European Journal of Ophthalmology / Vol. 13 no. 1, 2003 / pp. 74-79

Retinochoroiditis associated with congenital toxoplasmosis in children: IgG antibody profiles demonstrating the synthesis of local antibodies

R. DE MARCO[2], R.CECCARELLI[1], R. FRULIO[1], C. PALMERO[1], P. VITTONE[2]

[1]Department of Infectious Diseases, University of Genova, Genova
[2]Department of Ophthalmology, G. Gaslini Institute, Genova - Italy

PURPOSE. *Retinochoroiditis is generally diagnosed after the first year of life and the association with congenital toxoplasmosis presents a diagnostic dilemma. The detection of local intraocular specific antibodies could be useful for diagnosis.*
METHODS. *We studied six patients (mean age 7±5 years) with retinochoroiditis which appeared after the first year of life. Aqueous and serum were analysed by immunoblotting for anti* T. gondii *IgG to diagnose toxoplasmosis.*
RESULTS. *All serum samples were positive only for anti* T. gondii *IgG. The retinochoroiditis was active in three patients and inactive in the others. Immunoblot analysis of serum and aqueous from the patients with active lesions showed IgG versus the specific antigen of* T. gondii. *In the patients with inactive lesions the pattern was the same in the two compartments. In active forms, aqueous and serum Western blot patterns differed in proteins lower than 16kDa and higher than 116kDa: in aqueous the findings were typically positive for 30kDa.*
CONCLUSIONS. *Aqueous humour analysis by the Western blot technique may be useful in the diagnosis of congenital toxoplasmosis. In the present small series, we nevertheless detected different patterns for inactive and active retinochoroiditis, confirming the diagnosis in the latter. Aqueous humour paracentesis may be indicated in a child with active retinochoroiditis with unusual clinical features, appearing after the first year of life, and with no clinical or serological evidence of congenital infection. (Eur J Ophthalmol 2003; 13: 74-9)*

KEY WORDS. *Immunoblotting, Retinochoroiditis, Congenital toxoplasmosis, Children*

Accepted: May 20, 2002

INTRODUCTION

Toxoplasmic retinochoroiditis or ocular toxoplasmosisis (OT) is one of the major causes of visual impairment in children and accounts for about 25% of all cases with uveitis, or 30-50% of posterior uveitis (1-4). Most diagnoses of ocular toxoplasmosis should be considered presumptive since it is not possible to examine histopathologic sections in children.

Most infants with congenital infection are asymptomatic at birth but develop retinal or neurological damage later in life, leading to loss of vision. The most frequent ocular lesion is focal necrotizing retinochoroiditis and the diagnosis relies on the presence of these lesions and on serum tests in the infant and mother.

1120-6721/074-06$03.00/0

This paper has been presented as oral communication to EVER 2000 Palma de Mallorca, Spain

When the typical signs are present, the diagnosis of recurrent OT is easy for the ophthalmologist: the retina has an irregular oval yellowish lesion with an adjacent old scar (satellite lesion), segmentary perivasculitis with overlying exudation of the vitreous. However, the diagnosis is not always straightforward, especially in cases lacking serologic confirmation of the infection (antitoxoplasma IgG titers positive and IgM negative), when there is no information about the mother during pregnancy, or when chorioretinitis does not show typical clinical features. There are in fact many atypical forms of ocular toxoplasmosis and the differential diagnosis may include different etiological agents such as human cytomegalovirus, herpes simplex virus, Epstein Barr virus, *Treponema pallidum*, mycobacterium and fungi.

Since the treatment of congenital toxoplasmosis may involve multiple side-effects in children, we should consider therapy only when the diagnosis is confirmed. In cases where serum tests give no conclusive result, analysis of intraocular parasite-specific antibody production (4, 5) can help reach a firm diagnosis of ocular toxoplasmosis.

The simultaneous detection of pathogens and consequent antibodies in serum and aqueous humour and the calculation of a ratio between these values in the two compartments (Goldmann-Witmer coefficient (6, 7)) makes it possible to determine the local production of antibodies against toxoplasma and provides quantitative information about the intraocular antibody response (7).

As regards qualitative information about local antibody production it is important to consider the different stages of toxoplasma which changes from a rapidly replicating tachyzoite lytic form to a slow-growing bradyzoite inside long-lasting tissue cysts. These two stages are antigenically distinct and therefore the antibody response is likely to be highly stage-specific (Tab. VI) (8-10). An immunoblotting technique can be employed to analyze the antigen specificity of locally produced antibodies, comparing aqueous humour with serum samples (11, 12).

