized against toxoplasma (C). Peak heights were directly proportional to the numbers of unagglutinated particles. □, untreated; ◆, pepsin; △, trypsin; ◇, lipase; ■, proteinase K.

K, in contrast to pepsin, lipase, and trypsin, abolished the latex agglutinability by IgG antibodies completely (Fig. 1A) but reinforced the agglutinability by IgM antibodies (Fig. 1B). Proteinase K also decreased the agglutinability by rabbit antitoxoplasma antibodies (Fig. 1C). Pronase had the same effect as proteinase K (data not shown). The optimal concentration of proteinase K for the treatment of antigen-coated latex (50 μl of the 10% [wt/vol] suspension in a final volume of 1 ml) was between 20 and 200 μg/ml (Fig. 2). For the present work, we used a 200-μg/ml concentration of proteinase K. When a solution of toxoplasma antigen was first treated by proteinase K and then used to coat latex, the

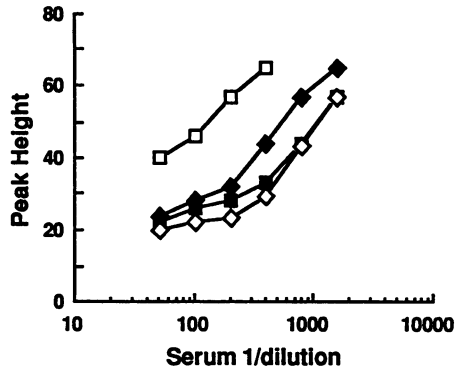

FIG. 2. Agglutination curves of latex coated with toxoplasma antigen. The particles were treated as indicated by increasing concentrations of proteinase K. □, 0 mg/ml; ◆, 4 mg/ml; ■, 20 mg/ml; ◇, 200 mg/ml. The agglutinator was a human serum containing IgM antitoxoplasma antibodies. Peak heights were directly proportional to the numbers of unagglutinated particles.

results were the same as those given by the digestion of the antigen coupled to latex. When antigen-coated latex was treated with proteinase K and then oxidized with sodium periodate, it lost its agglutinability by IgM antibodies.

Properties of toxoplasma antigens. We measured the agglutinating activity of our antigen preparation on latex coated with $F(ab')_2$ fragments of rabbit antitoxoplasma antibodies. When the antigens were treated with proteinase K, they lost a significant amount of their agglutinating activity (Fig. 3).

We also studied the specificities of IgM and IgG antibodies by inhibition experiments in which antigen-coated latex was agglutinated by either IgM or IgG antibodies in the presence of inhibiting concentrations of antigens. No difference between the inhibitory capacities of untreated and proteinase K-treated antigen toward the agglutinating activity of human IgM antibodies on proteinase K-treated antigen-coated latex was observed (Fig. 4A). When untreated antigen-coated latex was agglutinated by human IgG antibodies, proteinase K treatment of the soluble antigen affected its inhibiting capacity markedly (Fig. 4B). When the same latex preparation was agglutinated by human IgM antibodies, inhibition by proteinase K-treated antigen was incomplete (Fig. 4C), indicating that part of the antigen present on latex and

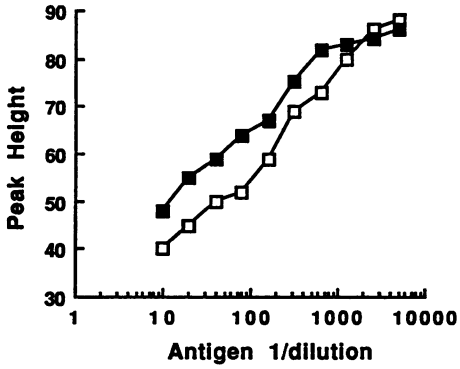

FIG. 3. Agglutination curves of latex coated with the $F(ab')_2$ fragments of rabbit antitoxoplasma antibodies. The agglutinator was the toxoplasma antigen untreated (□) or treated (■) with proteinase K. Peak heights were directly proportional to the numbers of unagglutinated particles.

