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Immunodiagnosis of ocular toxocariasis using Western-blot for the detection of specific anti-*Toxocara* IgG and CAP™ for the measurement of specific anti-*Toxocara* IgE

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Abstract

A prospective multicentric study was carried out to assess both the performance of Western-blot (WB) detecting specific anti-*Toxocara* IgG and that of CAP™ measuring specific IgE titre for the immunodiagnosis of ocular toxocariasis. For 14 outpatients presenting ophthalmic symptoms (choroiditis, chorioretinitis, papillar oedema, hyalitis, retinal detachment and/or uveitis), samples of serum and aqueous fluid (AF) were sent to the Department of Parasitology, University Hospitals, Toulouse, France. All patients but two tested positive with WB on the serum; 13 WB tests were performed on the AF, 12 of which were positive. The two patients who had a negative WB serum result tested positive for the AF. Specific IgE detection was considered as a complementary test of WB. Two patients showed a greater specific IgE titre in the AF than in the serum, and one had a positive result in the AF, but not in the serum. These six patients were considered as clear cases of ocular toxocariasis. Western-blot coupled with specific anti-*Toxocara* IgE detection appeared therefore to be an accurate procedure for the immunodiagnosis of ocular toxocariasis, provided the testing was simultaneously performed on the serum and AF.

Introduction

Toxocariasis is a worldwide helminthic zoonosis (Barriga, 1988) due to human infection by larvae of *Toxocara canis* (Beaver *et al.*, 1952), the common ascarid of dogs, and also by the cat ascarid *T. cati* (Petithory & Beddock, 1997).

The ocular form of the disease, termed ocular larva migrans (OLM), was primarily described by Wilder (1950). Ocular larva migrans is apparently a rare disease, since its incidence has been found at approximately 1 per 100,000 (Maetz *et al.*, 1987). Typically, OLM occurs unilaterally in toddlers, schoolchildren, teenagers and young adults (Glickman & Schantz, 1981), often exhibiting a history of pica and/or close contact with undewormed puppies (Glickman, 1993). The most common symptom is visual loss, with onset over a period of days to weeks. With funduscopy and biomicroscopic examination, uveitis, endophthalmitis, papillitis (Gass & Braunstein, 1983; Beerlandt *et al.*, 1995) and/or retinal granulomatous lesions (Gillespie *et al.*, 1993), or inflammatory masses (snow-banks) in the peripheral vitreous (Tran *et al.*, 1999), are observed. In some individuals these signs may wax and wane over a period of years. Ocular infection may also be subclinical and only detected during a routine eye examination.

The clinical diagnosis of OLM has been improved by the use of medical imaging techniques. In a group of 11 patients, ultrasonography of the eye showed high-reflective peripheral mass, vitreous band or membrane, and traction retinal detachment (Wan *et al.*, 1991), all results consistent with those yielded by computerized tomography or magnetic resonance imaging (Templeton & Rao, 1987; Mafee *et al.*, 1989).

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In the past, the laboratory diagnosis of OLM was achieved by pathological examination of enucleated eyes (Neafie & Connor, 1976). For very few patients, a mobile larva could be directly observed under the retina (De Souza & Nakashima, 1995; Meyer-Riemann *et al.*, 1999). However, such direct parasitological assessment is awkward and uncommon, and serological methods are now the mainstay for laboratory diagnosis.

The so-called TEX-ELISA (ELISA using the excretory–secretory antigens of *T. canis*, or TEX-Ag), was primarily described by De Savigny *et al.* (1979). When this test, which detects specific IgG in serum, was applied to the diagnosis of OLM, it was found to have less sensitivity than in the detection of the other forms of the disease (Glickman *et al.*, 1986). Moreover, since human toxocariasis is most often a benign, asymptomatic and self-limiting disease, most seropositivities correspond with the presence of residual antibodies, without any pathological significance. Serum immunodiagnosis alone is therefore insufficient to ascertain the toxocaral origin of observed ophthalmic problems.

A distinct improvement came from performing the immunodiagnosis on aqueous fluid (AF) obtained by puncture of the anterior chamber. In this humour, the titre of anti-*Toxocara* antibodies was found to be more elevated than in the serum (Brasseur *et al.*, 1984; Benitez del Castillo *et al.*, 1995). With reference to other immunoglobulin isotypes, Genchi *et al.* (1988) demonstrated that in four of five OLM cases the level of specific anti-*Toxocara* IgE in AF was greater than in the serum.

In the Department of Parasitology of the University Hospitals in Toulouse (CHU), France, routine imunodiagnosis of toxocariasis relies upon the simultaneous detection of both specific anti-*Toxocara* IgG by a Western-blotting procedure (WB) using TEX-Ag, and specific anti-*Toxocara* IgE by CAP™ Pharmacia with the same antigens. These tests are approved by the French Ministry of Health, and listed in the French Nomenclature of Laboratory Acts.

