Detection of Specific Immunoglobulin E in Patients with Toxoplasmosis

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An immunocapture assay was developed to detect *Toxoplasma gondii*-specific immunoglobulin E (IgE) in sera from adults with acute acquired infection or reactivation and from babies with congenital toxoplasmosis. The components of this assay were monoclonal antibody to human IgE, samples from patients, and *T. gondii* tachyzoites treated with Formalin. When *T. gondii*-specific IgE antibodies were present, visually detectable agglutination occurred. Sera, umbilical cord blood, fetal blood, cerebrospinal fluid, and amniotic fluid were tested by this method. Specific IgE antibodies were detected in sera from 25 (86%) of 29 adults who developed specific IgG antibody during pregnancy or had specific IgA and IgM antibodies. Specific IgE was present early during infection, at the time that IgM antibodies were present, and slightly preceding the presence of specific IgA antibodies. In 23 patients tested serially, IgE antibodies never persisted for longer than 4 months. No nonspecific anti-*T. gondii* IgE was detected in sera from uninfected individuals. Maternal IgE antibodies did not cross the placenta. In sera of patients with congenital toxoplasmosis, specific IgE antibodies were found at birth, during the first year of life, and during immunologic recrudescence following discontinuation of pyrimethamine-sulfonamide therapy. The IgE immunocapture assay is simple to perform. It is especially useful for determining when *T. gondii* was acquired by recently infected pregnant women.

We were interested in studying the specific immunoglobulin E (IgE) response in patients with toxoplasmosis because there are few studies on anti-*Toxoplasma gondii* IgE (1, 13, 19) and there are many methods used to detect other antigen-specific isotypes (2–4, 11, 15, 18). Also, immunopathologic mechanisms during human and experimental toxoplasmosis (5–7, 12, 19, 21, 22) could involve IgE antibodies. In addition, confirmation of recent infections of pregnant women with *T. gondii* can be difficult, despite concomitant studies of specific IgG, IgM, and IgA antibodies. The rapid disappearance of IgE antibodies occurs in certain viral infections (20), and this observation provides a precedent for the study of specific IgE antibody in sera of pregnant women with recently acquired *T. gondii* infections. Thus, a simple and sensitive IgE immunocapture assay was developed. Optimal conditions were defined, and specific IgE responses in adults with recently acquired *T. gondii* infection, immunocompromised patients, and babies with congenital toxoplasmosis were studied.

MATERIALS AND METHODS

Samples. A total of 713 samples from 350 patients were examined, as follows: sera ($n = 676$), umbilical cord blood ($n = 27$), cerebrospinal fluid ($n = 2$), amniotic fluid ($n = 4$), and fetal blood obtained from the umbilical cord with ultrasound guidance ($n = 4$). A total of 137 subjects, adults and pregnant women ($n = 85$) and newborn babies ($n = 52$), were uninfected. One hundred and ten subjects had chronic toxoplasmosis with stable or low titers of anti-*T. gondii* IgG without specific IgM or IgA antibodies. Seven adults showed reactivation of toxoplasmosis, with elevation of IgG antibodies after kidney ($n = 3$) and heart ($n = 1$) transplants or toxoplasmic encephalitis ($n = 3$) in patients with acquired immunodeficiency syndrome (AIDS). Twenty-nine pregnant women routinely tested every month had a recently acquired

infection (3). Twenty-seven of these women were asymptomatic and two other women had cervical lymphadenopathies. Forty-one children contracted congenital toxoplasmosis, as ascertained by previously defined parasitologic and/or immunologic criteria (3, 13). We also studied 26 subjects with rheumatoid factor ($n = 13$), anti-nuclear antibodies ($n = 12$), or acute infectious mononucleosis ($n = 1$) with fever, cervical lymphadenopathy, a positive Paul-Bunnell-Davidson test, and a negative cytomegalovirus IgG enzyme-linked immunosorbent assay (ELISA).