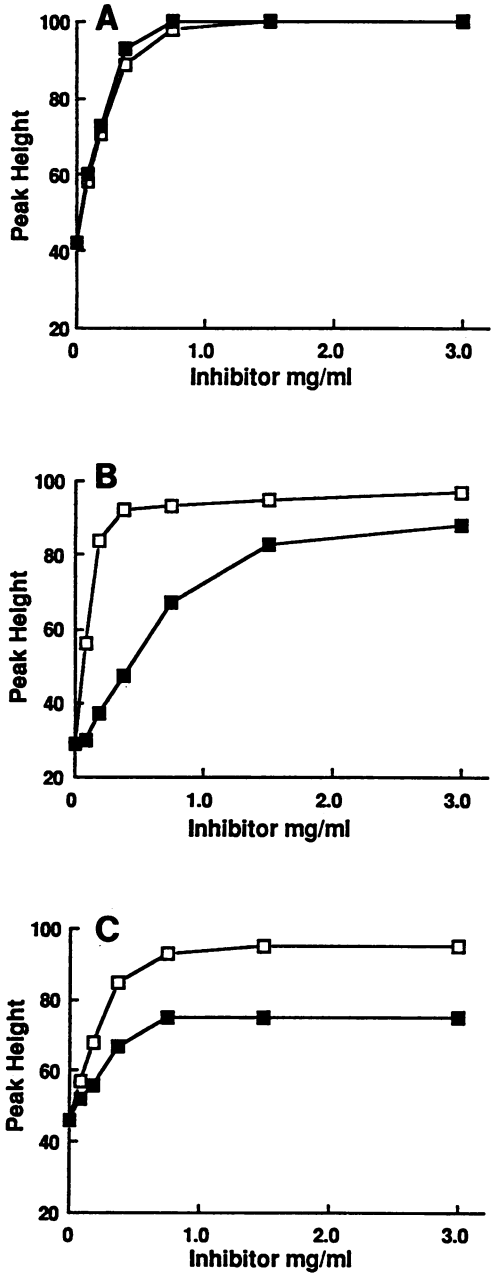

FIG. 4. Inhibition curves of latex agglutination. The inhibitor was either proteinase K-treated (■) or untreated (□) toxoplasma antigen. Peak heights were directly proportional to the numbers of unagglutinated particles. (A) The agglutinator was human serum containing IgM antitoxoplasma antibodies, and the particles were coated with toxoplasma antigen treated with proteinase K. (B) The agglutinator was human serum containing IgG antitoxoplasma antibodies, and the particles were coated with toxoplasma antigen not treated with proteinase K. (C) The agglutinator was human serum containing IgM antitoxoplasma antibodies, and the particles were coated with toxoplasma antigen not treated with proteinase K.

recognized by IgM antibodies had been destroyed by proteinase K.

Effect of proteinase K on ELISA. Digestion of the antigen coating the plates by proteinase K substantially reduced the binding of both IgM and IgG antibodies (Table 1), but to different extents: 40- to 160-fold for IgG antibodies and 4- to 16-fold for IgM antibodies. Similar results were obtained after treatment of the antigen with proteinase K prior to adsorption on microplates.

TABLE 1. Binding of human IgM and IgG antibodies to proteinase K-treated *T. gondii* antigens as measured by ELISA[a]

Serum sample	Titer of IgM antibodies		Ratio	Serum sample	Titer of IgG antibodies		Ratio
	Untreated	Treated			Untreated	Treated	
1	1/64,000	1/4,000	16	7	1/32,000	1/200	160
2	1/128,000	1/8,000	16	8	1/16,000	1/200	80
3	1/32,000	1/2,000	16	9	1/64,000	1/1,000	64
4	1/64,000	1/4,000	16	10	1/8,000	1/100	80
5	1/32,000	1/2,000	16	11	1/128,000	1/2,000	64
6	1/16,000	1/4,000	4	12	1/4,000	1/100	40

[a] Antigens were either untreated or treated with proteinase K as described in the text. Ratios are of titers of IgM or IgG antibodies with untreated antigens to those with proteinase K-treated antigens.

Determination of IgM antibodies. Equal volumes (30 μl) of the sample to be analyzed (diluted 1/250 in GBS-BSA), of mouse ascites containing anti-Fcμ MAb (diluted 1/100), and of proteinase K-treated antigen-coated latex (0.05% [wt/vol]) were vortex mixed together for 20 min at 37°C. The reaction was stopped by the addition of 750 μl of GBS-EDTA (pH 6.5), and the remaining unagglutinated particles were counted. The concentration of IgM antibodies was inversely proportional to the concentration of the free latex particles. When the agglutination was measured by turbidimetry, a 5- to 10-fold decrease in sensitivity, which could be compensated by a lower dilution of the samples, was observed.

