Diagnosis of Toxoplasmosis by Joint Detection of Immunoglobulin A and Immunoglobulin M

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An indirect immunofluorescence test with total anti-human immunoglobulin conjugate (IgG,A,M-IIF) can be used for joint detection of immunoglobulin A (IgA) and IgM antibodies, provided serum IgG is previously absorbed with anti-human IgG. To determine the validity of the IgG,A,M-IIF assay with absorbed sera, the results obtained were compared with those obtained by methods routinely used for the detection of acute-phase markers, IgA and IgM IIF and enzyme immunoassay. Accordingly, 114 serum samples were selected from patients showing titers of $\geq 1:1,024$ by IgG,A,M-IIF. (i) In 90 of the samples, neither IgA nor IgM was detected by any of the methods employed; (ii) the remaining 24 samples showed IgA and/or IgM. In all cases, the IgG,A,M-IIF assay with absorbed sera was positive. These comparative data support the use of IgG,A,M-IIF, performed with absorbed and unabsorbed sera simultaneously, for determining the presence of specific IgG, IgA, and IgM by employing a single technique (IIF), one conjugate (anti-IgG,A,M), and only one sample (with and without previous absorption), thus providing a useful initial tool for the diagnosis of toxoplasmosis.

Infection by *Toxoplasma gondii* is distributed worldwide, benign, and generally asymptomatic in an immunocompetent individual and confers immunity for life (2, 4, 30, 45). However, it can produce serious disorders in an immunosuppressed subject (20, 27, 28, 35, 40) and in a fetus when the mother acquires primary infection during pregnancy (8, 13, 16, 17, 22, 24, 26, 34, 39, 41). For this reason, the search for antibodies against *T. gondii* is a routine test in the follow-up of pregnant women and a diagnosis of acute infection poses a challenge for an obstetrician.

Therapeutic success is obviously greater when treatment is provided without delay (1, 2, 25, 36), so early detection of infection during pregnancy is essential.

Several reports have indicated the role that specific immunoglobulin A (IgA) plays in the acute-infection process (6, 7, 18, 19, 21, 38, 42), stressing the need to detect specific IgG, IgA, and IgM antibodies (33, 37, 47–49) in order to increase diagnostic sensitivity.

The aim of this work was to demonstrate the validity of using as an initial step an indirect immunofluorescence test (IIF) with absorbed IgG and unabsorbed sera instead of carrying out three determinations for detecting IgG, IgA, and IgM antibodies by routine techniques (IIF and enzyme immunoassay [EIA]) (3, 5, 12, 32, 44, 46). The usefulness of implementing this follow-up with pregnant women is evident in the following. (i) Most women with chronic infections fail to present IgA and/or IgM antibody persistence, and by this procedure, the chronic stage of infection may be diagnosed. (ii) Cases where both absorbed and unabsorbed sera are positive must be studied more thoroughly by either confirmatory testing or serial serum specimens in order to make a differential diagnosis between acute infection and chronic infection with IgA and/or IgM antibody persistence.

MATERIALS AND METHODS

Experimental principle. Total immunoglobulin detection by IIF (IgG,A,M-IIF) allows simultaneous evaluation of three specific immunoglobulin classes. If serum IgG is eliminated, only anti-toxoplasma antibodies are detected when the patient presents IgA and/or IgM. Therefore, when the IgG,A,M-IIF assay is carried out after total serum IgG removal, IgA and IgM may be jointly detected when present.

Serum IgG removal. When IgM or IgA IIF is carried out, false-negative results due to the presence of high levels of specific IgG (10) and false-positive results (11, 29, 31) due to the presence of antinuclear antibodies and/or rheumatoid factor, which react with specific human IgG to produce immunocomplexes which bind to the antigen (23), may occur. Therefore, to detect acute-infection markers by IIF (either in an individual step or as a whole), it is necessary to eliminate total serum IgG (43). For this reason, all the sera used to detect acute-infection markers were previously absorbed at a 1:10 ratio with goat anti-human IgG sera (The Binding Site, Birmingham, London). The suspension was gently mixed several times and after 30 min at room temperature was centrifuged to eliminate precipitated immunocomplexes.

