Role of Specific Immunoglobulin E in Diagnosis of Acute Toxoplasma Infection and Toxoplasmosis

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Toxoplasma immunoglobulin E (IgE) antibodies were evaluated in an immunosorbent agglutination assay (ISAGA) and an enzyme-linked immunosorbent assay (ELISA) to determine their usefulness in the diagnosis of acute infection with *Toxoplasma gondii*. IgE antibodies were not detected in serum specimens from otherwise seronegative individuals, individuals with chronic toxoplasma infection, or infants without congenital toxoplasmosis. In contrast, they were detected in pregnant women who seroconverted during gestation (100% by ELISA, 63% by ISAGA), patients with toxoplasmic lymphadenopathy (96% by ELISA, 88% by ISAGA), infants with signs of congenital toxoplasmosis which prompted serologic testing in the postnatal period (92% by ELISA, 67% by ISAGA), children and adults with toxoplasmic chorioretinitis (36% by ELISA, 18% by ISAGA), and adult patients with AIDS and toxoplasmic encephalitis (33% by ELISA, 25% by ISAGA). In many of the serum specimens, the titer of IgE antibodies detected by the ISAGA were close to or at the positive cutoff value. The duration of detectable IgE antibodies in patients with acute infections varied considerably among individuals but showed a trend toward a briefer duration by the ISAGA than by the ELISA. These results reveal that recrudescence of IgE antibodies in patients with reactivated chronic infection (toxoplasmic chorioretinitis and toxoplasmic encephalitis) may be useful diagnostically and that demonstration of toxoplasma IgE antibodies is a useful adjunct to currently available serologic tests for the diagnosis of acute toxoplasma infection and toxoplasmosis.

Although the definitive diagnosis of toxoplasma infection and toxoplasmosis is established by demonstration of the tachyzoite form of the parasite either histologically or by isolation procedures, improvements in serologic diagnosis now usually obviate the need for biopsy and isolation studies, except in some patients with congenital toxoplasma infection and when disease occurs in immunocompromised patients. We and others have previously reported on the use of specific immunoglobulin M (IgM) (6, 9), IgA (4, 11, 16), and low-avidity IgG (8) antibodies and the differential agglutination (AC/HS) test (3, 17, 18) for the serologic diagnosis of acute toxoplasma infection during pregnancy, toxoplasmic lymphadenopathy, congenital toxoplasma infection, and toxoplasmic encephalitis (TE). A frequent clinical problem associated with many of these serologic tests is the persistence of toxoplasma IgM (2, 5, 19) and IgA (19) antibodies for months or even more than 1 year after the acute infection. For this reason and because of the variation in individual serologic responses to toxoplasma infection and toxoplasmosis, there has been a continued search for and development of serologic techniques that are both sensitive and specific and that will allow clinicians to appropriately date the approximate time that the infection was acquired.

Pinon et al. (10, 12) and Poirriez et al. (13) reported the presence of specific IgE antibodies in patients with acute toxoplasma infection, congenital toxoplasmosis, or toxo-

plasmic chorioretinitis using enzyme-linked immunofiltration assays (10, 13) and immunosorbent agglutination (IgE) assays (ISAGAs) (12). Because of their promising results, we developed an IgE ISAGA and IgE enzyme-linked immunosorbent assay (IgE ELISA) and examined these methods for testing sera from patients with a variety of clinical manifestations associated with toxoplasma infection.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from 10 groups of patients as follows. Group A ($n = 42$ serum specimens) consisted of eight women who seroconverted during the first and second trimesters of pregnancy (14). The last negative serum sample prior to seroconversion is referred to as the preconversion sample. The serum sample in which seroconversion was documented is referred to as the seroconversion sample. The time interval between the preconversion and the seroconversion samples was rounded off to the nearest week and was less than 8 weeks for each of the patients. Group B consisted of 17 patients (average age, 34 years; range, 17 to 69 years) with toxoplasmic lymphadenopathy diagnosed histologically by lymph node biopsy and whose serum samples were obtained within 12 weeks of onset of clinical illness (7). Group C consisted of 11 patients of more than 2 years of age (average age, 29 years; range, 3 to 52 years) with symptomatic eye disease diagnosed by clinical history and ophthalmologic examination to have toxoplasmic chorioretinitis. Each of these patients either had

