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Differential Agglutination Test for Diagnosis of Recently Acquired Infection with *Toxoplasma gondii*

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We evaluated the recently described differential agglutination test (HS/AC test) to differentiate recently acquired toxoplasma infections from those acquired in the more distant past in sera obtained from 38 patients with carefully defined symptomatic and asymptomatic infections. AC antigens detect acute-phase-specific immunoglobulin G (IgG) antibodies against *Toxoplasma gondii* tachyzoites that are formed only during the acute stage of infection in humans. The HS/AC test correctly identified recently acquired infections in patients with toxoplasmic lymphadenopathy or asymptomatic infections (including infections in 7 women who seroconverted during gestation) in 31 of 33 patients. We also studied 15 individuals who had been infected for at least 2 years. In that group, only 13% had an acute pattern in the HS/AC test. However, the wide range in times from infection (from 2 to 14 years) did not allow for an estimate of when the pattern in the HS/AC test changed from acute to not acute. These results reveal that in the appropriate clinical situation, when both IgG and IgM tests are positive and a question still remains about the acuteness of infection, the HS/AC test may be useful for differentiating between toxoplasma infections acquired recently and those acquired in the more distant past.

The introduction of prenatal diagnosis for detection of toxoplasma infection in the fetus has served to emphasize and focus on the importance of making an early diagnosis of recently acquired toxoplasma infections in pregnant women (2, 5). This has become even more compelling because of recent data which suggest that treatment of the infection in the fetus can be accomplished with gratifying results (11, 15). It should also be recognized that abortions cannot be performed after the second trimester and that early prenatal diagnosis of an infection in the fetus is necessary for appropriate decisions to be made regarding abortion versus treatment (2, 15).

Physicians in France, where the initial studies with prenatal diagnosis were performed, routinely and by law screen sera from pregnant women. Women without serologic evidence of prior toxoplasma infections are screened during gestation to detect seroconversion. For women with antibodies to toxoplasma on initial blood testing, additional blood is drawn 3 to 4 weeks later to determine whether the infection was recently acquired or chronic. Although the incidence of seroconversion during pregnancy and of congenital toxoplasma infection has been estimated to be essentially the same in the United States as it is in France (15), screening of pregnant women in the United States for toxoplasma antibodies or for seroconversion is rarely practiced (R. McCabe and J. S. Remington, Editorial, N. Engl. J. Med. **318**:313–315, 1988). When a serum sample for toxoplasma serology is obtained, most often it is drawn relatively late in gestation. If antibodies are detected, it is frequently difficult to define whether the infection was recent or old. If the serum sample is negative for toxoplasma antibodies, additional testing for seroconversion should be determined at later dates, yet the necessary follow-up serum samples are rarely obtained (13).

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Thus, serologic methods which are simple and inexpensive to perform and which can differentiate between recently acquired and chronic toxoplasma infections in a single serum sample are needed. Investigators in our laboratories have previously described a method which appears to be promising for this purpose (18). The method is a differential agglutination test which compares the titers obtained with Formalin-fixed tachyzoites (HS antigen) with those obtained with acetone- or methanol-fixed tachyzoites (AC antigen). The AC antigen preparation contains stage-specific antigens which are recognized by immunoglobulin G (IgG) antibodies formed against toxoplasma tachyzoites early in infection (17). These antibodies have specificities different from those formed later in infection (17). We set out to determine whether the pattern (acute or not acute) of the HS/AC test alone would be helpful in the diagnosis of recently acquired toxoplasma infections in pregnant women in the United States. To mimic the situation as it exists in the United States, where sequential serum samples are rarely available, we focused on results obtained for a single sample obtained from patients with antibodies to toxoplasma.

MATERIALS AND METHODS

Patients. Fifty-seven serum samples from 38 patients were studied. Sera from pregnant and nonpregnant patients with recently acquired toxoplasma infections or with toxoplasma infections acquired in the more distant past were obtained from our serum banks, where they were stored at −70°C. Patients were selected on the basis of clinical history, known seroconversion during a systematic screening performed in pregnant women, availability of follow-up sera, and absence of any evidence of an immunologic deficit. The time interval between the dates on which the sera were obtained was rounded to the nearest number of months. Except for cases of seroconversion, serologic test results alone were not used as a selection criterion. Ten patients without a history of

previous infection with toxoplasma and who were seronegative for toxoplasma antibodies served as seronegative controls.