Monoclonal antibodies against human IgE, IgM, and IgA. Anti-IgE monoclonal antibody (MAb; ANA B16; lots 001 and 003; Clonatec Laboratories Department, Biosoft, Paris, France) was prepared by using BALB/c mice. The specificity of the anti-IgE antibody (1 mg/ml) was verified by a radioimmunoassay with monospecific anti-IgG, anti-IgM, or anti-IgA MAbs and competition with labeled IgE (Clonatec Laboratories Department). Anti-IgM and anti-IgA MAbs (1 mg/ml) were also from Clonatec Laboratories Department.

IgE immunocapture assay. The IgE immunocapture assay was adapted from tests that were used for the detection of anti-*T. gondii* IgM or IgA which depend on the same immunocapture principle (3, 15). To carry out the different immunocapture assays simultaneously under the simplest standard conditions, we looked for parameters that would allow us to use the same serum dilutions or the same concentrations of *Toxoplasma* antigen for the detection of specific IgM, IgA, and IgE.

Briefly, 96-well microtiter plates (262 162 301 182 1; Nunc, Roskilde, Denmark) were coated with 100 μ l of anti-human IgE MAb diluted to 2.2 μ g/ml in a solution of pyruvic aldehyde (252; Sigma Chemical Co., St. Louis, Mo.) in acetate buffer (15). After 18 h of incubation at 4°C, the plates were saturated for at least 5 days with a solution of phosphate-buffered saline (pH 7.2; 7551.1; Bio-Mérieux)—bovine serum albumin (1%)—sodium azide (0.1%). Before use, the plates were washed three times with phosphate-buffered

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saline. Each serum sample, which was diluted 25-fold in phosphate-buffered saline–0.4% bovine serum albumin, was distributed as 100 μ l in each of three adjacent wells. After 2.5 h of incubation at 37°C, the plates were washed in phosphate-buffered saline–0.5% Tween 20 (522.184; Merck). A suspension of formalinized tachyzoites, which were prepared in our laboratory as described previously (3, 4) and adjusted in BABS buffer (7333.1; Bio-Mérieux) to a concentration of 1.5×10^7 /ml, was added at 100, 150, or 200 μ l to the three wells corresponding to the same serum samples described above (15). After 18 h at 20°C, the results were read visually against a black background. A value of 0 was given for negative agglutination. Complete agglutination as a film was considered positive and was assigned a value of 4.

Intermediate diameters (25, 50, and 75% complete agglutination) were assigned values of 1, 2, and 3, respectively. Thus, the sum of the values of the three samples of the same serum sample varied between 0 and 12. The results could also be read automatically with a spectrophotometer (MR 5000; Dynatech). Each test included two positive controls (values 11 and 6) and one negative control (value 0).

Other methods. The samples were tested for specific IgG by highly sensitive direct agglutination (HSDA), and results were expressed as units per milliliter. The suspension of tachyzoites was prepared in our laboratory as described previously (4) for the immunocapture assays. To detect IgM and IgA antibodies, the immunocapture assays were similar to those used for IgE (3, 15). The only differences were the serum dilutions (1/100) and the coating concentrations (1 μ g/ml) of the anti-IgM or anti-IgA MAb. The IgE immunocapture assay results for serum specimens ($n = 114$) tested by the different techniques allowed us to distinguish six groups: A (IgE[−], IgG⁺); B (IgE[−], IgA⁺, IgG⁺); C (IgE[−], IgM⁺, IgG⁺); D (IgE⁺, IgA[−], IgG⁺); E (IgE⁺, IgM[−], IgG⁺); F (IgM, ≤ 6 IU/ml; IgG, <10 U/ml). These 114 serum specimens were selected by using only immunologic criteria, independently of any associated physical state or clinical syndrome (see Fig. 1, groups A through F).