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a prior clinical episode of chorioretinitis or had evidence of new retinal lesions in an area of old retinal scarring. None of these patients was known to be seropositive for human immunodeficiency virus, and all sera from patients in this group were positive in the Sabin-Feldman dye test (dye test). Group D consisted of 12 adult patients with AIDS and biopsy-proven TE. The sera from these patients were positive in the dye test (range 20 to 600 IU/ml) and negative for IgM antibodies by ELISA. Group E consisted of 13 infants (average age, 2 weeks; range, 1 to 7 weeks) with congenital toxoplasmosis diagnosed by the presence of neurologic disease including hydrocephalus, chorioretinitis, and/or the presence of cerebral calcifications and whose serum specimens were obtained within 8 weeks of birth. The diagnosis was confirmed by isolation of the parasite from blood, placenta, and/or amniotic fluid and by positive serologic tests for toxoplasma IgM and/or IgA antibodies (14). Group F consisted of six clinically normal uninfected infants born to mothers who had serologic test results that suggested that they acquired acute *Toxoplasma gondii* infection during pregnancy. Serum samples from these infants were obtained within 8 weeks of birth (14). These infants were followed clinically and serologically (by the dye test, IgM ISAGA, and IgA ELISA) for at least 6 months, had no clinical evidence of congenital toxoplasmosis, and had falling dye test titers. Group G consisted of 10 random infants without clinical or serologic evidence of congenital toxoplasmosis whose serum specimens were sent to our laboratory for routine screening. Sera from six of these infants were positive in the dye test (range, 10 to 150 IU/ml). Sera from the other four infants were negative in the dye test. Each of these sera was also negative in the IgM ISAGA (6) and IgA ELISA (16). Group H consisted of 15 adults without clinical disease who were known to be positive for toxoplasma antibodies for more than 2 years before serum samples were drawn for the present study. Group I consisted of 33 random asymptomatic pregnant patients whose serum samples were sent to our laboratory for antenatal screening. Each of these serum specimens was positive in the dye test (titer range, 5 to 150 IU/ml) and negative in the IgM ELISA and IgA ELISA. Group J consisted of 24 asymptomatic seronegative pregnant patients sent to our laboratory for antenatal screening. Each of the serum specimens from patients in the group was negative in the dye test (less than 5 IU/ml) and IgM ELISA. In our laboratory, IgA ELISA is not routinely performed on sera from asymptomatic pregnant women whose sera are otherwise negative in the dye test and IgM ELISA.

Serological tests. The dye test (14), IgM ELISA (9, 15), IgM ISAGA (6), differential agglutination test (AC/HS) (3, 18), IgA ELISA (16), and IgE ISAGA (12) were performed as described previously. In our laboratory, our IgM ELISA is considered positive if the value is ≥ 2.0 , negative if the value is < 1.7 , and equivocal if the value is 1.7 through 1.9. For sera from infants less than 6 months of age, it is considered positive if the value is ≥ 0.2 and negative if the value is < 0.2 . Our IgA ELISA is considered positive for sera from adults if the value is ≥ 1.4 and negative if the value is < 1.4 . For sera from infants less than 6 months of age, the corresponding positive and negative values for the IgA ELISA are ≥ 1 and < 1 , respectively. For sera from adults, our IgM ISAGA result is considered positive if the value is ≥ 7 , negative if the value is ≤ 3 , and equivocal if the value is 4 through 6. For sera from newborns, our IgM ISAGA is considered positive if the value is ≥ 6 and negative if the value is ≤ 2 ; a value of 3 through 5 is considered suspicious

of congenital toxoplasmosis and requires additional testing in the IgA ELISA. For sera from adults and infants, the IgE ISAGA is considered positive if values of ≥ 4 are obtained and negative if values of ≤ 2 are obtained. An IgE ISAGA value of 3 is considered a low-positive, equivocal result. For the AC/HS, interpretation of the test is based on the pattern of serum agglutination titers when two different sets of toxoplasma antigens are used (3). Acute and nonacute refer to interpretation of the pattern of the AC/HS test and not to whether the patient actually had a recently acquired infection.

MAbs against IgE. Anti-human IgE (clone ANA B16, mouse IgG2a) monoclonal antibody (MAb) was purchased from Sera-Lab, Sussex, England. The specificity was determined by radioimmunoassay and displayed no cross-reactivity with IgA1, IgA2, IgG1, IgG2, IgG3, IgG4, IgD, or IgM. This MAb was used at a concentration of 2.2 $\mu\text{g/ml}$ to coat the wells for both the IgE ISAGA and the IgE ELISA. This concentration was determined in pilot experiments to be optimal; it was also the concentration that was recommended by the manufacturer.

Toxoplasma antigen preparation. Antigen for the IgE ISAGA was a suspension of formalinized tachyzoites which were prepared in the laboratory of one of us (P.T.) as described previously (6). Antigen for the IgE ELISA was prepared from *T. gondii* tachyzoites of the RH strain as described previously (9), with minor modifications. Briefly, peritoneal fluid was obtained from outbred Swiss Webster mice (Simonsen Laboratories, Gilroy, Calif.) infected intraperitoneally 2 days earlier with the RH strain. The peritoneal fluid was collected in cold (4°C) phosphate-buffered saline (PBS; pH 7.2) containing heparin (10 U/ml), and the mixture was filtered through a polycarbonate membrane (pore size, 3 μm ; Nuclepore Corp., Pleasanton, Calif.). The tachyzoite suspension was centrifuged at $850 \times g$ for 30 min; the supernatant was removed and used as the source of antigen. The protein concentration of the supernatant was determined by the Lowry method (BCA protein assay reagent; Pierce, Rockford, Ill.), and antigen was used at a concentration of approximately 0.8 mg/ml. The optimal concentration of the initial lot of antigen was determined by preliminary testing, and subsequent lots of antigen were titrated to ensure reproducibility of results. Pilot experiments in which a sonicate of *T. gondii* was used as the source of antigen revealed poor reproducibility of results.