Patient groups. (i) Patients with recently acquired infections. Recently acquired infection was defined by the following clinical and laboratory findings: (i) biopsy-proven toxoplasmic lymphadenopathy (7), (ii) lymphadenopathy with Sabin-Feldman dye and double-sandwich (DS) IgM enzyme-linked immunosorbent assay (ELISA) results suggestive of recently acquired toxoplasma infection (1), (iii) pregnant women who gave birth to a congenitally infected infant (15), and (iv) seroconversion during gestation (McCabe and Remington, Editorial).

For the purposes of this study, sera were dated from the reported onset of clinical signs (e.g., lymphadenopathy) or from seroconversion in the dye test. In cases of asymptomatic infections that resulted in the birth of a congenitally infected infant, we arbitrarily dated maternal serum from the midpoint of gestation (1). Although this approach may introduce small errors (1 to 4 months), it eliminates potential investigator bias when dating is based on the clinical severity of congenital infection.

(ii) Pregnant patients with recently acquired infections. There were 19 pregnant women who acquired infections during pregnancy. Four patients (patients 3, 10, 13, and 15) had lymphadenopathy and characteristic histologic findings at the time of lymph node biopsy. Their sera were obtained an average of 4 months (range, 1 to 6 months) after the onset of lymphadenopathy. Three additional patients (patients 4, 11, and 12) with lymphadenopathy, but who did not undergo lymph node biopsy, were also studied. Serologic test results on serum obtained an average of 3 months (range, 1 to 4 months) after the onset of lymphadenopathy were suggestive of recently acquired toxoplasmosis in these three patients. Two of the three patients (patients 4 and 12) gave birth to congenitally infected infants; the third patient underwent a therapeutic abortion, and attempts to isolate toxoplasma from fetal tissue were not done. Five women (patients 14, 16, 17, 18, and 19) who gave birth to congenitally infected infants, but who did not have a clinical illness suggestive of toxoplasmosis during their pregnancies, were also studied. Their sera were obtained an average of 6 months (range, 5 to 7 months) after midgestation.

Seven pregnant women (patients 1, 2, 5, 6, 7, 8, and 9) who were monitored in a systematic screening program in Paris, France, and who seroconverted in the dye test were also studied (8). Their sera were obtained an average of 1 month (range, 0 to 1 month) after seroconversion. Because antitoxoplasma therapy may affect serologic test results, except where noted (see Table 2), none of the patients received therapy.

(iii) Nonpregnant patients with recently acquired infections. There were 14 individuals (patients 20 to 33; 4 males; 10 females) with characteristic histologic findings of toxoplasmic lymphadenopathy at the time of lymph node biopsy. Their sera were obtained an average of 2 months (range, 1 to 7 months) after the onset of lymphadenopathy.

(iv) Patients with infection of more than 2 years in duration. There were three groups of patients with infection of more than 2 years in duration: (i) nine patients from whom follow-up sera were obtained at least 2 years after biopsy-proven toxoplasmic lymphadenopathy; (ii) two patients who experienced lymphadenopathy and seroconverted at least 2 years before serum sampling; and (iii) four patients with more than 2 years of follow-up sera, stable dye test titers, and stable or decreasing IgM antibody test results.

Sera from the first two groups were available both early following the onset of clinical disease and later. Early-onset sera were not available from the third group. Each of the individuals in the third group was known to have been seropositive for greater than 10 years. Serum samples were dated from the onset of clinical signs of the initial infection or from the date of the first available sample.

Fifteen individuals (six males; nine females) were studied. Nine patients (patients 10, 15, 20, 22, 25, 26, 27, 29, and 31) had biopsy-proven toxoplasmic lymphadenitis and were studied an average of 43 months (range, 24 to 63 months) after the reported onset of lymphadenopathy. One patient (patient 11) had lymphadenopathy during her initial pregnancy and seroconverted. Three patients (patients 36, 37, and 38) had no history of toxoplasma infection but had at least 10 years (range, 10 to 14 years) of serologic follow-up. One patient (patient 34) experienced lymphadenopathy and had seroconverted and was studied 56 months after seroconversion. The last patient (patient 35) was a congenitally infected child who received antitoxoplasma drug therapy during the first year of life. He was studied at the age of 7 years.