The method of compared immunologic profiles was carried out by an enzyme-linked immunofiltration assay to study sera (group G) from subjects with congenital infections (subgroup G₁; $n = 9$) or acute toxoplasmosis (subgroup G₂; $n = 21$) (12). All these sera had *T. gondii*-specific IgG, IgM, and IgA antibodies (Fig. 1, G₁ and G₂).

RESULTS

Serum dilutions and standardization. We chose eight serum specimens (two negative and six positive with values from 3 to 12) which were tested as twofold dilutions from 1/25 to 1/3,200. As there were sudden drops in values from the dilution 1/100 or 1/50, we chose 1/25 as the optimal dilution (Table 1). The addition of 0.4% bovine serum albumin in the phosphate-buffered saline used for dilutions aided in reading the test results. Under the preparation conditions described above, the plates could be kept for at least 6 months at 4°C. From one batch (lot 001) to another (lot 003) of anti-IgE MAb, 2.2 μ g/ml remained the optimal concentration to coat plates to produce similar results.

Repetition and reproducibility of the immunocapture assay. The reproducibility and homogeneity of the coating were evaluated by using four selected serum specimens (values of 11, 4, 1, and 0). Each serum specimen was tested simultaneously 20 times on the same plate. The values obtained for the four serum specimens were 11 ($n = 19$) and 11 + 1 ($n = 1$), 4 ($n = 18$) and 4 $\pm$ 1 ($n = 2$), 1 ($n = 20$), and 0 ($n = 20$),

TABLE 1. Variations in results of the IgE immunocapture assay based on the serum dilution

Serum specimen no.	Value at the following serum dilution:							
	1/25	1/50	1/100	1/200	1/400	1/800	1/1,600	1/3,200
1	12	12	12	11	9	7	3	2
2	12	11	9	4	1	0	0	0
3	12	11	7	2	0	0	0	0
4	12	9	4	1	0	0	0	0
5	11	7	2	0	0	0	0	0
6	3	1	0	0	0	0	0	0

respectively. The reproducibility and the stability of the coating were evaluated by using three controls (values of 12, 4, and 0) tested on 10 different plates that were sensitized in parallel by the same lot of MAb and studied by using *Toxoplasma* suspensions from a single production batch. The values obtained for the three controls were 12 ($n = 9$) and 12 − 1 ($n = 1$), 4 ($n = 8$) and 4 $\pm$ 1 ($n = 2$), and 0 ($n = 9$) and 0 + 1 ($n = 1$).

Specificity of the immunocapture assay. The specificity of the immunocapture assay was verified by using 114 serum specimens (Fig. 1, groups A through F). Group A (IgE[−], IgG⁺) consisted of 30 serum specimens with positive IgG HSDA values at dilutions of 1/25 to 1/100,000. The IgE immunocapture assay results obtained were all negative (mean, 0). The IgE immunocapture assay values for serum specimens ($n = 10$) from group B (IgE[−], IgA⁺, IgG⁺) were also less than 1 (mean, 0), with IgA immunocapture assay values of 2 to 11. The results for serum specimens ($n = 10$) belonging to group C (IgE[−], IgM⁺, IgG⁺) were all negative, with IgM immunocapture assay values of from 2 to 12 for sera from children with congenital toxoplasmosis or 7 to 12 for adults with acquired toxoplasmosis. For group D (IgE⁺, IgA[−], IgG⁺) serum specimens ($n = 8$), values were 2 to 6 (mean, 3). For group E (IgE⁺, IgM[−], IgG⁺) serum specimens ($n = 6$), the IgE immunocapture assay values were 2 to 11 (mean, 4.8). Of 50 serum specimens from group F (IgM, ≤ 6 IU/ml; IgG, <10 U/ml), 49 had IgE immunocapture assay values of less than 1 whether the sera came from children or from adults with or without natural IgM (Fig. 1, group F). In one case, the IgE immunocapture assay was positive (value of 3) (see Discussion) (Table 2). The 26 serum specimens were positive with low or stable IgG HSDA titers and contained rheumatoid factor, antinuclear antibodies, or antibodies specific for infectious mononucleosis and were all negative when tested by the IgE immunocapture assay. These results showed the specificity of the IgE immunocapture assay.