Since WB has been demonstrated to have a better sensitivity than TEX-ELISA from commercial kits (Gueglio *et al.*, 1994; Courtade *et al.*, 1995), the aim of the present study was to assess the value of the above-cited procedure (WB plus CAP™) for the immunodiagnosis of OLM in patients presenting clinical ophthalmic disturbances consistent with this aetiology.

Materials and methods

Fourteen outpatients, who attended a specialized consultation in the Department of Ophthalmology of the general or university hospitals in Angoulême (one patient), Caen (eight), Strasbourg (one), Tarbes (one) and Toulouse (three), were found to have clinical symptoms consistent with OLM, and their sera and AF were referred to the Department of Parasitology in Toulouse CHU from 1996 to 1999. Demographic, clinical and laboratory data were collected by the same investigator (LM).

Given the very low amount of fluid (about 50 μl) provided by anterior chamber puncture, no parallel testing was done using the more conventional TEX-ELISA for specific IgG.

The WB procedure was performed according to Magnaval *et al.* (1991). TEX-Ag were produced according to de Savigny's prototype method (De Savigny, 1975) modified by Bowman *et al.* (1987). With Western blotting, animal and human reference sera from toxocariasis cases showed a typical pattern where seven bands were split into two groups, the first group including four low-molecular weight bands, the second three high-molecular weight bands. Statistical analysis of results from all sera has previously demonstrated that these seven-band patterns were significantly correlated with cases of toxocariasis. Sera under test were diluted at 1:100, and AF at 1:10.

Specific IgE raised against TEX-Ag were detected and the IgE levels quantified as previously described by Magnaval *et al.* (1992). This technique was subsequently adapted to the CAP™ FEIA Pharmacia system (Pharmacia, Orsay, France). Briefly, 96-well microtitration polystyrene plates (Immunoplate™ Nunc, Poly-Labo Block, Strasbourg, France) were loaded with a TEX-Ag solution (50 μl per well) containing 5.0 μg protein ml^{-1} in a 0.1 M pH 9.6 carbonate–bicarbonate buffer. The plates were maintained for 18 h at 25°C in a moist chamber. The antigenic solution was then discarded and the plates were washed three times with PBS Tween (Tween 20: 0.05%) pH 7.2 buffer, dried and finally stored at −70°C. Sera and AF from patients were tested undiluted, according to Pharmacia's procedure. Results were expressed in *Toxocara* Units (TU) per litre, with 2.0 TU l^{-1} as a cut-off value.

Results and Discussion

Table 1 displays demographic data, as well as the ophthalmic symptoms of the patients. Table 2 shows the results from the blood eosinophil count and serological tests. The different WB patterns which were observed are shown in fig. 1.

Twelve of 14 patients exhibited a positive WB result from the serum, correlating with ten subjects with a similar positivity in the AF. Since WB is not a quantitative method, it could be argued that these specific IgG were not synthesized inside the eye, but were due to a leakage from blood provoked by the inflammation. The detection of specific IgE yielded higher levels in the AF than in the serum for patients nos 3, 9 and 11, thus suggesting an intraocular origin for these antibodies. These OLM cases were considered as having had an infectious metastasis during a generalized toxocariasis.

Patient no. 8 was especially interesting, and demonstrated a positive WB result from the serum, but not from the AF, while the specific IgE result from the AF was sharply positive (58 TU l^{-1}). A blood AF barrier breakdown would have elicited a similar leakage of IgG and IgE, and was therefore excluded. The diagnostic conclusion which was reached classified this patient as presenting both a generalized and ocular toxocariasis with a predominant specific IgE synthesis in the eye.

Two patients (no. 7 and no. 12) displayed a positivity relating only to the higher molecular weight bands. This

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Table 1. Demographic data and clinical symptoms of 14 patients referred to the Department of Parasitology, Toulouse University Hospital.

No.	Sex*	Age (in years)	Ophthalmic symptoms
1	F	41	Choroiditis, hyalitis
2	M	19	Chorioretinitis, hyalitis, papillar oedema
3	F	17	Chorioretinitis, hyalitis
4	M	46	Chorioretinitis
5	F	45	Choroiditis, hyalitis
6	M	35	Chorioretinitis
7	F	39	Chorioretinitis, hyalitis, retinal detachment
8	M	25	Chorioretinitis, hyalitis
9	F	10	Chorioretinitis, hyalitis
10	F	50	Chorioretinitis, hyalitis
11	M	33	Chorioretinitis, hyalitis, papillar oedema
12	M	47	Anterior uveitis, hyalitis
13	F	36	Chorioretinitis
14	M	21	Chorioretinitis, hyalitis

*F, female; M, male.

pattern could indicate a cross-reaction with another helminthiasis (Magnaval *et al.*, 1991), or a recent infection (Akao *et al.*, 1983). For patient no. 7, the WB result from the AF was lacking, and therefore a definitive diagnosis could not be made.