(v) Patients studied more than once following recently acquired infections. Follow-up serum samples, which were obtained 1 to 57 months after the first sample, were available from 13 patients. These follow-up serum samples can be identified in Tables 3 and 4 by the numbers assigned to each patient (for example, patient 15 was studied 6 [Table 2], 11 [Table 3], 20 [Table 3], and 63 [Table 4] months after toxoplasmic lymphadenopathy).

Serologic tests. A micromodification of the Sabin-Feldman dye test was performed with fourfold dilutions of sera (8). The IgM ELISA was performed by the single-dilution method, as described previously (14, 16). Values of ≥ 2.0 were considered to represent significant amounts of IgM toxoplasma antibody. The IgM immunosorbent agglutination assay was performed as described previously (4). The HS and AC tests were run with Formalin-fixed (HS test) and acetone-fixed (AC test) tachyzoites (3×10^7 organisms per ml) suspended in alkaline buffer (pH 8.7) containing 1% bovine serum albumin as described previously (18). A total of 50 μl of the suspension was added to 50 μl of twofold dilutions of sera in microdilution polystyrene plates (Dynatech Laboratories, Inc., Chantilly, Va.). Sera were diluted in phosphate-buffered saline containing 0.2 M 2-mercaptoethanol. The first dilution was 1:2,000 which, if positive, was a titer of 100 IU/ml. The plates were read after overnight incubation at room temperature, as described previously (6). Acetone-fixed (AC) tachyzoites were suspended in the same alkaline buffer and used in the same numbers as the Formalin-fixed organisms were. Sera were diluted in the alkaline buffer containing 2-mercaptoethanol. The first dilution was 1:100 which, if positive, was a titer of 50 μl. Each serum sample was first evaluated in the conventional agglutination test by using Formalin-fixed parasites, beginning with a dilution of 1:36 (6). Sera that were negative in the conventional agglutination test were not evaluable in the HS/AC test. Thus, an HS test result of <100 IU/ml was positive with serum dilutions from 1:36 to <1:2,000.

HS antigen was obtained from *Toxoplasma gondii* RH as described previously (6). The procedure described above was performed to prepare the AC antigen, but instead of Formalin, acetone was used as the fixative. Briefly, after trypsin treatment (6), tachyzoites were washed by centrifugation and suspended in phosphate-buffered saline (pH 7.2), containing 1% bovine serum albumin. Under continuous

TABLE 1. Criteria used for interpretation of HS/AC test results[a]

HS test result (IU/ml)	Interpretation with the following AC test result (IU/ml)[b]:							
	<50	50	100	200	400	800	1,600	>1,600
<100	NA[c]	NA[c]	A	A	A[d]	A[d]	A[d]	A[d]
100	NA	NA	A	A	A	A	A[d]	A[d]
200	NA	NA	A	A	A	A	A	A[d]
400	NA	NA	A	A	A	A	A	A
800	NA	NA	NA	A	A	A	A	A
1,600	NA	NA	NA	NA	A	A	A	A
3,200	NA	NA	NA	NA	NA	A	A	A
>3,200	NA	NA	NA	NA	NA	NA	A	A

[a] The HS/AC test was performed as described in the text.
[b] A, Acute pattern; NA, not acute pattern.
[c] HS titer of >0 but <100; this pattern may be seen in the earliest stages of infection. Follow-up sera are necessary to clarify whether the infection is acute.
[d] Results were not observed in routine use of the test.

agitation, acetone was immediately added to provide a final concentration of 25%. Agitation was maintained for 10 min. Thereafter, the solution was kept at 4°C for 36 h. The parasites were then washed twice in phosphate-buffered saline containing 1% bovine serum albumin, at 4,000 × *g*, and suspended in the same buffer. AC antigen was stored at 4°C until use.