IgE ELISA. F(ab)2' fragments of the IgG fraction of rabbit antiserum to *T. gondii* were prepared as described previously (1). These fragments, at a protein concentration of 1.25 mg/ml, were conjugated to alkaline phosphatase (1). The optimal dilution of conjugate was determined in preliminary experiments and was 1:2,000. This enzyme conjugate was also used for the IgM ELISA (15) and the IgA ELISA (16).

The IgE ELISA was adapted from the ELISAs used in our laboratory for the detection of IgM and IgA antibodies (15, 16). In brief, flat-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 μl of an anti-human IgE MAb diluted to 2.2 $\mu\text{g/ml}$ in 0.1 M carbonate buffer (pH 9.8). After overnight incubation at 4°C, the plates were washed three times with PBS containing 0.05% Tween 20 (PBS-T; Sigma Chemical, St. Louis, Mo.) and were postcoated with 1% bovine serum albumin (BSA; Sigma) in 0.1 M carbonate buffer (pH 9.8) for 2 h at 37°C. After three washes with PBS-T, the plates were used immediately or were stored in an airtight wrap at -20°C. Each serum sample was diluted in 1% BSA in PBS-T. Sera from infants less than 6 months of age were used at a dilution of 1:10; all other sera

were used at a dilution of 1:20. Dilutions were chosen from results of pilot experiments with sera from infants and adults. Each diluted serum sample was added in a volume of 100 μ l into four wells. A positive control serum sample with a high level of toxoplasma IgE antibodies and five control serum samples (dye test negative) were each placed in three wells on each ELISA plate. Four of the negative control serum specimens consisted of four individual serum specimens from four individual patients. The fifth control serum specimen was a pool of serum specimens from three other individuals. After 2.5 h of incubation at 37°C, the wells were washed three times with PBS-T. Antigen was added in a volume of 100 μ l into three of the wells for each serum sample, and the plates were incubated at 4°C for 18 h. Diluent without antigen was added to the fourth well for each serum sample. The antigen-free well of each set of wells for the serum sample served as the internal standard, to control for nonspecific IgE binding to the conjugate. If there was nonspecific IgE binding to the conjugate, the test was repeated. Pilot experiments determined that the positive and negative control sera did not bind nonspecifically to the conjugate. Thus, antigen-free wells were not required for the control sera. After washing the wells three times with PBS-T, 100 μ l of alkaline phosphatase-conjugated rabbit anti-toxoplasma F(ab)2' at a dilution of 1:2,000 was added. After 3 h of incubation at room temperature, wells were washed three times with PBS-T. *p*-Nitrophenyl phosphate disodium (1 mg/ml; Sigma) in 0.05 M carbonate buffer (pH 9.8) containing 0.001 M MgCl₂ was then added as substrate in a volume of 200 μ l to each well. Following a 1-h incubation at 37°C, the A_{405} values were read on an ELISA reader (Dynatech MR5000; Dynatech Laboratories, Chantilly, Va.). The results are expressed as the mean of the A_{405} values for the three wells containing each serum sample to which antigen was added. For the purposes of the present study, we considered a reaction to be positive if the mean A_{405} of the test sample was greater than the mean of the A_{405} of the five negative controls plus 3 standard deviations (SDs) in that ELISA plate; a result was considered to be negative if the A_{405} value was less than the mean of the A_{405} values of the negative control sera plus 2 SDs and equivocal if the A_{405} values were between the defined positive and negative results.

RESULTS

Reproducibility of the IgE assays. To compare the reproducibility of the IgE ELISA, 10 serum specimens (5 positive and 5 negative on preliminary testing) were each tested on 3 separate days. On repeat testing, consistent results were found for each of the negative and positive sera with respect to their negative and positive results, respectively (data not shown).

Sera were exchanged with J. M. Pinon, Reims, France, and tested in the IgE ISAGA in his (12) and our laboratories. Comparable quantitative results were obtained in both laboratories.