Comparison of IgM, IgA, and IgE immunocapture assay values in patients with congenital or acute toxoplasmosis. We tested the group G sera (IgE⁺, IgM⁺, IgA⁺, IgG⁺), and the range of values (0 to 12) was the same for the three assays. Nine sera came from subjects with congenital toxoplasmosis (subgroup G₁). In those cases, the IgE results varied from 2 to 11 (mean, 4.8) (Fig. 1, group G₁). These values were equal to those for IgM ($n = 1$) or IgA ($n = 3$) and less than those for IgM ($n = 8$) or IgA ($n = 6$). Subgroup G₂ consisted of serum specimens ($n = 21$) taken from adults with acute toxoplasmosis. In these cases, the IgE results varied from 3 to 12 (mean, 9.57) (Fig. 1, group G₂) and were greater than the IgM ($n = 7$) or IgA ($n = 15$) values, equivalent to the IgM ($n = 5$) or IgA ($n = 1$) values, and less than the IgM ($n = 9$) or IgA ($n = 5$) values.

Evolution of IgE antibodies in subjects with seroconver-

FIG. 1. Results of the IgE immunocapture assay (values 0 to 12) in 144 serum specimens. The numbers of serum specimens tested in each group were as follows: A (IgE⁻, IgG⁺), 30; B (IgE⁻, IgA⁺, IgG⁺), 10; C (IgE⁻, IgM⁺, IgG⁺), 10; D (IgE⁺, IgA⁻, IgG⁺), 8; E (IgE⁺, IgM⁻, IgG⁺), 6; F (IgG⁻), 50 (total of groups A through F, 114); G (IgE⁺, IgM⁺, IgA⁺, IgG⁺), 30 (G₁ [subjects with congenital toxoplasmosis], 9; G₂ [adults with recently acquired toxoplasmosis], 21).

sions, recently acquired toxoplasmosis, and reactivations. We followed serum specimens ($n = 118$) from 20 subjects with seroconversions (passage from negative to positive; $n = 88$) and from 9 subjects with recent acute toxoplasmosis (increase in IgG antibody with positive IgM antibody, IgA antibody, or both; $n = 30$). All these samples were taken from 29 pregnant women. Specific IgE was found in 16 of 20 subjects (27 of 88 serum specimens) during seroconversion and in 9 of 9 subjects (15 of 30 serum specimens) with recent acute toxoplasmosis as defined above. Pregnant women with cervical lymphadenopathy ($n = 2$) had specific IgE antibodies. The specific IgE antibody appeared early, as did the IgM or IgA antibodies, but it persisted for less than 4 months in all serially tested patients ($n = 23$). In the cases of reactivation manifested after kidney transplantation ($n = 3$), we detected an increase only of anti-*T. gondii* IgG antibody without IgE antibody. In serum specimens ($n = 5$) from one heart transplant recipient, we observed a rise of specific IgG antibody (from 25 to 6,400 U/ml), with the synthesis of IgE antibodies (values of 0, 4, 11, 9, and 8) and IgA antibodies

TABLE 2. Comparative evolution of IgG antibody by HSDA and IgM, IgA, and IgE antibodies detected by the immunocapture assay^a

Date	IgG HSDA titer (U/ml)	Value for the following antibodies in the immunocapture assay:		
		IgM ^b	IgA	IgE
6 January	Negative	<6	0	3
3 February	10	12	11	9
23 February	25	12	11	7
16 March	200	12	10	0
20 April	400	12	10	0

^a Toxoplasmic seroconversion was monitored in a woman who had been pregnant for 2 months.