Calibration curve. Antibody concentrations were expressed in arbitrary units by reference to a serum rich in IgM antibodies and to which a value of 500 U/ml had been ascribed. The curve relating the peak heights or numbers of unagglutinated particles to the units of IgM per milliliter was sigmoidal in the range of 0.04 to 2.5 U/ml (Fig. 5). Because serum samples were diluted 250-fold, the actual range of sensitivity was 10 to 625 U/ml and the lower limit of detection was fixed statistically at 20 U/ml.

Specificity of the assay. The specificity of the reaction was confirmed by inhibition experiments; 42 agglutinating serum samples were totally inhibited by the addition of 10 μl of 1/10-diluted soluble antigen. Reduction with DTT com-

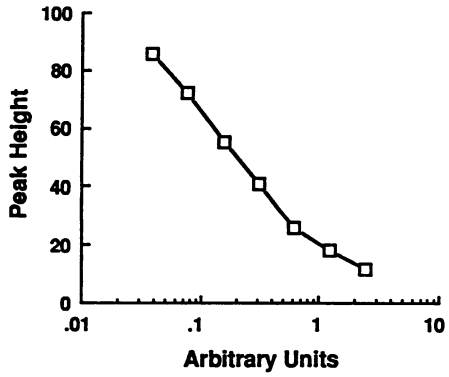

FIG. 5. Calibration curve for the determination of IgM antitoxoplasma antibodies. Peak heights were directly proportional to the numbers of unagglutinated particles.

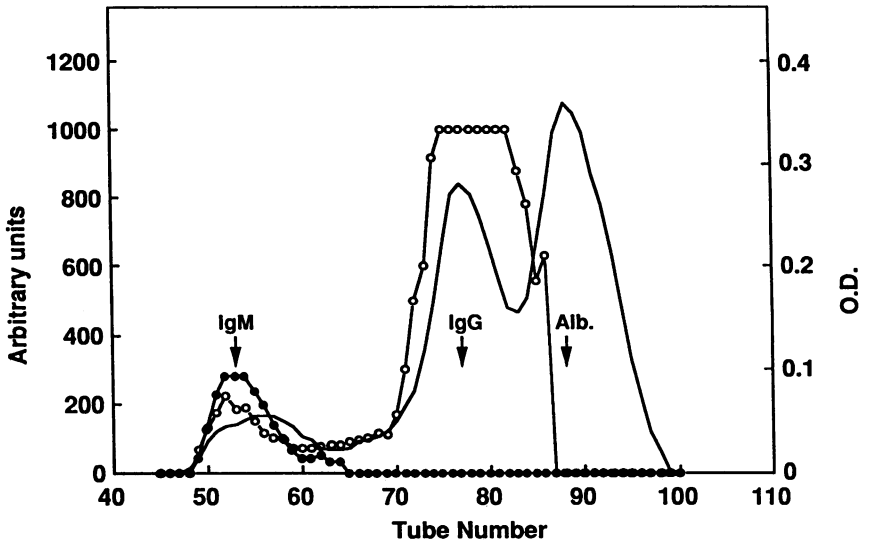

FIG. 6. Gel filtration chromatography on Ultrogel AcA 3-4 of 3 ml of a human serum containing IgG and IgM antitoxoplasma antibodies. Individual fractions were tested for their agglutinating activity toward toxoplasma antigen-coated latex, which was either untreated (○) or treated with proteinase K (●). The agglutinating activity is expressed in the arbitrary units used to define the concentration of IgM antitoxoplasma antibodies. Optical density (OD) at 280 nm is indicated by a continuous line.

pletely abolished the agglutinating activity of the same 42 serum samples, indicating that the agglutinator pertained to the IgM class. More direct evidence of the specificity of the assay for IgM antibodies was obtained by molecular sieve chromatography of a human serum rich in IgM and IgG antitoxoplasma antibodies. The test revealed an agglutinating activity only in the IgM fraction. When the antigen-coated latex was not treated with proteinase K, the agglutinating activity was found in both the IgM and the IgG fractions (Fig. 6).

Possible interfering factors. IgG antibodies could cause false-positive results by agglutinating latex or false-negative results by reacting with antigens on latex and masking them to IgM antibodies. Sera (n = 21) rich in antitoxoplasma IgG and containing DTT-inactivated IgM were found to be unable to agglutinate the antigen-coated latex. To check the possibility of competition between IgG and IgM antibodies, we mixed a serum sample containing IgM antibodies either with a DTT-treated serum rich in IgG antibodies or with a serum sample without antitoxoplasma antibodies (control). The titers of IgM antibodies in both samples were found to be similar, indicating that IgG antibodies did not interfere in the agglutinating activity of the IgM antibodies.