Control of serum IgG removal. (i) In order to confirm complete elimination of total IgG, radial immunodiffusion for low-level IgG (RID_{LL}-IgG), which detects up to 12 mg of total human IgG (Quantiplate; Kallestad, Chasca, Minn.) per dl (1 IU/ml), was performed; (ii) in order to confirm complete elimination of specific IgG, IgG-IIF, employing fluorescein isothiocyanate (FITC)-labeled goat anti- γ -chain serum (Pasteur, Marnes-la-Coquette, France), was performed.

Antigen for IIF. The antigen for IIF testing (BioMérieux, Marcy-l'Étoile, France) was a lyophilized suspension of formalin-treated toxoplasma organisms obtained from mouse ascitic fluid. It was diluted in phosphate-buffered saline (PBS) (pH 7.2), and 10 μ l was applied in each slide circle to obtain 40 microorganisms in each field with a 40 $\times$ objective. Slides were allowed to sediment for 15 min, dried in an incubator at 37°C, and stored at –70°C until used.

EIA. EIA was performed by double-sandwich IgM EIA (DS-EIA-IgM) (Abbott, North Chicago, Ill.) and reverse IgA EIA (R-EIA-IgA) (Sorin-Biomédica, Vercelli, Italy).

IIF. Semiquantitative IgG,A,M-IIF assays were performed with unabsorbed sera. The conjugate used was FITC-labeled goat anti-total human immunoglobulin serum (Pasteur). This technique was used (i) to determine the percentage of the population that was infected and (ii) to select patients presenting toxoplasmosis titers of $\geq 1:1,024$ in order to investigate the presence of acute-infection markers. The starting serum dilution was 1:16, and successive 1:4 dilutions were made. Qualitative IgG,A,M-IIF assays with absorbed sera (IgA and IgM) were performed with serum samples presenting titers of $\geq 1:1,024$. The conditions of this reaction were identical to those employed with unabsorbed sera. The same anti-total human immunoglobulin conjugate was used, but sera were previously absorbed at a 1:10 ratio with anti-human IgG. When positive results were obtained, successive 1:4 dilutions with PBS were made.

The presence of IgA and IgM antibodies was investigated by IIF. In both cases, the conjugate used was FITC-labeled goat anti- α - and anti- μ -chain serum (Kallestad, respectively, starting with a 1:10 dilution in human IgG absorbent, con-

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TABLE 1. Comparison of results^a by different techniques for four patients with only IgA antibodies by monospecific IIF

Unabsorbed sera			Absorbed sera ^b			No. of patients
IgG,A,M-IIF	DS-EIA-IgM	R-EIA-IgA	IgM-IIF	IgA-IIF	IgG,A,M-IIF ^c	
+ ^d	—	+	—	+ ^e	+ ^e	2
+ ^f	+	+	—	+ ^g	+ ^h	2

^a +, positive; —, negative.^b Without IgG; 1/10 absorbed sera with anti-human IgG.^c IgA and IgM were detected simultaneously by IIF.^d Titer, 1:4,096.^e Titer, ≥1:40.^f Titer, 1:8,192.^g Titer, ≥1:640.^h Titer, ≥1:1,280.

sidered the minimum significant titer, and successive 1:4 dilutions with PBS were made.

Patients. The presence of anti-*T. gondii* antibodies was investigated in a population of 1,343 patients at the Hospital de Clínicas José de San Martín (University of Buenos Aires) from August 1993 to July 1994; 1,211 of these patients were pregnant women at different gestational stages (usually asymptomatic patients), and 132 were men who presented with lymphadenopathy.

Human sera. A total of 114 samples with IgG,A,M-IIF titers of ≥1:1,024 were selected; 15 were from men, and 99 were from pregnant women.