^b For the IgM immunocapture assay, values of ≤ 6 were not significant in adults.

(values of 0, 11, 12, 12, and 12) but without significant anti-*T. gondii* IgM levels (values of 5). Specific IgA and IgE antibodies were detected in two of three patients with AIDS who had toxoplasmic encephalitis (Table 3).

Systematic search. We studied serum specimens taken at birth from 10 infants free of *Toxoplasma* infection whose mothers acquired the organism near the end of their pregnancies and still had specific IgE antibodies at the time of delivery. The sera of all these children were negative by the IgE immunocapture assay.

We also carried out a systematic search for IgE antibodies of 163 serum specimens that were examined routinely during the monitoring of newborn children or obligatory tests for toxoplasmosis among pregnant women. The IgE immunocapture assay, which was carried out simultaneously with the IgG HSDA, the IgM immunocapture assay, and the IgA immunocapture assay, was positive in three patients. One (value of 4) was from a child with congenital toxoplasmosis. The others (values of 6 and 11) were from two pregnant women. All these sera had anti-*T. gondii* IgG and IgM or IgA antibodies.

The studies of cerebrospinal fluid, fetal blood, or amniotic fluid by the immunocapture assay did not reveal specific IgE.

DISCUSSION

We developed an IgE immunocapture assay in which we used an anti-human IgE mouse Mab to coat the microdilution plates and a suspension of tachyzoites to demonstrate *T. gondii*-specific IgE antibody. This test is simple to perform. The reproducibility is satisfactory, since the variations observed were not greater than ± 1 on a scale of 0 to 12. Initially, reading of the results was more difficult than in the IgM or the IgA immunocapture assay. However, this problem was overcome by adding bovine serum albumin to the phosphate-buffered saline that was used to dilute the sera. The coated plates were stable for at least 6 months. There were no significant variations in the results of tests carried out with two independently produced batches of anti-IgE MAbs.

The specificity of the assay was studied with sera which were positive by IgG HSDA at dilutions of 1/50,000 or

TABLE 3. Comparative evolution of IgG antibody and IgM, IgA, and IgE antibodies detected by the immunocapture assay^a

Patient no.	Date	IgG HSDA titer (U/ml)	Value for the following antibodies in the immunocapture assay:		
			IgM ^b	IgA	IgE
1	20 January	400	3	0.5	0
	23 February	1,600	3	10	4
	23 March	25,000	3	10	4
2	22 December	1,600	0.5	0	0
	22 February	800	0.5	0	0
	14 March	1,600	0.5	0	0
3	9 December	200	7	1.5	0
	11 July	25,000	8	7	11
	19 August	25,000	7	6	8
	16 January	6,400	7	5	2
	13 March	6,400	7	5	1
	17 May	3,200	7	5	0

^a Toxoplasmic encephalitis was monitored in three patients with AIDS.

^b For the IgM immunocapture assay, values of < 6 were not significant in adults.

greater (titers, >12,800 U/ml) and which gave values of less than 2 by the IgE immunocapture assay (Fig. 1, group A). The same negative results were obtained with sera that were highly positive by the IgM or IgA immunocapture assay, or both (Fig. 1, groups B and C). The negative control sera never had values greater than 1, and there did not seem to be natural anti-*T. gondii* IgE (Fig. 1, group F). The IgE immunocapture assay cutoff value was 3, which was the same as that in the IgA immunocapture assay. This was much lower than that of the IgM immunocapture assay, in which in subjects over 6 months of age, the cutoff value was 7, which excluded false-positive test results because of natural IgM (3). Maternal IgE antibodies do not normally cross the placenta. Although specific IgE antibodies were detected in maternal sera at the time of birth, they were not found in newborn babies who were not infected with *T. gondii*. This is in contrast to some previous observations of the transmission of maternal IgM (14). The presence of rheumatoid factor or antinuclear antibodies did not alter the specificity of the test. It has been shown previously (15, 17) that utilization of a mouse MAb avoids the disadvantages described for other immunocapture assays with rabbit antibodies.