TABLE I - PROFILES OF PATIENTS WITH OCULAR TOXOPLASMOSIS

	Active			**Inactive**		
Test (*)	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
Serum IgG vs. *T.gondii* (IU/ml)	71	334	139	11	67	118
Aqueous humour						
IgG vs. *T.gondii* (IU/ml)	2	4	4	1	1	1
C-coefficient (Rate)	9.8	9.5	12.5	3.4	3.2	3.1

(*) No patient was positive for IgM and IgA in serum and aqueous humour

TABLE II - IgG ANTIBODY PATTERNS IN SERUM SAMPLES (identifiable antigens)

	Active			**Inactive**		
Antigen M.w.	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
16	-	-	-	-	-	-
27	+	+	+	+	+	+
30	-	-	-	-	-	-
35	-	-	-	-	+	+
40	+	+	+	+	+	+
60	-	-	-	-	-	-
67	-	-	-	-	-	-
70	+	+	+	+	+	+
88	-	-	-	-	-	-
94	-	-	-	-	+	-
110	-	-	-	-	-	-

PATIENTS AND METHODS

From April 1999 to October 2001, 329 children were admitted to our center for suspected congenital toxoplasmosis. Children were considered congenitally infected if specific IgM or IgA was detected in serum following the classification of the European Research Network on Congenital Toxoplasmosis. However, a negative IgM does not exclude this diagnosis as children with specific IgG persisting for more than one year should also be considered congenitally infected.

Of these patients 188 were children whose mothers had been followed for toxoplasmosis infection during pregnancy: only one of them developed clinical

TABLE III - IgG ANTIBODY PATTERNS IN SERUM SAMPLES (unidentifiable antigens)

	Active			Inactive		
Antigen M.w.	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
<16	+	+	+	-	-	-
17-26	+	+	-	+	-	+
31-34	+	+	+	+	+	+
36-39	+	+	+	+	+	+
41-59	+	+	+	+	+	+
61-66	+	+	+	+	+	+
68-69	+	+	+	+	+	+
71-87	-	-	+	-	-	-
>110	+	+	+	-	-	-

TABLE IV - IgG ANTIBODY PATTERNS IN AQUEOUS SAMPLES (identifiable antigens)

	Active			Inactive		
Antigen M.w.	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
16	-	-	-	-	-	-
27	+	+	+	+	+	+
30	+	+	+	-	-	-
35	-	-	-	-	-	-
40	-	-	-	-	-	-
60	-	-	-	-	-	-
67	-	-	-	-	-	-
70	-	-	-	-	-	-
88	-	-	-	-	-	+
94	-	-	-	-	-	-
110	-	-	-	-	-	-

TABLE V - IgG ANTIBODY PATTERNS IN AQUEOUS SAMPLES (unidentifiable antigens)

	Active			Inactive		
Antigen M.w.	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
<16	+	+	+	-	-	-
17-26	+	+	+	+	+	+
31-34	+	+	+	+	+	+
36-39	+	+	+	+	+	+
41-59	+	+	+	-	-	-
61-66	+	+	+	-	-	-
68-69	+	+	+	+	+	+
71-87	-	+	-	-	-	-
>110	+	+	+	-	-	-

signs of retinochoroiditis during the period of clinical observation. We saw 141 for the first time after one year of age and their mother's serological profile during pregnancy was not known. Among 49 of these patients (34.8%) with signs of ocular toxoplasmosis, 36 had inactive retinochoroiditis with chorioretinal scars in the macular, juxtapapillary or peripheral areas and 13 had active retinochoroiditis. In 29 of these patients, we were able to confirm the diagnosis of retinochoroiditis due to congenital toxoplasmosis (59.2%), while in the other 20 (8 with active retinochoroiditis and 12 with inactive retinochoroiditis) both the clinical history and maternal serological data during pregnancy were lacking and the retinochoroiditis showed atypical clinical features, so diagnosis could not be confirmed.

Only in six cases out of 20 were we able, (under general anesthesia necessary for magnetic resonance imaging), to do aqueous humour paracentesis for Western Blot analysis.

Paired samples of aqueous humor and serum from six children (mean age ± standard deviation 7±5 years) (Tab. I) were analyzed. In all these cases, it was not possible to confirm the diagnosis of congenital toxoplasmosis. Toxoplasmic retinochoroiditis was suspected and considered in the differential diagnosis but the clinical presentation was not diagnostic and the response to treatment was inadequate. The clinical picture of retinochoroiditis was not clearly suggestive of toxoplasmosis and, at the time of the examination, the typical clinical picture of a focus of necrotizing retinochoroiditis adjacent to a pigmented retinochoroiditic scar was not present.