Evaluation of sera from control groups (groups G, H, I, and J). Sera from groups H, I, and J served as controls for evaluation of IgE antibodies as a serologic marker of acute toxoplasma infection and toxoplasmosis in infants and adults. IgE antibodies were not detected by IgE ELISA in any of the 48 serum specimens from the 48 chronically infected individuals in groups H and I. Each of the 15 serum specimens from the 15 patients in group H had IgE ISAGA values of zero. Sera from 13 of the 33 individuals in group I

were tested by IgE ISAGA, and all had results of zero. Sera from the 24 seronegative patients in group J were negative in the IgE ELISA; sera from 14 of them had values of zero in the IgE ISAGA.

Group G sera were from infants who had no clinical or serologic evidence of congenital toxoplasmosis; they served as controls for the infants in groups E and F. Each of the 10 serum specimens from the 10 infants were negative by IgE ELISA and had values of zero in the IgE ISAGA.

Seroconverters during pregnancy (group A). In each of the eight women who had acquired acute *T. gondii* infection during pregnancy, the IgE ELISA was as sensitive as the dye test, IgM ELISA, and IgM ISAGA for the detection of seroconversion in the first serum sample in which seroconversion was initially detected (Table 1). Detection of IgE antibodies in the seroconversion samples by ISAGA was as sensitive as that by ELISA but in five of eight patients (patients 3, 4, 5, 7, and 8), the ISAGA values were at the cutoff value of 3. In patient 5, detection of IgA antibodies by ELISA was delayed, and IgA antibodies were not detected in any of the sera from another two patients (patients 3 and 4). The AC/HS test revealed an acute infection pattern in all the patients but was delayed in detecting seroconversion in five of them (patients 2, 3, 4, 5, and 6).

In two patients (patients 4 and 6), the IgE ISAGA was positive for the preconversion (see Materials and Methods for definition) samples obtained 2 to 6 weeks before seroconversion was documented by the other tests including IgE ELISA. Repeat testing confirmed that these results were reproducible, suggesting that in some patients, IgE antibodies detected by ISAGA may be the first serologic marker of acute infection.

The duration of time in which toxoplasma IgE antibodies were detected varied remarkably. In three of eight patients (patients 3, 5, and 6), the duration detected by ISAGA was shorter than that detected by ELISA. In no instance was the duration of toxoplasma IgE antibodies detected by ISAGA longer than that detected by ELISA.

When results from individual patients were analyzed, there was variability in the duration of time when the different classes of antibodies were detected. For example, for patient 1, the duration of detectable IgA and IgM antibodies (by ELISA) was brief when compared with the duration of detectable IgE antibodies detected by ELISA and ISAGA. In contrast, for patient 5, the duration of detectable IgE and IgM antibodies was briefer than that for IgA antibodies, which were demonstrable for as long as 45 weeks (the longest time tested).

In three of the eight patients (patients 1, 4, and 5), the titer of IgE antibodies varied in serial samples. All serum samples from each individual were run in parallel, and repeat testing revealed consistent results. Although we cannot exclude a clerical error in coding the samples prior to storage, we consider it a more likely explanation that the observed changes in titers are a true reflection of changes in IgE antibody levels in an individual patient during acute infection; such changes were also demonstrable in patients with reactivation of their infections (see sections on group C and D patients and Discussion).

Toxoplasmic lymphadenopathy (group B). The serologic data for the 17 patients with acute toxoplasmic lymphadenopathy are given in Table 2. The initial serum samples from 16 of the 17 patients were positive in the IgE ELISA. One patient had a low-positive or equivocal result. Of interest is the absorbance values for the initial serum specimens from 12 of these 17 patients; they were greater than those for the

TABLE 1. Toxoplasma serology in women who seroconverted during pregnancy (group A)^a

Patient no.	Time (wk) ^b	Dye test (IU/ml)	IgM ELISA	IgM ISAGA	IgA ELISA	AC/HS ^c	IgE ISAGA	IgE ELISA (no. of SDs) ^d
1	0		—	—	—	NA	0	<2
	8	1,600	+	+	+	A	6	>5
	13	3,200	+	+	—	A	3	>5
	18	6,400	—	+	—	A	3	>5
	22	6,400	—	+	—	A	3	<2
	26	6,400	—	+	—	A	3	4
2	0		—	—	—	NA	0	<2
	4	1,300	+	+	+	NA	4	>5
	18	1,600	+	+	—	A	0	E
3	0		—	—	—	NA	0	<2
	8	1,600	+	+	—	NA	3	3
	15	3,200	+	+	—	A	0	3
	31	800	—	+	—	NA	0	<2
4	0		—	—	—	NA	3	<2
	6	640	+	+	—	NA	3	3
	11	640	+	+	—	NA	3	<2
	17	12,000	+	+	—	A	3	>5
	37	6,400	—	—	—	A	0	<2
5	0		—	+	—	NA	0	<2
	3	2.5	+	+	—	NA	3	>5
	9	150	+	+	+	A	3	3
	16	600	+	+	+	A	3	E
	22	600	+	+	+	E	3	3
	38	20	—	+	+	A	0	<2
	45	20	—	+	+	A	0	E
6	0		—	QNS	QNS	QNS	6	<2
	2	20	+	+	+	NA	9	>5
	22	1,200	+	+	+	A	6	>5
	26	600	+	+	+	A	6	>5
	39	300	+	+	—	A	3	3
	78	80	+	+	—	E	0	E
7	0		ND	—	—	NA	0	E
	8	20	ND	+	+	A	3	>5
	10	40	ND	+	+	A	3	>5
	12	200	ND	+	+	A	5	>5
	15	1,600	ND	+	+	A	9	>5
8	0		QNS	—	—	QNS	QNS	<2
	7	400	ND	+	+	ND	3	>5
	10	800	ND	+	+	A	5	>5
	14	3,200	ND	+	+	A	4	>5
	18	3,200	ND	+	+	A	3	>5
	22	1,600	ND	+	+	A	3	>5