We also checked whether IgM rheumatoid factor or other autoantibodies like those occurring in systemic lupus erythematosus could interfere in the assay. The toxoplasma antigen-coated latex was agglutinated neither by 7 rheumatoid serum samples with agglutination titers of >500 in the Behringwerke rheumatoid factor latex test nor by 10 lupus serum samples with antinuclear antibodies. However, when the samples contained both IgG antitoxoplasma antibodies and IgM rheumatoid factor, an agglutination of antigen-coated latex was observed, despite the proteinase K digestion of the antigen. At a dilution of 1/50 (rather than of 1/250), sera with concentrations of IgG antibodies of >4,000 IU/ml agglutinated antigen-coated latex in the presence of high concentrations of rheumatoid factor (titers of >500). Human aggregated IgG (1 mg/ml) totally inhibited this reaction (data not shown).

Since an increase in the concentration of total IgM could decrease the enhancing effect of the anti-Fcμ MAb on agglutination by IgM antibodies, we raised the concentration of total IgM to 300% of the normal value, but we did not find any significant effect.

Reproducibility. The positive control serum (500 U/ml) was diluted in normal serum to give four different concentrations (20, 40, 80, and 160 U/ml), and the intra-assay and interassay precisions were studied. The assays were repeated either 20 times on the same day or once each day for 20 days (Table 2).

Comparison with other methods. The results obtained for collection 1 are presented in Table 3. For group A (nonimmune patients), the three methods agreed with IFA: 100% of the serum samples were found to be negative. For group B (patients with previous toxoplasma infections), 30% of the serum samples gave values of >20 U/ml by the latex agglutination assay, whereas 50 and 5% were positive by ISAGA and ELISA, respectively. Group C showed a still larger disagreement between IFA and the three methods: 82% of the serum samples were positive (>20 U/ml) by the latex assay, 94% were positive by ISAGA, and 35% were positive by ELISA. For group D, which comprised patients with recent infections, as suggested by IgM-IFA and IgG-ELISA, the latex agglutination assay gave values of >20

TABLE 2. Reproducibility of latex agglutination assay for human IgM antitoxoplasma antibodies[a]

Serum sample	1 day		20 days	
	Mean (SD)	CV (%)	Mean (SD)	CV (%)
1	19.91 (0.78)	3.9	19.80 (2.18)	10.50
2	39.85 (1.53)	3.0	40.05 (2.53)	6.30
3	80.37 (3.56)	4.43	80.60 (4.50)	5.58
4	159.75 (13.16)	8.24	159.60 (12.73)	7.97

[a] Values are means (± standard deviations) of arbitrary units, as defined in the text. CV, coefficient of variation.

TABLE 3. Comparison of latex agglutination assay, ISAGA, and ELISA of human IgM antitoxoplasma antibodies[a]

Group	No. of serum samples	No. of results by					
		Latex agglutination assay		ISAGA		ELISA	
		<20 AU	>20 AU (mean ± SD [range])	−	+	−	+
A	35	35	0	35	0	35	0
B	72	50	22 (52 ± 26 (20–115])	36	36	68	4
C	17	3	14 (80 ± 125 [20–500])	1	16	11	6
D	13	0	13 (540 ± 342 [130–1,000])	0	13	2	11

[a] A, patients without IgM or IgG antibodies (nonimmune patients); B, patients with IgG antibodies but without IgM antibodies (previous infections); C, patients with levels of IgG antibodies that have changed with time (possible recent infections); D, patients with IgM antibodies with or without IgG antibodies (recent infections). AU, arbitrary units; −, negative; +, positive.

U/ml for 100% of the samples, in agreement with ISAGA, which also gave positive results for 100% of the samples, whereas by ELISA two serum samples were negative. The mean results (in units per milliliter) by the latex agglutination assay increased from group A to group D, indicating an inverse relationship between the titer of IgM antibodies and the time since the onset of the disease. However, the dispersion was very high, as indicated by the range of values and the variation in the maximal IgM response illustrated in Fig. 7, which shows longitudinal studies of patients with acute toxoplasmosis (collection 2). For some of the patients, the IgM antibody titers remained high even 6 to 7 months after the acute process.