Aqueous humor paracentesis gave paired samples of aqueous humor and serum, which were analyzed as follows:

- By radial immunodiffusion for total IgG concentration – 5 µL of undiluted serum and 5 µL of diluted aqueous humor (1/10 using a low-level plate).
- By ELISA for anti *T. gondii* IgG (ETI-TOXO G Sorin IT), 25 µL of undiluted IgM and IgA (ISAGA Plus Biomeriux FR) – 25 µL of undiluted serum and 40 µL of diluted aqueous humor (1/20).
- By immunoblotting for anti *T. gondii* IgG (Autoim-

TABLE VI - MAJOR PROTEINS IN RELATION TO THE DIFFERENT STAGES OF *TOXOPLASMA GONDII* (*)

	Gene	**Gene product**	
		MW (kDa)	**Location**
Tachyzoite specific	SAG1	30	Surface
	SAG2	22	Surface
Constitutive	SAG3	43	Surface
	GRA1	24	Intravacuolar - soluble
	GRA2	28	Intravacuolar
	GRA3	30	PVM(**)
	GRA4	36	Intravacuolar
	GRA5	21	PVM
	GRA6	32	Intravacuolar
	GRA7	29	PVM - Intravacuolar
	GRA8	38	PVM
Bradyzoite specific	SAG4	18	surface
	BSR4	36	surface
	BAG1	28	cytoplasm
	MAG1	65	matrix
	LDH2	35	cytoplasm
	CST1	116	cyst wall
	-	29	matrix
	-p34	34	surface
	-p21	21	surface

* The rhoptry proteins (ROP1-2-3-4-8) and the microneme proteins (MIC 1-2-3) are similar in bradyzoites and tachyzoites.
** Parasitophorous vacuole membrane.

mun Diagnostika Labor Strassberg D) (dilution 1/100 for serum and 1/20 for aqueous humor).

The Goldmann-Witmer ratio was calculated as follows:

(anti *T. gondii* IgG/ total IgG) (aqueous humor);

(anti *T. gondii* IgG / total IgG) (serum);

Patients were considered positive when the C coefficient was >3 (13).

RESULTS

Three patients had active retinochoroiditis and anterior uveitis and in the other patients the disease was inactive. The bands that were clearly recognised in every serum sample corresponded to a molecular weight of 27, 40, and 70kDa. These were considered specific for *Toxoplasma gondii* tachyzoite stage-specific antigens. No patient showed an antibody response to 30 kDa proteins (Tab. II). In every serum sample, bands were between 31-34 kDa (34 kDa bradyzoite-specific surface protein), 36-39 kDa (36 kDa bradyzoite-specific surface protein), 41-59 kDa and 61-66 kDa. Only patients with active ocular toxoplasmosis showed bands lower than 16 kDa and higher than 110 kDa (Tab. III).

Every aqueous humor sample presented an antibody response to 27 kDa but the antibody response to 30 kDa was only present in the aqueous humor of patients with active ocular toxoplasmosis (Tab. IV), with bands between 61 and 66 kDa, higher than 110 kDa and lower than 16 kDa. No such findings were seen in patients with inactive ocular toxoplasmosis. Only in the active group was there a 41-59 kDa band (Tab. V).

DISCUSSION

In 20 of the 49 children (40.8%), we could not confirm the diagnosis of toxoplasmic retinochoroiditis. We did aqueous humor paracentesis in three of 12 patients with inactive disease but Western blot analysis of serum and aqueous humour did not show any intraocular antibody neosynthesis. However, in three of the eight patients with active retinochoroiditis this analysis brought to light a response to antigens with molecular weight lower than 16kDa and higher than 110 kDa (116 kDa - cyst wall bradyzoite-specific).

The detection of low-molecular-weight antigens is diagnostic of active disease (14) and high molecular weight might be the result of the release of bradyzoites from ruptured cysts. In the patients with active ocular toxoplasmosis,a local antibody response to 30 kDa (surface tachyzoite-specific) was only detected in the aqueous humor. This protein may be related to the tachyzoite form and consequently to active retinochoroiditis. Only in active retinochoroiditis was there an antibody response to 61-66 kDa (65 kDa matrix bradyzoite-specific) and 41-59 kDa. The presence of this latter band might be related to a local antibody response to 43 kDa (constitutive surface protein) or could indicate an antiretinal immunological response to h-SAg (15).