^a Abbreviations: —, negative; +, positive; ND, test not performed; QNS, insufficient quantity of serum to perform further tests; E, equivocal or low-positive result; A, acute pattern on AC/HS testing; NA, nonacute pattern on AC/HS testing.

^b The preconversion sample was arbitrarily designated as 0 weeks, and the time in weeks thereafter was calculated.

^c Acute (A) and nonacute (NA) refer to interpretation of the pattern of the AC/HS test and not to whether the patient actually had a recently acquired infection (sera of patients seronegative [by the dye test] and patients with chronic infection had a nonacute pattern in the AC/HS test).

^d The results of the IgE ELISA were expressed as the number of SDs above the negative controls.

negative pool plus 5 SDs. Fifteen of the initial serum samples from the 17 patients had detectable IgE antibodies by ISAGA, and for each of these 15 serum samples, the values ranged from 7 to 12. For the serum samples from the other two patients, one had an equivocal result and the other was negative by IgE ISAGA; the corresponding IgE ELISA results were positive. IgM antibodies were detected in the sera from all 17 patients, and the AC/HS test revealed a pattern consistent with acute infection for 16 of them. The IgA ELISA was negative for two patients (patients 3 and 8)

and was equivocal for two others (patients 1 and 16). The serologic response in patient 16 is of particular interest, because at the time of clinical presentation, her IgE ELISA titer was clearly positive at a time when her IgA ELISA titer was equivocal and her dye test result and IgM ELISA titers were low. Two months later, repeat serology revealed a high dye test titer, equivocal results in the IgE ELISA and IgM ELISA, and negative results in the IgA ELISA and IgE ISAGA. The serologic response of this patient differed from those of all other patients with toxoplasmic lymphadenopa-

TABLE 2. Toxoplasma serology of patients in groups B and C

Group and patient designation	Symptom duration (wk)	Dye test (IU/ml)	IgM ELISA	IgA ELISA	AC/HS ^b	IgE ISAGA	IgE ELISA (no. of SDs) ^c
Group B							
1	2	600	+	E	A	12	3
2	3	80	+	+	A	7	E
3	3	300	+	—	A	12	>5
4	4	2,400	+	+	A	12	>5
5	8	>4,800	+	+	A	12	>5
6	8	1,200	+	+	A	12	>5
7	10	1,200	+	+	A	12	>5
8	12	2,400	+	—	A	7	4
9	12	2,400	+	+	A	0	3
10	12	2,400	+	+	A	12	>5
11	12	1,200	+	+	A	7	4
12	12	1,200	+	+	A	11	>5
13	12	2,400	+	+	A	12	>5
14	12	600	+	+	E	12	>5
15	12	2,400	+	+	A	12	>5
16	8	10	+	E	A	3	>5
17	17	300	+	—	A	0	E
	3	600	+	+	A	12	>5
	7	600	+	+	A	6	3
	13	600	+	E	A	6	3
	18	600	+	—	A	0	E
	23	600	+	—	A	0	E
	28	150	—	—	A	0	<2
	34	150	—	—	A	0	<2
	44	300	—	—	A	0	<2
	48	150	—	—	A	0	<2
Group C							
A		600	E	—	NA	3	>5
B		1,200	—	—	NA	12	>5
C		1,200	E	—	E	12	>5
D		40	—	—	NA	0	E
E		20	—	—	NA	0	<2
F		40	—	—	NA	0	<2
G		40	—	—	NA	3	<2
H		20	—	—	NA	0	<2
I		300	—	E	A	0	>5
J		40	—	—	NA	0	<2
K		40	—	—	NA	0	E

^a Abbreviations: —, negative; +, positive; ND, test not performed; QNS, insufficient quantity of serum to perform further tests; E, equivocal or low-positive result; A, acute pattern on AC/HS testing. NA, nonacute pattern on AC/HS testing.

