Frequency of Specific anti-*Toxoplasma gondii* IgM, IgA and IgE in Colombian Patients with Acute and Chronic Ocular Toxoplasmosis

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*We studied the frequency of specific anti-*Toxoplasma *IgM, IgA and IgE antibodies in serum of 28 immunocompetent Colombian patients, selected by ophthalmologists and with lesions that were compatible with ocular toxoplasmosis. Patients were classified in three groups: (i) group 1 consisted of ten patients with a first episode; (ii) group 2, with seven patients with a recurrence and (iii) group 3, consisted of eleven patients with chronic chorioretinal lesion without uveitis. We found that 10/28 (35%) of Colombian patients with ocular toxoplasmosis possessed at least one serological marker for* Toxoplasma *infection different from IgG. In group 1 (first episode), we found simultaneous presence of specific IgM plus IgA plus IgE in 1/10 (10%). In group 2 (recurrences) in 1/7 (14%) we found IgM and IgA test positives and in 1/7 (14%) we found IgM and IgE tests positives. In group 3 (toxoplasmic chorioretinal scar) the IgA serological test was positive in 2/11 (18%). These results show that serum IgM or IgA or IgE can be present during recurrences.*

Key words: toxoplasmosis - human ocular toxoplasmosis - IgG - IgM - IgA - IgE

Ocular toxoplasmosis is one of the main clinical manifestations of the human infection by the protozoan parasite *Toxoplasma gondii* (Remington et al. 1995). The retina is the primary site of *T. gondii* infection in the eye (Holland et al. 1996). The lesions cause retinal scars and the loss of visual function when the macula region is involved. Once a scar is established it remains for the whole life; recurrence lesions around scars are common and can aggravate the visual deficiency (Holland et al. 1996). Toxoplasmic chorioretinitis has been found to be the most common recognizable cause of posterior uveitis (intraocular inflammatory syndrome) in many surveys (Henderly et al. 1987). In a recent study in Dutch patients, toxoplasmic uveitis was the most frequent cause of unilateral visual loss (Rothova et al. 1996). In Colombia, ocular toxoplasmosis represents 4% of consultation in ophthalmology (Varela 1986). The ocular involvement of toxoplasmosis has been considered a recurrent manifestation of congenital infection. However, for many years an infrequently reported, but important, subgroup of patients was that with toxoplasmic chorioretinitis as the only manifestation of recently acquired toxoplasmosis. Recent reports show that this form of presentation in non-immunosuppressed patients is more common than believed (Nussenblatt & Belfort 1994, Ronday et al. 1995). Montoya and Remington (1996) reported 22 adult cases with serological profile presumptive of acute acquired toxoplasmosis. Such patients had detectable IgM antibodies and/or acute pattern by a differential agglutination test but did not have lymphadenopathy or other nonocular clinical disease. Most of these patients were non-im-

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munosuppressed, however the authors did not include chronic toxoplasmic chorioretinitis without inflammatory signs. We report here the frequency of IgM, IgA and IgE specific anti-*T. gondii* antibodies in sera of Colombian patients with toxoplasmic chorioretinitis during the acute symptomatic setting (first episode or recurrence) and during the non-inflammatory status.

MATERIALS AND METHODS

Patients - We performed serological studies in consecutively non-immunosuppressed Colombian patients with ocular toxoplasmosis referred by opthalmologists during the period of June to September 1994 and March to June 1996. The patients presented retinal inflammatory lesions consistent with ocular toxoplasmosis and diagnosis was made using the criteria defined previously (Holland et al. 1996). The patients were asked about similar episodes and classified according to their clinical ocular history and the result of ofthalmoscopic examination. Group 1 consisted of ten patients with signs of vitritis and a first episode (established by history) and without old retinal scars at examination. Group 2 consisted of seven patients with recurrence, they had signs of vitritis and history of a least one similar past episode or the presence of retinal scars indicating past episodes. Group 3 consisted of eleven patients with old scars and without retinal inflammatory signs at the moment of sampling. These former patients were discovered in a prevalence study of ocular toxoplasmosis in the Quindio region performed during January-August 1996. All patients were originating from the Quindio region (central Andean area of Colombia). The collection of clinical data was made by a questionnaire including clinical and epidemiological antecedents. All patients were asked about extra-ocular clinical manifestations and were also examined looking for extraocular signs.