Controls. Sera obtained from 10 healthy individuals were pooled to serve as a negative control. A commercial IgG control (Toxotrol; BioMérieux) was used at 250 IU/ml as a positive control in IgG,A,M-IIF assays with absorbed and unabsorbed sera. A serum sample obtained from one acutely infected individual with titers of 1:4,096 by IgG,A,M-IIF, 1:1,280 by IgM-IIF, and 1:640 by IgA-IIF and with positive results by DS-EIA-IgM and R-EIA-IgA was selected as a positive control for IgA-IIF and IgM-IIF assays.

RESULTS

Detection of toxoplasmic infection. Toxoplasmic antibodies were detected in 958 of 1,343 (71.3%) samples by IgG,A,M-IIF assay; 114 of 958 (11.9%) samples had titers of ≥1:1,024, and 844 of 958 (88.1%) samples had titers of ≤1/512. Follow-up of the latter failed to show any significant titer increases (with one exception). In 385 of 1,343 (28.7%) samples from noninfected patients, seroconversion was not detected within 6 months.

Joint detection of acute-infection markers (IgA and IgM). In 114 patients with IgG,A,M-IIF titers of ≥1:1,024, a joint search for acute-infection markers (IgG,A,M-IIF with absorbed sera) was carried out. The results were compared with those obtained by the tests routinely used for IgA and IgM detection (IIF and EIA).

IgG,A,M-IIF with absorbed sera versus IgA- and IgM-IIF and EIA. Of 114 patients with IgG,A,M-IIF titers of ≥1:1,024, 90 (78 women and 12 men) were classified as chronically infected individuals because they presented neither IgA nor IgM antibodies, as measured by both IIF and EIA. The IgG,A,M-IIF assay with absorbed sera was also negative, showing a 100% correlation with the previously obtained results. In the remaining 24 patients (21 women and 3 men), one or both acute-infection markers were detected by IIF. Four patients had only IgA antibodies (Table 1), 17 patients had only IgM antibodies (Table 2), and 3 patients presented both antibody classes (Table 3). When EIAs were carried out, 2 patients had only IgA antibodies (Table 1), 15 patients had only IgM antibodies (Table 2), and 7 patients had both classes (Tables 1 through 3). In every case, the IgG,A,M-IIF assay with absorbed sera was positive; thus, the joint IIF assay allowed the detection of the 24 sera presenting IgA and/or IgM antibodies, as measured by conventional IIF and/or EIA.

Control of serum IgG removal. The 114 absorbed samples were studied by IgG-IIF and RID_{LL}-IgG, with negative results

TABLE 2. Comparison of results^a by different techniques for 17 patients with only IgM antibodies by monospecific IIF

Unabsorbed sera			Absorbed sera ^b			No. of patients
IgG,A,M-IIF	DS-EIA-IgM	R-EIA-IgA	IgM-IIF	IgA-IIF	IgG,A,M-IIF ^c	
+ ^d	+	—	+ ^e	—	+ ^f	4
+ ^g	+	—	+ ^e	—	+ ^h	5
+ ⁱ	+	—	+ ^f	—	+ ^h	3
+ ^j	+	—	+ ^k	—	+ ^l	1
+ ^j	+	—	+ ^l	—	+ ^m	1
+ ^j	+	—	+ ⁿ	—	+ ⁿ	1
+ ^j	+	+	+ ^o	—	+ ^g	2

^a +, positive; —, negative.^b Without IgG; 1/10 absorbed sera with anti-human IgG.^c IgA and IgM were detected simultaneously by IIF.^d Titer, 1:1,024.^e Titer, ≥1:10.^f Titer, ≥1:20.^g Titer, 1:2,048.^h Titer, ≥1:40.ⁱ Titer, 1:4,096.^j Titer, 1:8,192.^k Titer, 1:40.^l Titer, 1:80.^m Titer, ≥1:160.ⁿ Titer, 1:160.^o Titer, 1:640.

by both techniques in every case, confirming the complete removal of both specific IgG and total IgG from these sera. One must take into account that there are exceptional cases of very high levels of total IgG where absorption is not totally effective. In fact, we recently evaluated a patient who presented monoclonal gammopathy with high total IgG levels, showing the presence of specific IgA and IgM antibodies by IgG,A,M-IIF assay with absorbed serum as well as that of total IgG by RID_{LL}-IgG with previously absorbed serum. Both DS-EIA-IgM and R-EIA-IgA rendered negative results.