An HS titer of <100/AC titer of <50 and an HS titer of <100/AC titer of 50 were seen in the earliest stages of infection (i.e., near seroconversion); follow-up sera were necessary to clarify whether the infection was recently acquired. Test results were reproducible on different days when the same samples were used (data not shown), and technician bias was reduced by use of sample codes. As described previously (17, 18), high titers in the AC test are associated with recently acquired infections, and high titers in the HS test are associated with chronic infections (6). Results are expressed in international units, and the criteria used for interpretation are shown in Table 1. These criteria were chosen to reflect how we interpret test results in our laboratories (18; J. S. Remington and P. Thulliez, unpublished data). We emphasize that the terms acute and not acute refer solely to the interpretation of the pattern of the HS/AC test and not to whether the patient actually had a recently acquired infection. The tests were run and read without prior knowledge of the clinical history of the patient.

In preliminary studies, we performed the HS/AC test on serum samples from 10 patients who had no prior clinical or serologic evidence of toxoplasma infection. These sera had no demonstrable antibodies in the dye, IgM ELISA, or conventional agglutination tests; and all sera were negative in the HS/AC test (data not shown).

Data analysis. For purposes of data analysis, HS results of <100 and AC results of <50 were recorded as 0; HS results of >3,200 and AC results of >1,600 were recorded as 3,200 and 1,600, respectively. In a similar fashion, a duration of infection of >10 years was recorded as 10 years. Geometric means were calculated by using the reciprocal of the titers obtained by the dye, HS, and AC tests.

RESULTS

Patients with recently acquired infections. The clinical characteristics, time of serum sampling, serologic test results, and our interpretation of the HS/AC test results in pregnant patients (patients 1 to 19) with recently acquired infections are given in Table 2. The HS/AC test result was considered acute in each of the 19 serum samples. Results for nonpregnant patients with recently acquired infections (patients 20 to 33) are also shown in Table 2. Interpretation of the HS/AC test was acute for 12 of these serum samples and not acute for 2 serum samples. Noteworthy is the fact that an HS titer of <100/AC titer of 50 may occur in the earliest stages of infection; this occurs when the conventional agglutination titer is low (P. Thulliez, unpublished data). An example of this was seen in patient 20 (Tables 2 and 3), in whom the AC and HS titers rose.

Duration of the acute pattern in the HS/AC test. To determine the duration of the acute pattern in the HS/AC test, we studied follow-up sera from 11 patients (patients 3, 4, 11, 15, 20, 22, 25, 26, 30, 32, and 33), from whom initial serum samples were obtained from 1 to 7 months (mean, 3 months) after the onset of lymphadenopathy. The second serum sample was obtained 2 to 12 months (mean, 4 months) after the onset of lymphadenopathy. The HS/AC pattern was acute in each of these follow-up serum samples (Table 3).

Serum samples obtained 14 to 21 months (mean, 17 months) after the onset of toxoplasmic lymphadenopathy were available from eight patients (patients 15, 20, 21, 22, 26, 30, 32, and 33), and serologic test results are shown in Table 3. The HS/AC test pattern was acute in sera from five (63%) of these eight patients. The durations of infection at the time that these sera were obtained were 15 months in two patients and 16, 20, and 21 months in the remaining three patients.

We also studied 15 patients who were infected for more than 2 years (Table 4). Earlier results for 7 (patients 10, 11, 15, 20, 22, 25, and 26) of the 15 patients are given in Table 2. The HS/AC pattern was not acute in 13 (87%) of these 15 patients.

Sensitivity and specificity of the HS/AC test. The sensitivity and specificity of the HS/AC test were calculated by using the data given in Tables 2 and 4. The sensitivity of the HS/AC test for the identification of recently acquired infections in pregnant women (Table 2) was 100%. The sensitivity of the HS/AC test for the identification of recently acquired toxoplasma infections in nonpregnant patients was 86% (12 of 14 patients). When these results were combined, the overall sensitivity of the HS/AC test for the identification of recently acquired infections was 94% (31 to 33 patients). The specificity of the HS/AC test was calculated by using data for all of the patients who were known to have been infected for 2 or more years (Table 4). After a mean of 64 months (range, 24 months to >12 years), the specificity of the HS/AC test was 87% (13 of 15 patients).