The similarities between latex assay and ISAGA results obtained for collection 1 prompted us to compare both methods on a larger number of randomly collected serum samples (collection 3). Of a total of 1,047 samples, 91.9% of the results agreed (934 serum samples [89.2%] were negative and 28 serum samples [2.7%] were positive by both methods), whereas 8.1% of the results disagreed (71 serum samples [6.8%] were positive by the latex assay only and 14 serum samples [1.3%] were positive only by ISAGA). The latter 14 serum samples were negative by IgM-IFA.

DISCUSSION

The technique that we have developed for the assay of IgM antitoxoplasma antibodies is based on the observation that latex coated with toxoplasma antigens, after treatment with proteinase K, becomes less agglutinable by IgG antibodies but more agglutinable by IgM antibodies. Mineo et al. (7) reported that human IgM antibodies reacted mainly with polysaccharidic antigens of toxoplasma, whereas human IgG antibodies were directed preferentially against proteins. Using protease digestion or periodate oxidation of the antigen, Naot et al. (8) confirmed this observation, but both IgM and IgG antibodies showed considerably reduced reactivities. Sharma et al. (12) failed to find toxoplasma antigens recognized only by IgM antibodies. In our study, treatment of the antigen with proteinase K before or after coating ELISA plates reduced the binding of IgG antibodies 40- to 160-fold and the binding of IgM antibodies 4- to 16-fold. A decrease of reactivity with IgM antibodies was also observed for the latex inhibition experiments (Fig. 4). Even if the decrease was weaker than that for IgG antibodies (see the ELISA results in Table 1), the paradox that, in the agglutination assay with antigen-coated particles, proteinase K treatment enhances agglutination by IgM antibodies must still be explained. One may conceive that stripping the protein coat from the particles changes their physical surface properties and makes them more agglutinable. This phenomenon would be seen only with IgM antibodies, because they react preferentially with the protease-resistant antigenic determinants. Another factor to be considered is the density of ligands on the particles. Sato et al. (11) had already noticed that agglutination occurred only with IgM antibodies at a certain density of toxoplasma antigen on the particles. In our experience, reducing the density of antigen (or of antibodies, in the reverse reaction) on the particles usually decreases their agglutinability; however, in an earlier experiment with antibody-coated particles (1), in which the antigen was the agglutinator, we observed that the particles loaded with the highest amounts of antibodies were less agglutinated, indicating that in some systems too much ligand on particles could decrease their agglutinability. Perhaps the protease digestion of the toxoplasma antigen by chance gave us the optimal density of determinants recognized by IgM antibodies.

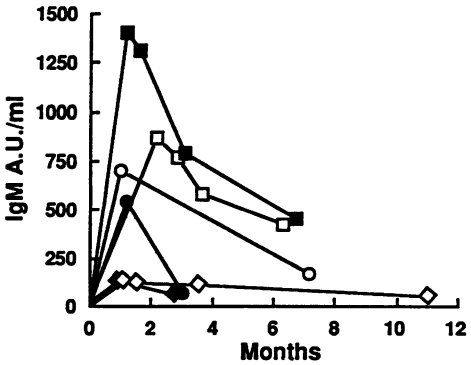

FIG. 7. Concentrations, expressed in arbitrary units (AU), of IgM antitoxoplasma antibodies as determined by the latex agglutination assay of serial samples from six patients.

The specificity of the assay that we are proposing was shown by testing for (i) complete inhibition by soluble antigen, (ii) enhancement of the reaction by anti-Fcμ MAb, (iii) the absence of reaction after reduction with DTT, and (iv) the parallelism between the agglutinating activity and the IgM elution profile in the gel filtration of a serum rich in IgG and IgM antitoxoplasma antibodies. Under the test conditions, high concentrations of IgG antitoxoplasma antibodies did not agglutinate and did not interfere with the agglutinating activity of IgM antibodies.

The results obtained with patients' sera indicated that our latex assay was as sensitive as or more sensitive than ISAGA and that both assays were more sensitive than ELISA. Therefore, the destruction of a part of the antigenic determinants by proteinase K did not affect sensitivity, which can be important for the detection of congenital diseases (13) and for deciding whether a result is really negative. Since the diagnosis of toxoplasmosis usually requires comparison of two serial results, their expression in arbitrary units and the reproducibility of the assay represent nonnegligible advantages.

Most of the present work was carried out with a special instrument which measures the level of agglutination by optical counting of the nonagglutinated particles. As this instrument is not easily available, we checked whether one could use turbidimetry. This less sensitive reading system

was found to be suitable, provided that the samples were 5 to 10 times less diluted.

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