^b Acute (A) and nonacute (NA) refer to interpretation of the pattern of the AC/HS test and not to whether the patient actually had a recently acquired infection (sera of patients seronegative by the dye test and patients with chronic infection had a nonacute pattern in the AC/HS test).

^c The results of the IgE ELISA were expressed as the number of SDs above the negative control.

thy examined in the present study and also from those of most of the patients described in previous reports (2, 5, 19), in which serologic test titers were usually markedly elevated at the time of clinical presentation. We had the opportunity to follow the kinetics of the antibody response for 11 months in patient 17. Her IgE antibody titers were positive by ISAGA and ELISA for 13 weeks after the onset of her clinical illness and then became undetectable at 18 weeks; her IgA antibody titers persisted for 13 weeks, whereas her IgM antibody titers persisted for at least 23 weeks.

Toxoplasmic chorioretinitis (group C). The 11 patients in group C were chosen specifically to characterize the toxoplasma serologic test response in patients who suffered from reactivation of latent toxoplasma infection of the eye (Table 2). IgE ELISA antibodies were detected in the sera of four patients, and for two others the results were equivocal. The IgE ISAGA was less sensitive; two patients had positive results and for two the results were equivocal. The IgM ELISA result was negative for 9 of the 11 patients and

equivocal for 2 patients. IgA antibodies were detected in only one patient and only at low titers. The AC/HS test revealed a nonacute pattern in sera from 9 of the 11 patients and equivocal for sera from 1 patient; an acute pattern was found for the sera from 1 patient.

Toxoplasmic encephalitis (group D). IgE antibodies were detected in the sera of 4 (33%) of the 12 patients by ELISA and in the sera of 3 (25%) of the 12 patients by ISAGA (data not shown). The results were equivocal or low-positive for sera from two patients by the IgE ELISA and seven patients by ISAGA. Sera from all the 12 patients did not have detectable IgA or IgM antibodies.

Congenital toxoplasmosis (group E). IgE antibodies were detected in the sera of 11 (92%) of the 12 infants by ELISA and in the sera of 8 (62%) of the 12 patients by ISAGA (Table 3). Results of the IgE ELISA were equivocal for one patient and negative for another. Of interest is that for sera from 11 of the 12 infants, the absorbance values in the IgE ELISA were greater than the mean absorbance of the negative

TABLE 3. Toxoplasma serology in infants in groups E and F^a

Group and patient designation	Age (wk)	Dye test (IU/ml)	IgM ELISA	IgM ISAGA	IgA ELISA	IgE ISAGA	IgE ELISA ^b
Group E							
1	1	4,800	+	ND	+	12	>5
2	1	300	ND	+	+	0	>5
3	1	300	ND	+	+	0	>5
4	1	9,600	ND	+	+	8	>5
5	1	1,200	+	+	+	5	>5
6	1	1,200	ND	+	+	12	>5
7	1	150	+	+	+	12	>5
8	2	600	+	E	+	0	>5
9	2	600	ND	+	+	0	<2
10	2	1,200	+	+	+	8	>5
11	3	1,200	+	ND	+	12	>5
12	7	1,200	ND	+	+	9	>5
Group F							
A	4	20	—	—	—	0	E
B	4	20	—	—	—	0	—
C	5	10	ND	—	—	0	—
D	6	20	—	—	—	0	—
E	7	20	ND	—	—	0	—
F	1	80	ND	—	—	0	>5
	5	80	ND	—	—	0	3
	10	80	ND	—	—	0	<2
	15	40	ND	—	—	0	<2
	26	10	ND	—	—	0	<2

^a Abbreviations: —, negative; +, positive; ND, test not performed; QNS, insufficient quantity of serum to perform further tests; E, equivocal or low-positive result.

^b The results of the IgE ELISA were expressed as the number of SDs above the negative controls.

controls plus 5 SDs. Serum from each of the infants had positive dye test titers, ranging from 150 to 1,200 IU/ml. Sera from all infants were positive in the IgM ISAGA and/or IgM ELISA. IgA antibodies were detectable in the sera from all the infants.

Sera from the mothers of 11 of these infants were obtained within 1 month of the time that corresponding samples were obtained from the infants. Each was positive in the IgE ELISA and 10 were positive in the IgE ISAGA (data not shown). Of interest is the serologic response in one of these mothers, who gave birth to two symptomatic twins (patients 2 and 3); the parasite was isolated from both of the infants. Her serum sample was positive in the IgE ELISA at an absorbance value between the negative pool plus 3 SDs and the negative pool plus 4 SDs and was negative in the IgE ISAGA. Sera from both of her children had IgE ELISA absorbance values of greater than the negative pool plus 5 SDs and were negative in the IgE ISAGA. These data further support our conclusion and that of Pinon et al. (12) that the IgE antibody responses in the infants were specific and not the result of maternal transfer.