Serological studies - All studied patients were positive for specific IgG anti-*T. gondii* by immunofluorescence antibody test (IFAT-IgG) (Instituto Nacional de Salud 1981). Briefly, the formaldehyde-treated tachyzoites obtained from peritoneal exudate of mice was fixed on glass slides. Two fold dilutions of serum samples were incubated 1 hr and washed twice with 150 mM phosphate buffer (pH 7.2) and then incubated with a fluorescein isothiocyanate-conjugated secondary antibody (Fluoline H, Biomérieux) diluted 1:320. The IFAT-IgG result was considered to be reactive when fluorescence of the entire parasite appeared at dilution of 1:8. Frenkel (1990) noted that dilutions of 1:8 can be useful in old infection as can occurs in ocular toxoplasmosis and that cross reactions with infections by others *Coccidia* can be considered minimal in humans. Specific IgM, IgA and IgE anti-*T. gondii* antibodies were studied by immunocapture assay (IC) as described previously (Pinon et al. 1995) and performed in the laboratory of "Hôpital Maison Blanche" in Reims (France). Briefly, each serum sample was diluted (1:100) and deposited in three adjacent wells of microplates sensitized with anti-IgM, anti-IgA or anti-IgE monoclonal antibodies. The plates were then incubated for 2.5 hr. After the plates were washed with 150 mM phosphate buffer (pH 7.2), a suspension of formaldehyde-treated tachyzoites was added at concentration of 1, 1.5 and 2 million parasites to the three wells respectively. Sedimentation was read by an automated system with specific software and the results expressed in points from 0 to 12 according to end-point agglutination (Pinon et al. 1995). The following results were considered positive in the IC assay: 9 points for specific IgM, 4 points for specific IgA and 1 point for IgE.

RESULTS

The clinical data are summarized in Tables I, II and III. The mean age in group 1 was 27.9 years (range 11-57), in group 2 was 32.2 years (range 25-45) and in group 3 was 33.5 years (range 19-54). The distribution by sex was 60% (6/10) of males in group 1, 14.2% (1/7) in group 2 and 36% (4/11) in group 3. Serological results are shown in Table IV, V and VI. We found that chronic and acute ocular toxoplasmosis in 10/28 (35%) of Colombian non-immunosuppressed patients was accompanied by serological markers others than specific IgG. More interesting, one patient (patient no. 7) aged 57 years-old and with a first episode presented specific anti-*T. gondii* IgM plus IgA plus IgE positives test (IgM 12, IgA 12, IgE 1) strongly indicating a recent acquired infection and not recurrence of a congenital infection. In one patient (patient no. 15) with a recurrence (the first episode was two years before) we found the IgM and IgA positive tests, but the IgE test was negative. In another patient (patient no. 11) with recurrence IgM and IgE test were positives. In patients from the group without inflammatory signs two of them (patients nos. 22 and 23) were IgA positive without high IgG titers (<1/128 by IFAT technique) and none of them had IgM or IgE positive tests.