TABLE 2. Clinical characteristics and toxoplasma serology in patients with recently acquired infections

Patient	Mo[a]	Sex[b]	Clinical signs[c]	Serologic test result[d]				HS/AC interpretation
				Dye test	IgM	HS	AC	
1	0	F	None[e]	2,048	12[f]	200	200	Acute
2	0	F	None[e]	2,048	12[f]	100	200	Acute
3	1	F	BX LN	2,048	4.9	400	800	Acute
4	1	F	LN[g,h]	4,096	7.3	200	200	Acute
5	1	F	None[e]	2,048	12[f]	<100	100	Acute
6	1	F	None[e]	4,096	12[f]	200	400	Acute
7	1	F	None[e]	4,096	12[f]	200	100	Acute
8	1	F	None[e]	4,096	12[f]	100	200	Acute
9	1	F	None[e]	4,096	12[f]	800	800	Acute
10	2	F	BX LN	32,000	4.3	100	100	Acute
11	3	F	LN[g]	2,048	3.2	400	800	Acute
12	4	F	LN[h]	8,000	2.2	1,600	>1,600	Acute
13	5	F	BX LN	4,096	4.1	400	200	Acute
14	5	F	None[h]	4,096	3.8	1,600	800	Acute
15	6	F	BX LN	32,000	1.7	200	100	Acute
16	6	F	None[h]	512	3.4	200	100	Acute
17	6	F	None[h]	8,000	2.7	800	400	Acute
18	6	F	None[h]	8,000	10.7	1,600	>1,600	Acute
19	7	F	None[h]	4,096	0.8	1,600	400	Acute
20	1	M	BX LN	1,024	5.7	<100	50	Not acute
21	1	F	BX LN	2,048	7.3	1,600	>1,600	Acute
22	1	M	BX LN	4,096	6.4	400	800	Acute
23	1	F	BX LN	4,096	8.9	400	200	Acute
24	1	F	BX LN	8,000	10.1	>3,200	>1,600	Acute
25	2	F	BX LN	2,048	10.4	400	800	Acute
26	2	F	BX LN	8,000	3.6	400	400	Acute
27	2	F	BX LN	16,000	4.3	800	800	Acute
28	2	F	BX LN	32,000	9.8	>3,200	>1,600	Acute
29	3	F	BX LN	8,000	2.2	<100	50	Not acute
30	3	F	BX LN	8,000	8.2	400	400	Acute
31	3	M	BX LN	8,000	8.8	400	>1,600	Acute
32	3	F	BX LN	32,000	7.1	1,600	>1,600	Acute
33	7	M	BX LN	1,024	8.4	800	800	Acute

[a] Months after the onset of lymphadenopathy, seroconversion, or midpoint of gestation.
[b] F, Female; M, male; female patients 1 to 20 were pregnant.
[c] BX LN, Biopsy-proven lymphadenopathy; LN, lymphadenopathy
[d] In pregnant patients, the arithmetic mean titer was 4.1 by DS IgM ELISA. The geometric mean titers for the other tests were as follows: dye test, 5,100; HS test, 500 IU; AC test, 476 IU. In nonpregnant patients, the mean titer was 7.2 by DS IgM ELISA. The geometric mean titers for the other tests were as follows: dye test, 5,700; HS test, 600 IU; AC test, 570 IU. Values for the dye, HS, and AC tests are reciprocals of the highest positive serum dilution. For IgM results are reported as 0 to 12, as determined by DS IgM ELISA.
[e] Seroconversion in a systematic screening program.
[f] Results are reported as 0 to 12, as determined by IgM immunosorbent agglutination assay.
[g] Patient received spiramycin during pregnancy.
[h] Pregnancy produced a congenitally infected infant.

DISCUSSION

Because toxoplasma infections in immunocompetent individuals are most often asymptomatic, determination of whether the infection occurred recently or was acquired in the more distant past is almost always based on serologic test results (15). Unfortunately, interpretation of such results is frequently complicated by the prevalence of latent toxoplasma infection and, thus, of toxoplasma antibodies. Accurate interpretation of serologic test results is particularly important in pregnant women with recently acquired toxoplasma infections because several diagnostic and therapeutic options are available. Fetal blood sampling can be used to determine whether the fetus is infected (2, 5), and treatment of pregnant women during gestation both to reduce the chance of giving birth to an infected infant and to decrease the severity of infection in the congenitally infected fetus can be considered (11, 15).