Uninfected infants born to mothers with serologic evidence of acute infection during gestation (group F). The six infants who did not have clinical or serologic evidence of congenital toxoplasmosis (group F) were born to mothers who had serologic evidence of acute infection that was most likely acquired during pregnancy. IgE antibodies were not detected by ISAGA in the sera from any of these infants. Sera from four of the infants were also negative by the ELISA (Table 3). For serum from patient A, the IgE ELISA result was equivocal and all subsequent serum samples were negative. For serum from patient F, IgE antibodies were detected at 1 and 5 weeks of age. At 1 year of age, both of these infants (patients A and F) remained without clinical

evidence of congenital toxoplasmosis, despite extensive investigations. They had received antitoxoplasma therapy for only 1 month. They continue to be under close follow-up by their respective physicians.

DISCUSSION

The purpose of the present study was to determine the value of demonstrating toxoplasma IgE antibodies in the sera of patients with different clinical problems associated with *T. gondii* infection. Seronegative individuals, asymptomatic individuals who were chronically infected with *T. gondii*, and infants without congenital toxoplasmosis (except for one infant in whom we considered it a false-positive result) did not have detectable IgE antibodies by either the ELISA or the ISAGA method.

IgE antibodies were detected (by ELISA or ISAGA) in 100% of women who seroconverted during pregnancy. These antibodies were demonstrable in the first serum sample in which seroconversion was documented (in the dye test, IgM ELISA, IgM ISAGA, and IgA ELISA) and extend the results of Pinon et al. (12), who noted that IgE antibodies were formed early after toxoplasma infection in women who seroconverted during pregnancy.

In the women who seroconverted during pregnancy, a trend toward a brief duration of toxoplasma IgE antibodies detected by ISAGA was observed when compared with the time that antibodies could be detected by the IgE ELISA, the IgM ELISA, or the AC/HS test. In the present study, the duration of detectable IgA antibodies was also brief, but the assay for IgA was less sensitive (75%) in detecting seroconversion in women who acquired the acute infection during gestation. In pregnant women for whom only one serum sample was tested during pregnancy, data on the duration of

time that the antibodies of the different classes were detectable are important for evaluating the risk of transmission of infection to the fetus. Pinon et al. (12) reported that IgE antibodies detected by ISAGA were present in 25 (86%) of 29 women who seroconverted during gestation and that these antibodies were not demonstrable by 4 months after infection. In the present study, we were able to detect a greater percentage of women who seroconverted during pregnancy (100%) in both of our IgE assays, but the decline of IgE antibodies (detected by ELISA and ISAGA) in individual patients varied considerably, and serum from one patient remained positive for IgE by both ELISA and ISAGA for 37 weeks after seroconversion was documented. The patient-to-patient variability in relation to the time at which IgE antibodies became undetectable was also observed for IgM and IgA antibodies. Although we detected a trend toward a shorter duration of detectable IgE antibodies in the IgE ISAGA for women who acquired the acute infection during gestation, we were unable to define a specific time period at which such antibodies would no longer be demonstrable by the assays performed in the present study.

Our data with the IgM antibody tests confirm those of others who reported on the longer duration that IgM antibodies could be detected by ISAGA compared with the duration that they could be detected by ELISA (5, 19). IgM antibodies detected by ISAGA were demonstrable up to the time that last serum sample was tested (range, 15 to 76 weeks) for the majority of the eight women who seroconverted during gestation and from whom serial serum samples were available. The greatest value of detection of IgM antibodies is in determining that an individual has not been recently infected. A negative result virtually rules out recently acquired infection unless the sera are tested so early after the acute stage of infection that an antibody response has not yet occurred (in which case, the acute infection would be identified in a screening program in which follow-up serology is performed for seronegative women). A single positive IgM test result is more difficult to evaluate unless a significant rise in IgG or IgM titer can be demonstrated when sera are run in parallel or when other tests including IgA ELISA, IgE ELISA, IgE ISAGA, and the AC/HS test suggest acute infection.

For patients from whom serial serum samples were available for analysis (those who had seroconverted during pregnancy and one patient with toxoplasmic lymphadenopathy), our study revealed a trend toward higher IgE antibody titers (by both ELISA and ISAGA) during the first 8 weeks after infection. However, in the sera of some patients, IgE antibodies were positive at low titers in the ISAGA (values of 3 to 4) and ELISA (absorbance values between the negative control plus 2 SDs and the negative control plus 3 SDs), whereas in the sera of others, high titers of IgE antibodies, in particular, those detected by ELISA (greater than the negative control plus 5 SDs), remained for up to 15 weeks (in one patient) after seroconversion was documented. For many of the patients who we studied, IgE antibody titers detected by ISAGA were low. In our limited experience, for the individual patient with a high IgE titer by ELISA in a single serum sample, infection was likely to have occurred within the past 15 weeks, whereas a low IgE antibody titer (in either the ISAGA or ELISA) did not exclude a recent infection. This is true not only for the IgE assays described here but also for the IgM ELISA and IgA ELISA that are routinely performed in our laboratories.