Patient no. 12 presented mainly an anterior uveitis, which is an uncommon symptom for ocular toxocariasis, along with a lack of specific IgE in the AF. Aetiologies of anterior uveitis are multiple, including systemic inflammatory diseases, often rheumatological in origin (Banares *et al.*, 1997). It was concluded that this patient presented a fortuitous association between a generalized toxocariasis and an anterior uveitis due to a non-parasitic inflammatory illness.

Patients nos 10 and 13 were typical OLM cases, since having only a positive WB result in the AF and also, for no. 10, the presence of specific IgE only in the AF.

In conclusion, WB detecting specific anti-*Toxocara* IgG, coupled with ELISA for specific IgE, appeared to be a convenient procedure for the immunodiagnosis of OLM, provided the testing was simultaneously performed on the serum and on the AF.

On the other hand, demographic data and blood eosinophil levels showed some substantial differences with the classic OLM profile, such as reported in the literature. (Glickman & Schantz, 1981; Gillespie *et al.*, 1993). If patients who definitely showed classic OLM symptoms are considered (nos 3, 8, 9, 10, 11 and 13), only nos 3 and 9 were under 20 years of age. This finding is

Table 2. Blood eosinophil counts and serological testing in 14 patients referred to the Department of Parasitology, Toulouse University Hospital.

	Blood			Aqueous fluid	
No.	Eosinophil count[a]	WB[b]	sIgE[c]	WB	SIgE
1	nd[d]	Positive	5.0	Positive	3.0
2	104	Positive	<1.0	Positive	<1.0
3	797	Positive	10	Positive	40
4	730	Positive	<1.0	Positive	<1.0
5	79	Positive	5.0	Positive	nd
6	200	Positive	<1.0	Positive	<1.0
7	500	Positive[e]	15	nd	9.0
8	480	Positive	78	Negative	58
9	1400	Positive	<1.0	Positive	10
10	nd	Negative	< 1.0	Positive	7.0
11	99	Positive	2.0	Positive	35
12	486	Positive[e]	35	positive	< 1.0
13	144	Negative	< 1.0	Positive	< 1.0
14	728	Positive	2.0	Positive	< 1.0

[a]Eosinophil cells per mm^3; [b]Western-blot; [c]level of specific anti-*Toxocara* IgE in *Toxocara* units per l; [d]not done; [e]positive for only higher molecular weight bands.

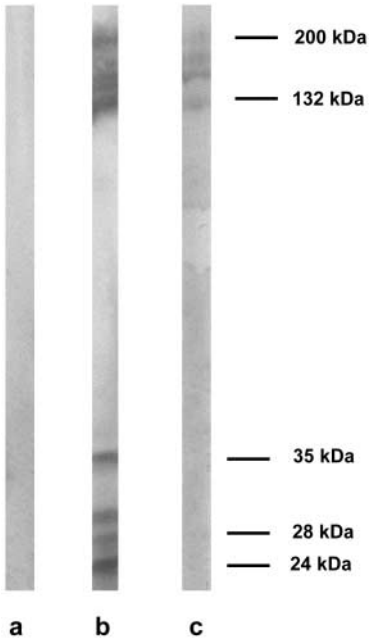

Fig. 1. Western-blot testing: (a) negative result, (b) full pattern and (c) pattern with only higher molecular weight bands.

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consistent with a more recent study concerning OLM by Yoshida *et al.* (1999) who reported that 89% of 38 OLM cases were older than 20 years of age. Regarding the pattern of occurrence of ocular toxocariasis, Glickman & Schantz (1981) stated that '*Toxocara* infection rarely results in concurrent ocular and systemic disease', a point which is at variance with the status of patients nos 3, 8, 9 and 11. Moreover, Glickman & Schantz (1981) underlined the absence of blood eosinophilia in OLM, a fact which is not consistent with the findings of the present study: out of six ascertained OLM patients, two (nos 3 and 9) had a blood eosinophil count over the generally accepted limit of 500 cells per mm^3 (Van Asseldeft, 1985).

The availability of accurate immunodiagnostic methods, together with an increase in the practice of anterior chamber puncture, is likely to allow the detection of many more OLM cases and, in turn, likely to change the spectrum of ocular toxocariasis.

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