Immunological analysis of the aqueous humour in candida endophthalmitis. II: Clinical study

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SUMMARY An anterior chamber paracentesis was carried out on six patients with candida endophthalmitis and on control subjects. We endeavoured to find out if there was local production of antibodies by evaluating the anti-candida antibody titres, the immunoglobulin G, and the albumin levels in the aqueous humour and in the serum. A significant difference in the level of specific antibodies between patients and controls was found. Local synthesis of anti-candida antibodies was discovered in five out of six patients with candida endophthalmitis. There would seem to be a correlation between the severity of uveitis and antibody titres.

Over the last two years the number of cases of candida endophthalmitis in Europe has increased, especially among heroin addicts.¹ While the clinical ocular findings are quite typical, in order to be absolutely sure of the diagnosis it is necessary to recover the candida from the vitreous body. However, this procedure at times proves unsuccessful. We therefore carried out anterior paracentesis on six patients presenting symptoms of candida endophthalmitis to determine whether local production of antibodies was present in the aqueous humour.

Materials and methods

Patients. Our study was carried out by comparing two groups of subjects: patients with candida endophthalmitis and control patients.

The six patients with candida endophthalmitis are described below.

CASE 1

A 21-year-old woman, a drug addict, complained of blurred vision. Her visual acuity was finger counting at 3 feet (1 m) (OD and OS). A dense cellular reaction was observed in the anterior chamber and in the vitreous. It was associated with a massive vitreous exudation in both eyes. The fundus examination,

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difficult to perform, revealed in each eye a paramacular white mass extending into the vitreous. Blood cultures were negative. Candidosis serological results were: immunofluorescence 1:320; haemagglutination 1:20 480; immunoelectrophoresis against *Candida albicans* antigenic extract 6 precipitin lines. Scalp culture revealed *Candida albicans* colonies.

CASE 2

A 33-year-old man developed bilateral blindness following neurosurgery. Examination revealed severe anterior uveitis in both eyes as well as considerable vitreous organisation, making fundus examination impossible. Blood cultures were negative. Candidosis serological results were: immunofluorescence 1:80; haemagglutination 1:320; immunoelectrophoresis against *Candida albicans* antigenic extract 1 precipitin line.

CASE 3

A 28-year-old man, a drug addict, consulted us for decrease of visual acuity in the left eye. Ocular examination revealed left side visual acuity of 1/10, severe anterior uveitis with many iris lens synechiae, and dense hyalitis obscuring retinal detail. The right eye was normal. Candidosis serological results were: immunofluorescence 1:160; haemagglutination 1:320; immunoelectrophoresis against *Candida albicans* antigenic extract 3 precipitin lines. Attempts to

recover candida organisms from the blood and scalp were unsuccessful.

CASE 4

A 39-year-old man suffered from a candida septicaemia induced by intestinal perforation owing to sigmoid diverticulitis. The ocular symptoms developed one month later while he was convalescing. His visual acuity was obviously normal. The left eye had grade 1+ cells in the anterior chamber and two exudative retinal lesions in the posterior pole. His right eye was normal on examination. Candidosis serological results were: immunofluorescence 1:320; haemagglutination 1:160; immunoelectrophoresis against *Candida albicans* antigenic extract 5 precipitin lines. Attempts to recover candida organisms from the blood and scalp were unsuccessful.

CASE 5

A 21-year-old man, a drug addict, presented with decreased visual acuity in the right eye. Ophthalmic examination revealed a visual acuity of 1/10, a Tyndall light phenomenon in the anterior chamber (1+ cells), a choroiditis paramacular lesion with local vitreous haze, and macular oedema. A search for candida from the scalp was negative. Candidosis serological results were: immunofluorescence 1:320; haemagglutination 1:20 480; immunoelectrophoresis against *Candida albicans* antigenic extract 2 precipitin lines. Blood cultures yielded many *Candida albicans* colonies.

CASE 6

A 30-year-old woman, a drug addict, had a visual acuity of 8/10 in both eyes with a normal anterior segment. A fundus examination revealed two white, round, jutting paramacular lesions. Scalp culture yielded *Candida albicans* colonies. Blood cultures were negative. Candidosis serological results were: immunofluorescence 1:320; haemagglutination 1:160; immunoelectrophoresis against *Candida albicans* antigenic extract 2 precipitin lines.

The control group consisted of 14 patients (six males and eight females) undergoing routine cataract extraction or retinal detachment surgery. Their mean age was 42 years, range 24 to 72 years. All these patients had raised serum anti-candida antibody titres—above 80 by immunofluorescence, above 160 by haemagglutination, or at least 1 precipitin line in immunoelectrophoresis.

Methods. The same immunological studies were performed on the patients suffering from candida endophthalmitis as on the control group. In each patient in order to determine whether intraocular synthesis occurred it was necessary to evaluate the anti-candida antibody titre and the IgG level in the

aqueous humour and in the serum. All the investigations were performed according to the methods described in the experimental study (anti-candida antibodies were analysed by indirect immunofluorescence according to Ambroise-Thomas²). Serum and aqueous humour albumin and immunoglobulin G concentrations were analysed by laser immunonephelometry (Behring). The only differences were: for immunofluorescence a goat antihuman immunoglobulin conjugated antibody (bioMérieux) was diluted to 1:100; for albumin analysis sera were diluted to 1:2000; aqueous humour was diluted to 1:10. For IgG analysis serum was diluted to 1:200, and aqueous humour was tested undiluted. In both cases concentrations were determined by comparison with dilutions of a standard human serum (standard protein Behring) analysed simultaneously under the same experimental conditions.