The IgE ELISA appeared to be more sensitive than the

IgE ISAGA (85 versus 62%, respectively) for the diagnosis of congenital toxoplasmosis in infants. Of note, such antibodies were detected within a mean of 2 weeks after birth and therefore may be a useful diagnostic aid for early detection of congenital toxoplasmosis. Whether congenital toxoplasmosis would have been detected in more infants by testing for IgE antibodies (by both assays) if testing was performed at a later age is unknown. IgE ELISA and IgE ISAGA are routinely performed at the Palo Alto facility as part of a battery of serologic tests in all infants suspected of having congenital toxoplasmosis. Whether these tests will improve on the sensitivity of currently available serologic methods for testing for this condition remains to be determined.

In patients with toxoplasmic lymphadenopathy, high IgE antibody titers were detected by ELISA and ISAGA in virtually all patients within 12 weeks of the onset of clinical illness. These data confirm the results of previous reports on the usefulness of serologic tests in the diagnosis of toxoplasmic lymphadenopathy (2, 5).

Interestingly, in our study, toxoplasma IgE antibodies were detected in patients with toxoplasmic chorioretinitis which had resulted from reactivation of chronic (latent) infection. When low-positive results were included, more patients with toxoplasmic chorioretinitis had detectable IgE antibodies by ELISA (54%) than by ISAGA (36%). These results are similar to the 46% reported by Pinon et al. (12, 13) in patients with congenital toxoplasmic chorioretinitis. In a separate study of sera from nine patients who had serologic evidence of acute infection (by the dye test, IgM ELISA, and IgA ELISA) and ocular manifestations including uveitis and chorioretinitis, all had high IgE titers by ELISA (absorbance values greater than negative control plus 5 SDs), eight of nine had high ISAGA values (values greater than 9), and one had an ISAGA value of 6.

Pinon et al. (12) previously reported that toxoplasma IgE antibodies were detected in two of three patients with AIDS and TE by the ISAGA technique. In our study, when low-positive results were included, IgE antibodies were detected in 83% of the sera of 12 adult patients with AIDS and TE by ISAGA and in 50% by ELISA. These data are promising and warrant further study of the use of these methods for the diagnosis of TE in this patient population. Whether similar cutoffs for positivity in these assays should be used for both immunocompetent and immunocompromised patients and patients with reactivated disease remains to be clarified. In patients with AIDS and suspected TE, a panel of serologic tests (e.g., including the dye test, AC/HS test, agglutination test, IgE ISAGA, and IgE ELISA) should provide a more accurate means of assessing whether the infection is presently active or quiescent.

In a separate study on bone marrow transplant patients with toxoplasmosis at the Fred Hutchinson Cancer Research Center, Slavin and colleagues (15a) reported that sera of 2 of 12 patients had positive IgE antibodies by ELISA. The two patients whose sera had positive IgE antibodies also had serologic evidence (by IgA ELISA and IgM ELISA) that was consistent with recently acquired primary infection at the time of clinical presentation.

In the majority of patients with toxoplasmic chorioretinitis and those with TE, reactivation of a latent infection is the postulated pathogenetic mechanism of the disease. Our data are consistent with our hypothesis that recrudescence of IgE antibodies in patients with disease caused by reactivation may be a marker for the study of immune regulation of immunoglobulin switching in patients with this infection.

The data derived in the present study suggest that the different methods used for detection of toxoplasma IgE antibodies may be optimized for use in different clinical circumstances. In patients with congenital toxoplasmosis, toxoplasmic lymphadenopathy, and toxoplasmic chorioretinitis, the IgE ELISA appears to be more sensitive and may be the more appropriate test. In contrast, the IgE ISAGA may be more useful in pregnant women. Specific IgE antibodies were detected earlier by ISAGA than by ELISA in the sera of two of eight pregnant women who seroconverted during pregnancy. In addition, there was a trend toward a more rapid decline of IgE antibodies detected by ISAGA, and this may be useful in estimating the approximate time that infection occurred. The individual variabilities in the antibody responses of the different immunoglobulin classes to toxoplasma infection that we observed in the present study lend further support to our view that reliance on only one or two serologic tests for diagnosis may be insufficient. The use of a panel of tests is advisable to improve sensitivity and specificity.

Detection of toxoplasma IgE antibodies for the diagnosis of acute toxoplasma infection (seroconversion during pregnancy and toxoplasmic lymphadenopathy) and for congenital toxoplasma infection serves as a useful adjunct to currently available tests. Detection of IgE toxoplasma antibodies also appears to be associated with reactivated chronic (latent) infection and may be important for the diagnosis of these conditions.

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