In the United States, where there is no mandate for systematic serologic screening of pregnant women, the ability to make a serologic diagnosis of recently acquired toxoplasma infection by using a single test of a single serum sample becomes critical. This fact, along with the limitations of currently available serologic tests for the diagnosis of recently acquired infections (1, 15), served as the impetus for us to study the ability of the HS/AC test to differentiate recently acquired infections from those acquired in the more distant past.

The limitations of some currently used serologic tests for the diagnosis of recently acquired infections are given in Tables 2 to 4. For example, among those patients with recently acquired toxoplasma infections, in whom the IgM ELISA was performed, titers were <4.0 in 35% of the cases. Furthermore, 14% of patients known to have been infected 8 to 24 months earlier had IgM ELISA titers of >3.0. Thus, although a titer of 2.0 was positive in our IgM ELISA, we consider a titer of >4 to be suggestive of a more recently acquired infection (1; J. S. Remington, unpublished data). Although titers of >1:1,000 in the dye test may suggest a

TABLE 3. Toxoplasma serology 2 to 12 and 14 to 21 months after toxoplasmic lymphadenopathy

Patient	Serologic test results[a]											
	Second sample (2–12 mo)						Third sample (14–21 mo)					
	Mo[b]	Dye test	DS IgM ELISA	HS	AC	HS/AC interpretation	Mo[b]	Dye test	DS IgM ELISA	HS	AC	HS/AC interpretation
3	12	256	1.4	800	800	Acute						
4	10	8,000	3.1	1,600	800	Acute						
11	11	512	1.5	100	400	Acute						
15	11[c]	16,000	0.9	200	400	Acute	20	4,096	0.7	200	100	Acute
20	4	4,096	2.2	200	800	Acute	14	4,096	0.7	800	50	Not acute
21	—[d]						15	128	1.8	200	100	Acute
22	2	4,096	6	400	400	Acute	21	1,024	0.4	<100	<50	Not acute
25	4	1,024	5.5	200	100	Acute						
26	5	8,000	1.2	400	200	Acute	17	8,000	1.7	1,600	<50	Not acute
30	4	8,000	8.6	1,600	>1,600	Acute	21	2,048	3.9	100	200	Acute
32	9	2,048	5.1	800	200	Acute	15	1,024	3.5	200	200	Acute
33	8	4,096	5.6	800	800	Acute	16	2,048	1.6	800	400	Acute

[a] For the second serum samples, the arithmetic mean titer was 3.8 by DS IgM ELISA. The geometric mean titers for the other tests were as follows: dye test, 3,000; HS test, 450 IU; AC test, 450 IU. For the third serum samples, the arithmetic mean titer was 1.8 by DS IgM ELISA. The geometric mean titers for the other tests were as follows: dye test, 1,100; HS test, 270 IU; AC test, 100 IU. Values for the dye, HS, and AC tests are reciprocals of the highest positive serum dilution. For IgM, results are reported as 0 to 12, as determined by DS IgM ELISA.
[b] Months after the onset of lymphadenopathy.
[c] The patient was pregnant.
[d] —, Sample was not available.

recently acquired infection, such titers are not uncommon in the general population of individuals seropositive for toxoplasma (15).

The results of the present study reveal that, in the appropriate clinical situation, the HS/AC test performed on a single serum sample is useful for differentiating between toxoplasma infections acquired recently or those acquired in the more distant past. In practice, results of other serologic tests on this same serum sample are considered when making a clinical decision. Thus, the HS/AC test is only used in those adults who have both IgG and IgM antibodies and in whom there is a question as to whether the infection was recently acquired. The HS/AC test correctly identified all the pregnant and almost all of the nonpregnant patients with recently acquired infections. Pertinent to the role of the HS/AC test for diagnosis by using a single serum sample was the finding that in the seven women who seroconverted during gestation, each had an acute pattern in the HS/AC test within 0 to 8 weeks after seroconversion was noted in the dye test.