We can conclude that the local antibody response in ocular retinochoroiditis is:

1) *Toxoplasma gondii* antigen-specific
2) Different in active and inactive forms
3) Highly stage-specific for tachyzoites and bradyzoites

Though this was a purely preliminary study the findings illustrate the utility of aqueous humor analysis to determine local antibody production, particularly in children with no serological confirmation of the diagnosis of active Toxoplasma retinochoroiditis.

In our series,16.3% had active retinochoroiditis involving the macular area, potentially leading to severe permanent visual impairment. Analysis of aqueous humor antibody production against Toxoplasma helped confirm the diagnosis of infection, making it possible to start early treatment. Aqueous humor paracentesis in children is widely considered an invasive diagnostic approach as it usually requires general anesthesia, and our data suggest it is only needed in children with active ocular lesions.

Reprint requests to:
Riccardo de Marco, MD
Via Casaregis 5A/13
Genova 16129, Italy
Riccardo_de_Marco@hotmail.com

REFERENCES

1. McCannel CA, Holland GN, Helm CJ, Cornell PJ, Winston JV, Rimmer TG. Causes of uveitis in the general practice of ophthalmology. Am J Ophthalmol 1996; 121: 35-46.
2. Roberts F, McLeod R. Pathogenesis of toxoplasmic retinochoroiditis. Parasitology Today 1999; 15: 51-8.
3. Mets MB, Holfels E, Boyer KM, et al. Eye manifestations of congenital toxoplasmosis. Am J Ophthalmol 1996; 122: 309-24.
4. Quentin CD, Reiber H. Analysis of aqueous humour in intraocular toxoplasmosis. Ophthalmologe 1997; 94: 728-31
5. Liekfeld A, Schweig F, Jaeckel C, Wernecke KD, Hartmann C, Pleyer U. Intraocular antibody production in intraocular inflammation. Graefes Arch Clin Exp Ophthalmol 2000; 238: 222-7
6. Goldmann H, Wittmer R. Antikorper im kammerwasser. Ophthalmologica 1954; 127: 323-30.
7. Kijlstra A, Luyendijk L, Baarsma GS, et al. Aqueous humour analysis as diagnostic tool in Toxoplasma uveitis. Int Ophthalmol 1989; 13: 383-86.
8. Soete M, Dubremetz JF. *Toxoplasma gondii*: kinetics of stage specific protein expression during Tachyzoite-Bradyzoite conversion *in vitro*. In: Current Topics in Microbiology and Immunology, Gross U, ed. Berlin: Springer - Verlag, 1996; 76-80.
9. Bohne W, Paemley SF, Yang S, Gross U. Bradyzoite-Specific genes. In: Current Topics Microbiology and Immunology, Gross U, ed. Berlin: Springer - Verlag, 1996; 81-94.
10. Soête M, Dubremetz J. *Toxoplasma gondii*: kinetics of stage-specific protein expression during tachyzoite-bradyzoite conversion *in vitro*. Curr Top Microbiol Immunol 1996; 219: 76-80.
11. Klaren VNA, Van Doornik CEM, Ongkosuwito J, Feron EJ, Kijlstra A. Differences between intraocular and serum antibody responses in patients with ocular toxoplasmosis. Am J Ophthalmol 1998; 5: 699-706.
12. Riss JM, Carboni ME, Frank JY, Mary CJ, Dumon H, Ridings B. Ocular toxoplasmosis: value of immunoblotting for the detection of an intra-ocular synthesis of antibodies. Pathol Biol 1995; 9: 772-8.
13. Luyendijk L, de Boer JH, Rothova A, et al. Sensitivity and specificity of anti-viral antibody determination in the aqueous or vitreous of uveitis patients. In: Dernouchamps JP, Verougstreate I, Casper Velu I, Tassignon MJ, eds. Recent advances in uveitis. Amsterdam: Kugler, 1993; 265-7.
14. Ashburn D, Davidson MM, Joss AW, Pennington TH, Ho-Yen DO. Improved diagnosis of reactivated toxoplasmosis. Mol Pathol 1998; 51: 105-9.
15. Whittle RM, Wallace GR, Whiston RA, Dumonde DC, Stanford MR. Human retinal antibodies in Toxoplasma retinochoroiditis. Br J Ophthalmol 1998; 82: 1017-21.