C coefficient. As the presence of candida antibodies in the aqueous humour could be due either to a transudation of serum antibodies in the anterior chamber through the blood aqueous barrier, or to local synthesis, we calculated the C coefficient as we did in the experimental candida endophthalmitis.

In interpreting the results we followed Desmonts' formula,³ considering negative a C coefficient less than 2, significant a C from 2 to 4, and positive a C greater than 4.

Results

The results of aqueous humour and serum samples obtained from patients with candida endophthalmitis are presented in Table 1. Anti-candida antibodies were found in the aqueous humour of five patients. Concentrations in all patients of albumin and immunoglobulin G were high in the aqueous humour. The C coefficient was positive in three cases, significant in two cases, and negative in one. In the patients with a positive coefficient there was very intense anterior segment inflammation. Two other patients with slight local production showed less serious anterior uveitis. The only patient with a normal anterior segment presented no local production of antibodies.

The results of immunological studies in the control group are presented in Table 2. Despite raised anti-candida antibody titres in the serum of each patient, specific antibodies were found in the aqueous humour in only seven patients. All cases showed a C coefficient equal to or less than 1.

Discussion

Clinical examination may make us fairly certain of

Table 1 Results of tests on the aqueous humour and the serum: patients with candida endophthalmitis

Biological data	Patients						
	1	2	3	4	5	6	
	Before cure		After cure				
Immunoglobulin G							
Aqueous humour (mg/l)	410	25	68	240	50	24	12.7
Serum (mg/l)	18 270	11 510	9 300	12 870	14 800	16 890	15 050
Albumin							
Aqueous humour (mg/l)	2 050	220	206	1 200	475	95	100
Serum (mg/l)	32 290	40 220	36 280	32 610	41 430	43 800	42 900
Candida antibodies							
Aqueous humour (titre)	32	No antibody	8	20	2	Positive not diluted	No antibody
Serum (titre)	320	80	80	160	320	320	320
C coefficient	4.5	No local production	13.6	7	2	2	No local production

the presence of candida endophthalmitis. But confirmation depends on biological tests. However, recovering candida from an extraocular sample is by no means easy; in addition the interpretation of serological results causes some difficulty. In any case, neither a positive extraocular cultured site nor a serology suggesting recent visceral candidosis allows us to hold the candida organism responsible for the uveitis. Recovering candida organism from the eye is the only proof that candida infestation has actually occurred. Culture of aqueous humour^{4,6} has never revealed candida except in the case of an aphakic patient.⁷ However, a vitreous tap^{8,9} or a vitrectomy^{5,10,11} does enable the collection of the candida organism. Recovering candida from the vitreous cavity is thus the best diagnostic method, though it

may at times prove unsuccessful. In fact, vitreous tap is not always positive,^{10,12} and vitrectomy may be negative despite concentration of the specimens, particularly after antifungal treatment.¹⁴ Thus anterior chamber paracentesis, while presenting only presumptive diagnostic evidence, seems to be an interesting technique. Testing for production of intraocular specific antibodies by anterior chamber paracentesis can be used to diagnose uveitis caused by toxoplasmosis,³ herpes,¹³ and *Toxocara canis*.¹⁴ So far as we know it has never been used to confirm a diagnosis of candida endophthalmitis.

Immunological tests of the aqueous humour in candida endophthalmitis appear to be helpful. In fact numerous control studies have shown good reproducibility of these tests. Testing for local produc-

Table 2 Results of tests on the aqueous humour and the serum: control patients

Anti-candida antibodies			Immunoglobulin G		Albumin		C Coefficient
AG (titre)	Serum (titre)		AH (mg/l)	Serum (mg/l)	AH (mg/l)	Serum (mg/l)	
Patient 1	0	80	74	10 440	620	32 190	0
Patient 2	0	160	25	11 410	210	34 940	0
Patient 3	1	160	15	7 280	148	36 320	0.3
Patient 4	0	80	30	11 240	224	46 020	0
Patient 5	1	160	93	15 020	130	32 250	1
Patient 6	0	80	26	9 260	260	31 680	0
Patient 7	1	160	110	10 620	489	31 790	0.6
Patient 8	0	80	20	11 020	150	33 150	0
Patient 9	1	160	80	12 450	540	50 700	0.9
Patient 10	0	80	40	11 430	180	37 390	0
Patient 11	1	160	120	10 140	502	42 990	0.5
Patient 12	1	160	73	11 250	250	41 250	0.9
Patient 13	0	80	40	16 800	205	47 700	0
Patient 14	1	160	105	11 234	512	35 420	0.6

AH=aqueous humour.

tion of anti-candida antibodies has enabled us to express strong support for the diagnosis of ocular candidosis in five out of six patients.

Moreover, it would seem to be justifiable to note that the more severe the anterior inflammation, the more revealing the anterior chamber paracentesis actually is. Testing for local production of anti-candida antibodies has shown different immune responses: some of the results were significant, while others were only slightly positive or even negative. The last result may be explained either by a leak of specific serum antibodies through the blood-eye barrier which has been modified by inflammation, or by a tap performed too soon, as suggested by the results of our experimental study.

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