Although the pattern in the HS/AC test was still acute in most patients who were evaluated 14 to 21 months following the clinical onset of toxoplasmic lymphadenopathy, the exact duration of the acute pattern in these patients could

TABLE 4. Clinical characteristics and toxoplasma serology in patients with infection of more than 2 years in duration

Patient	Mo[a]	Sex[b]	Initial clinical signs[c]	Serologic test results[d]				
				Dye test	DS IgM ELISA	HS	AC	HS/AC interpretation
10	24	F	BX LN	256	1	<100	50	Not acute
11	56	F	LN	64	0.5	<100	100	Acute
15	63	F	BX LN	4,096	0.4	<100	50	Not acute
20	51	M	BX LN	256	0	200	50	Not acute
22	41	M	BX LN	512	0.1	<100	<50	Not acute
25	24	F	BX LN	32	2	100	<50	Not acute
26	51	F	BX LN	1,024	0.6	<100	<50	Not acute
27	28	F	BX LN	1,024	1.8	100	<50	Not acute
29	48	F	BX LN	128	0.8	100	<50	Not acute
31	29	M	BX LN	256	3.6	100	100	Acute
34	56	M	LN	64	0.1	<100	<50	Not acute
35	84	M	Congenital infection[e]	256	0.3	<100	50	Not acute
36	>168	F	Employee screen	128	−0.2	200	<50	Not acute
37	>120	F	Retinal scars	64	−0.2	200	50	Not acute
38	>120	M	Employee screen	32	0.1	200	<50	Not acute

[a] Months after clinical symptoms.
[b] F, Female; M, male; patients 10, 11, and 15 were pregnant.
[c] BX LN, Biopsy-proven lymphadenopathy; LN, lymphadenopathy.
[d] In pregnant patients, the arithmetic mean titer was 0.6 by DS IgM ELISA. The geometric mean titers for the other tests were as follows: dye test, 400; HS test, 100 IU; AC test, 63 IU. In nonpregnant patients, the mean titer was 0.8 by DS IgM ELISA. The geometric mean titers for the other tests were as follows: dye test, 170; HS test, 120 IU; AC test, 53 IU. Values for the dye, HS, and AC tests are reciprocals of the highest positive serum dilution. For IgM results are reported as 0 to 12, as determined by DS IgM ELISA.
[e] The patient received antitoxoplasma drug therapy during the first year of life.

not be determined because of the lack of additional follow-up serum samples. It is noteworthy that the AC titers were <200 IU in all of the patients known to be infected for 14 months or longer (except for patient 33, who had a titer of 400 IU). In contrast, only 8 of 26 patients with more recently acquired infection had titers of <200 IU. In the present study, we also included individuals whose infections were acquired more than 24 months earlier. In this group, only 13% had an acute pattern in the HS/AC test. However, the wide range in times (from 2 to 14 years) (Table 4) did not allow for an estimate of when the pattern in the HS/AC test changed from acute to not acute.

Recently, we performed a series of studies to define the antigen(s) of *T. gondii* which is detected by IgG antibodies that are present only during the acute stage of the infection (17). Sera of mice immunized with AC antigens and sera from individuals with recently acquired, but not chronic, infections recognized predominantly 10 tachyzoite antigens by immunoblot analysis. AC antibodies reacted with the cell membranes of tachyzoites, but not those of bradyzoites, whereas HS antibodies reacted with cell membranes of both forms of the organism in an indirect fluorescent-antibody test. These results suggest that in the acute stage of infection in humans, the IgG antibody response is directed against antigens which are different from those which stimulate the IgG antibody response during the chronic stage of the infection and that AC antigens can detect these IgG acute-phase-specific antibodies. In an earlier study by Thulliez et al. (18), two monoclonal antibodies developed by Handman and Remington (9), 2G11 and 1E11, which react against toxoplasma cell surface antigens with approximate molecular weights of 22,000 and 30,000, respectively, were used to study the HS and AC antigen preparations. Their results suggest that the IgG antibodies present during the acute phase of infection in humans are directed against a surface antigen that is recognized by monoclonal antibody 1E11. In separate studies, Derouin et al. (3) and Huskinson et al. (12) have reported that IgG1 antibodies are the predominant IgG antibody subclass present in sera from patients with both recently acquired and chronic toxoplasma infections (3, 12).

Hedman et al. (10) have recently developed a new method for the diagnosis of recently acquired infections that is also based on acute-phase IgG antibodies. Their assay is based on their observation of the low avidity of certain IgG toxoplasma antibodies that are present soon after the acute phase of infection. In their test, toxoplasma IgG antibodies are measured as a function of hydrogen bond dissociation. The finding that acute-phase IgG was eluted off the antigen much more easily than was IgG of prior immunity permitted the development of this promising new diagnostic test.

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