DISCUSSION

The only reliable way to ascertain a recently acquired *T. gondii* infection by serological tests is by documenting seroconversion. However, this finding can be difficult to make when a patient visits the ophthalmologist weeks or months after *T. gondii* infection. An alternative strategy to distinguish a recently acquired from a congenitally acquired ocular toxoplasmosis is by measuring *T. gondii*-specific immunoglobulins (other than IgG) like IgM, IgA or

TABLE I

Clinical findings and outcome for ten Colombian patients with a first episode of ocular toxoplasmosis

Patient no.	Sex	Age	Eye (s) involved	Localization of lesion	Visual acuity at sampling	Visual acuity at end of episode and complications	Duration of uveitis at the time of sampling	Extra-ocular symptoms	Treatment
1.	M	11	L	Vitritis	20/80	20/20	6 days	No	Cstd.
2.	M	19	L	Vitritis	20/70 at 2 m	20/70 at 3 m Persistent vitritis	30 days	No	Cstd. + P-S
3.	F	24	R	Peripherical chorioret.	20/20	20/20	5 months	No	None
4.	M	26	R	Vitritis	20/200	Only ligth Persistent vitritis	3 months	No	Cst.
5.	M	45	L	Peripapilar chorioret.	20/20	20/20	3 months	No	None
6.	F	54	R-L	R: macular chorioret. L: macularchorioret.	R 20/200 L 20/400	R:20/200 L: 20/400	2 months	No	None
7.	M	57	R	Vitritis	20/80	20/40 Persistent vitritis	20 days	No	Cstd.
8.	F	12	L	Macular chorioret.	CF at 3 m	CF at 3 m	40 days	No	Cst. + P-S.
9.	F	11	R	Vitritis	20/200	20/200	45 days	No	Cstd.
10.	M	20	R	Peripherical chorioret.	20/30	20/30	30 days	No	Cstd + P.S.

M: male; F: female; R: right; L: left; CF: counting fingers; Chorioret.: chorioretinitis; Cstd.: corticosteroids; P-S: pyrimethamine-sulfadoxine; severity score: normal 20/20, moderate: 20/30-20/100, severe > or = 20/200 (Snell's visual acuity table).

TABLE II
Clinical findings and outcome for seven Colombian patients with a recidive of ocular toxoplasmosis

Patient no.	Sex	Age	Eye (s) involved	Localization of lesion	Visual acuity at sampling	Visual acuity at end of episode and complications	Duration of uveitis at the time of sampling	Time from the first episode	Extra-ocular symptoms	Treatment
11.	M	25	R-L	R: macular chorioret. L.: peripher. Chorioret.	R: forms at 6 m L.: 20/30	R: forms at 6 m	15 days	14 years	No	Cst
12.	F	25	R	Vitritis	Amaurotic	Amaurotic Iris sinechial	10 months	5 years	No	Cst.
13.	F	26	R	Macular chorioret.	20/140	20/100	20 days	4 years	No	Cstd.+ P-S
14.	F	32	L	Macular chorioret.	20/140	Lost follow up	30 days	9 years	No	Cstd.
15.	F	44	L	Macular chorioret.	20/100	20/60	60 days	2 years	No	None
16.	F	45	L	Vitritis Chorioret.	20/400	Amaurotic Persistent vitritis	60 days	3 years	No	Cstd.
17.	F	28	L	Macular chorioret.	CF at 3 m	20/200	35 days	12 years	No	Cstd. + P-S

M: male; F: female; R: right; L: left; CF: counting fingers; Chorioret.: chorioretinitis; Cstd.: corticosteroids; P-S: pyrimethamine-sulfadoxine; severity score: normal 20/20, moderate: 20/30-20/100, severe > or = 20/200 (Snell's visual acuity table).