DISCUSSION

During the course of toxoplasmosis, the kinetics of IgA antibodies are similar to those displayed by IgM antibodies (6, 36, 49); therefore, joint detection of IgA and IgM antibodies may be useful regardless of whether it may be discriminated at the expense of which antibody the reaction proved positive. The presence of either of them is sufficient to warn the attending physician to ascertain whether the studied patient is in the

TABLE 3. Comparison of results^a by different techniques for three patients with IgM and IgA antibodies

Unabsorbed sera			Absorbed sera ^b			No. of patients
IgG,A,M-IIF	DS-EIA-IgM	R-EIA-IgA	IgM-IIF	IgA-IIF	IgG,A,M-IIF ^c	
+ ^d	+	+	+ ^e	+ ^f	+ ^e	2
+ ^g	+	+	+ ^h	+ ⁱ	+ ^h	1

^a +, positive.^b Without IgG; 1/10 absorbed sera with anti-human IgG.^c IgA and IgM were detected simultaneously by IIF.^d Titer, 1:4,096.^e Titer, 1:2,560.^f Titer, 1:20.^g Titer, 1:8,192.^h Titer, 1:1,024.ⁱ Titer, 1:80.

FIG. 1. Algorithm for the detection of toxoplasma infection in pregnant women. IgA and IgM were detected simultaneously by IgG,A,M-IIF with absorbed sera; +, positive; -, negative.

acute or chronic stage of infection. It is widely documented that to increase the specificity and sensitivity of IgA and IgM detection, it is essential to perform preabsorption of serum IgG, but such treatment has not yet been considered to detect both immunoglobulins jointly.

The IgG,A,M-IIF assay with absorbed sera was validated against routine techniques, and the results allow the following conclusions to be drawn. (i) It may be used to detect IgA and IgM antibodies together, providing data similar to those of different tests (IIF and EIA) for detecting these immunoglobulins (IgA and IgM) individually. (ii) In patients with acute-infection markers, as determined by conventional IIF, it must be considered that not only can the presence of specific IgG mask the detection of specific IgM and/or IgA but also that high concentrations of specific IgA can inhibit the detection of small amounts of specific IgM and vice versa. By capture techniques (lacking interferences produced by high concentrations of other immunoglobulins, such as DS-IgM-EIA and R-EIA-IgA) (12, 14, 15, 44, 46), some sera produced negative IgM-IIF results and positive IgM-EIA results and others produced negative IgA-IIF results and positive IgA-EIA results, specially at high titers of the other acute-infection marker; joint detection of IgA and IgM by IgG,A,M-IIF assay with absorbed sera avoids this problem because at least one of these antibodies is

invariably detected. (iii) In an initial step, simultaneous IgG,A,M-IIF assays with absorbed and unabsorbed sera may be usefully implemented (Fig. 1). This follow-up is also valid when instead of the IgG,A,M-IIF assay, the detection of specific IgG is carried out by different techniques, including IgG-IIF, IgG-EIA, and direct agglutination with 2-mercaptoethanol (9). When a sample tested by IgG,A,M-IIF is positive with unabsorbed sera but negative with absorbed sera, such results reflect the absence of specific IgA and IgM, indicating chronic infection (in unusual cases, due to early infection, the specific antibodies have not yet been produced [49]). When a positive IgG,A,M-IIF assay is obtained with absorbed sera, serum IgG absorption must be checked by RID_{LL}-IgG before performing confirmatory tests or evaluating results in serial samples obtained at least 3 weeks apart and run in parallel.

In an initial stage, the proposed algorithm constitutes a tool for providing useful information on specific humoral immune responses by a test involving only one technique (IIF), one conjugate (FITC-anti-IgG,A,M), and only one sample (with and without previous absorption). This type of approach could be used in the detection of not only other parasitoses but also bacterial and viral infections. The same principle could be applied to EIAs which detect total immunoglobulins, widening

the possibilities of implementing this follow-up to large quantities of samples.

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