Total and specific IgE in subjects with acquired or congenital toxoplasmosis have not been the subject of many reports (1, 9). IgE antibodies have been studied by using soluble extracts of antigens either by a test of basophil degranulation (8) or by enzyme-linked immunofiltration assay (14) and ELISA (1) immunochemical methods. Immunocapture (or reverse) techniques were not used for IgE ELISAs (1). Competition phenomena with specific IgG antibodies (well demonstrated between specific IgG and IgM or IgA) (3, 11) may have hidden anti-*T. gondii* IgE antibodies. This could explain, at least partially, the scarcity of positive results described by direct IgE ELISA. During our study, anti-membrane-specific IgE antibodies were frequently demonstrated in subjects with acute toxoplasmosis, in whom this isotype was detected in 86% of the subjects. Anti-*T. gondii* IgE antibodies appear early in infection, practically simultaneously with IgM and IgA antibodies. In one subject from group F, specific IgE was even detected when the IgG antibody was still negative at 10 U/ml and IgM and IgA had not reached significant levels. Further studies showed this case to be the result of seroconversion (Table 2). Anti-*T. gondii* IgE antibodies were transitory. They did not persist for more than 4 months in serially tested patients ($n = 23$). These results agree with those described previously for subjects with acute viral infections (20). Specific IgE antibodies were detected by ELISA in subjects with chronic toxoplasmosis. This difference could be a result of using an antigen that is principally cytoplasmic by ELISA (1). The rapid evolution of IgE antibodies detected by the immunocapture assay could constitute useful criteria in the monitoring of pregnant women, in whom it can be difficult to determine whether an infection is recent, despite the concomitant study of specific IgG, IgM, and IgA. The latter two isotypes do not always reach high values in subjects with a recently acquired infection or, on the contrary, may persist at high levels for long periods of time (3, 14, 15, 17). The association of IgE antibodies would be suggestive of the recent acquisition of infection. In this context, the absence of natural IgE is an additional advantage compared with the possible presence of natural IgM (3). Concerning congenital toxoplasmosis, results of the present study confirm our previous results by the enzyme-linked immunofiltration assay (14). Anti-*T. gondii* IgE antibodies can be detected from birth during monitoring of children under treatment or during

an immunologic recrudescence after therapy is discontinued (data not shown). These IgE antibodies were frequently detected in sera of children with complications (hydrocephalus, chorioretinitis). The prolonged detection of specific IgE and IgM was followed in 46% of subjects by the appearance of chorioretinitis (data not shown) (16). Anti-*T. gondii* IgE did not always occur in conjunction with recognized immunopathology (5, 21), but it accompanied the acute or subacute state (e.g., serologic reactivation; data not shown). We have found (2, 10) anti-*T. gondii* IgE in immunocompromised patients (heart transplant recipients or patients with AIDS with toxoplasmic encephalitis) with serologic and clinical reactivations and neuroradiologic responses to specific treatment. We did not detect specific IgE in sera from individuals with renal transplants, cerebrospinal fluid, amniotic fluid, or fetal blood, but the numbers of samples were small.

In conclusion, the detection of specific anti-membrane IgE by the immunocapture assay may constitute an extremely useful complementary test in the diagnosis of *Toxoplasma* infections acquired during pregnancy and, possibly, of toxoplasmic reactivation in transplant recipients or patients with AIDS. In the case of pregnant women, this test, in association with IgG, IgM, and IgA immunocapture assays, may clarify the presumed date of acquisition. This may help to evaluate the risk to the fetus and decisions concerning management of the pregnancy. In the cases of children suffering from congenital toxoplasmosis, the search for IgE antibodies at birth and during the first year of life may contribute to diagnosis and prognosis, although additional correlative data are needed to confirm this observation.

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