IgE. However, there are cases of reinfection presenting IgM or IgA alone or even IgE specific isotypes that have been documented (Fortier et al. 1991, Pinon et al. 1995). But, to our knowledge, there are not reports of simultaneous presence of anti-*T. gondii* specific IgM, IgA and IgE in reinfections. Then, simultaneous presence of IgM, IgA and IgE can be considered a strong evidence for recent primary infection. If this assumption is true, in our study we found that 1/10 (10%) of new cases of ocular toxoplasmosis in Colombian patients from the Quindio region could be related to a recent infection (proportion of cases with specific anti-*T. gondii* IgM plus IgA plus IgE positive in a first episode). Like Montoya and Remington (1996), we agree that this finding indicates that recent acquired toxoplasmosis is more frequent than believed. Extraocular manifestations of toxoplasmosis were not found in our series. However, in one patient, with an old scar, she stated presence of lymphadenopathies during her first episode of uveitis occurred many years ago. In addition, we have observed in Colombia two other cases with symptoms like fever and adenopathies and serological tests results of recent acquired infection, one of them was a woman who transmitted congenital infection to her offspring (Castaño et al. 1991). This suggests that postnatal acquired toxoplasmosis can produce ocular lesion either accompanied by extraocular symptoms or not. The physiopathological reason for one or the other event remain to be determined. There are similar reports about the frequency of ocular involvement in three outbreaks of recently acquired toxoplasmosis. In an epidemic in Atlanta only one of 37 affected individuals (2.7%) developed eye disease after four years of follow-up (Akstein et al. 1982). In a well documented family outbreak in New York city, one of seven members (14.2%) developed toxoplasmic chorioretinitis 129 days after infection (Masur et al. 1978), and in a recent outbreak in British Columbia (Canada) the number of symptomatic patients with ocular toxoplasmosis as clinical presentation was 19 of 100 acute outbreak-related cases (19%) (Bowie et al. 1997). However, at present we do not have longitudinal studies that establish the exact frequency of toxoplasmic chorioretinitis after postnatal acquired infection and if the frequency of this form can vary from one site to another.

As we stated above, the problem estimating the frequency of postnatal acquired ocular toxoplasmosis based on serological findings, is that it is possible in recurrent cases to find presence of antibodies classically considered to be markers of primary infection. The cases, in the present report, of a recurrence of toxoplasmic chorioretinitis with the presence of specific IgM plus IgA, or IgM plus IgE in serum, effectively suggest that IgM plus IgA

TABLE III

Clinical findings and outcome for eleven Colombian patients with chronic inactive ocular toxoplasmosis

Patient no.	Sex	Age	Eye (s) involved	Localization of lesion	Visual acuity at at sampling	Time from the first episode	Extra-ocular Symptoms
18.	M	17	R	Macular Scar	20/140	2 years	No
19.	F	19	R	Peripherical scar	20/20	3 years	Yes
20.	F	28	R	Peripherical scar	20/50	2 years	No
21.	M	33	L	Macular scar	20/200	20 years	No
22.	F	37	R-L	R: perimacular scar L: peripherical scar	R: 20/20 L: 20/20	1st episode: 6 years 2nd episode: 1 year	No
23.	M	38	L	Peripherical scar	20/20	Asymptomatic	No
24.	F	20	R	Peripherical scar	20/20	5 years	No
25.	F	20	R	Peripherical scar	20/30	18 months	No
26.	F	31	L	Peripherical scar	20/400	5 years	No
27.	F	58	R	Macular scar	CF at 3 m	20 years	No
28.	M	54	L	Macular scar	20/20	7 years	No

M: male; F: female; R: right; L: left; CF: counting fingers; Chorioret.: chorioretinitis; Cstd.: corticosteroids; P-S: pyrimethamine-sulfadoxine; severity score: normal 20/20, moderate: 20/30-20/100, severe > or = 20/200 (Snell's visual acuity table).

TABLE IV

Serological results for ten Colombian patients with a first episode of ocular toxoplasmosis

Patient no.	Inverse titers IgG IFAT	IgM IC	IgA IC	IgE IC
1.	64	8	2.5	0
2.	512	8	0	0
3.	512	**9**	0	0
4.	128	4.5	0	0
5.	256	**10**	1.5	0
6.	64	**10**	1.5	0
7.	128	**12**	**12**	**1**
8.	128	7	0	0
9.	512	**10**	0	0
10.	64	**10**	1	0
Total positives		6/10 (60%)	1/10 (10%)	1/10 (10%)

Cutoff values: IgM > 9, IgA > 4, IgE > 1. positive results are shown in bold; IFAT: immunoflourescence antibody test; IC: immunocapture test.

TABLE V

Serological results for seven Colombian patients with a recidive of ocular toxoplasmosis

Patient no.	Inverse titers IgG IFAT	IgM IC	IgA IC	IgE IC
11.	64	**11**	0.5	**2**
12.	64	0	0	0
13.	32	6.5	0	0
14.	128	8.5	0.5	0
15.	512	**9.5**	**11**	0
16.	128	5.5	0	0
17.	64	4	0	0
Total positives:		2/7 (28%)	1/7 (14.2%)	1/7 (14.2%)

Cutoff values: IgM > 9, IgA > 4, IgE > 1. positive results are shown in bold; IFAT: immunoflourescence antibody test; IC: immunocapture test.

or IgM plus IgE may be present in recurrent clinical episodes. Specific IgA without specific IgM has been reported in a case of reinfection in a French women who had an abortion (Fortier et al. 1991). Similarly, Pinon et al. (1995) described that in a group of HIV-infected patients with evidence of previous exposure to *T. gondii*, but no clinical manifestations, IgA and IgE were detected in 11% and 4% of cases. It would be important to determine if recurrent episodes are due to endogenous reactivation of old infection or to a new reinfection because in this way effective preventive measures may vary, like antibiotic prophylaxis in the case of endogenous reactivation or hygiene measures for the case of exogenous reinfection.

We have to learn more about the pathogenesis of ocular toxoplasmosis in order to get better therapeutic and public health measures. It will be important to establish if cases of postnatally acquired toxoplasmosis are due to a clonal related strain. Recently we forwarded some hypotheses concerning this topic (Gomez et al. 1997). Montoya and Remington (1996) suggest that the congenital form of ocular toxoplasmosis have a more guarded prognosis than postnatally acquired toxoplasmosis but we found that ocular toxoplasmosis was compli-

TABLE VI

Serological results for eleven Colombian patients with chronic inactive ocular toxoplasmosis

Patient no.	Inverse titers IgG IFAT	IgM IC	IgA IC	IgE IC
18.	64	4	0	0
19.	64	4.5	0	0
20.	64	7	0	0
21.	128	5.5	0	0
22.	64	5.5	**4.5**	0
23.	8	8	**4**	ND
24.	16	4.5	0	0
25.	64	8	0	0
26.	64	8.5	1	0
27.	8	8.5	0	0
28.	8	7.5	0.5	0
Total positives		0/11 (0%)	2/11 (18%)	0/10 (0%)

Cutoff values: IgM > 9, IgA > 4, IgE > 1; positive results are shown in bold; IFAT: immunoflourescence antibody test, IC: immunocapture test, ND: not done.

cated with a persistent vitritis in our patient with serological evidence of recently acquired toxoplasmosis. The study of Ronday et al. (1995) also found severe complications in six of eigth patients with presumed acquired ocular toxoplasmosis. It would be important to test whether Colombian strains of *T. gondii* are different to strains from other geographical regions and if different strains have different pathogenic properties. In a national survey, 47% of the Colombian population possessed specific IgG antibodies indicating a high exposition of population to *T. gondii* (Juliao et al. 1983), but we do not know the real impact of their pathogenic manifestations and studies about the prevalence of ocular toxoplasmosis in the general population are presently going on.

In conclusion, we found that 10% of patients with a first episode of ocular toxoplasmosis have serological findings of recently acquired infection (simultaneous presence of IgM, IgA and IgE). During recurrences specific anti-*Toxoplasma* IgM/IgA and IgM/IgE were present and IgA alone was found in chronic setting. We need studies in order to determine the frequency of acquired ocular toxoplasmosis, if the reinfections can trigger recurrences (i.e. in the Colombian cases of recurrence with presence of IgM and IgA or IgM and IgE in serum) and the impact of strain diversity in the ocular presentation of human toxoplasmosis.

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