

Rose Bengal Plate Agglutination and Counterimmunoelectrophoresis Tests on Spinal Fluid in the Diagnosis of *Brucella* Meningitis

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Rose Bengal and counterimmunoelectrophoresis, two tests that detect antibodies against different structural antigens, when carried out on spinal fluid permitted rapid diagnosis of human *Brucella* meningitis. The Rose Bengal test was positive in five out of five patients studied, and counterimmunoelectrophoresis was positive in all but one. The *Brucella* meningitis was characterized by an increase of immunoglobulin G in the cerebrospinal fluid.

Since the diagnostic utility of the Rose Bengal (RB) and counterimmunoelectrophoresis (CIEP) tests in human brucellosis (5) is now well established, we were interested in whether these tests could be used to detect *Brucella* antibodies in cerebrospinal fluid (CSF) of patients with *Brucella* meningitis, to establish a rapid diagnosis of this complication. Several authors (2, 8-10) have reported that there is an increase of immunoglobulin G (IgG) in CSF of patients with purulent and tuberculous meningitis. To our knowledge no report has been made on the behavior of IgG in CSF of patients with *Brucella* meningitis. In this study, we have also investigated the concentration of IgG in CSF from such patients.

The CSF studied was obtained from five patients with brucellosis, confirmed by seroagglutination, Coombs, complement fixation (CF-B), RB, and CIEP tests. All these patients had, in addition, clinical evidence of meningitis. Control CSF was selected from seven patients with brucellosis, confirmed by blood cultures and by serology but not showing any symptoms of meningitis, and was used both in the serological test and for the determination of the concentration of IgG.

Brucella abortus 99 antigen was prepared and employed in seroagglutination Coombs, and CF-B tests according to Alton et al. (1). The RB test was carried out as described by Morgan et al. (7). The antigen for this test was obtained from the Central Veterinary Laboratory, Weybridge, England. The soluble antigens employed in immunodiffusion and CIEP were lipopolysaccharide (LPS) extracted from *B. melitensis* 16M and protein antigen (P) extracted from *B. meli-*

tensis 115; methods of extraction, gel diffusion, and CIEP have been described previously (5). Complement fixation test with P antigen (CF-P) was also carried out, using 5 µg of P antigen in each antigen-serum mixture.

IgG concentration was determined by the radial immunodiffusion method of Mancini (6), using plates of ultra-low concentration obtained from ICL Scientific, Foundation Valley, Calif. The range of IgG concentration that could be detected varied between 3 and 15 mg/100 ml. The CSF samples of patients with symptoms of meningitis were diluted to produce results within the range of the reference serum, and the results were multiplied by the dilution factor. For anti-*Brucella* antibody adsorption, heat-killed cell suspensions of *B. melitensis* 16M and *B. ovis* were centrifuged, and packed volumes of 0.1 ml were mixed with 0.5 ml of CSF and placed at 37°C for 2 h. The mixture was centrifuged, and the supernatant CSF was removed and tested. In the same way, the CSF was adsorbed by mixing 0.5 ml with 2.5 mg of lyophilized P antigen.

Table 1 summarizes the results obtained by the serological methods and the concentration of IgG in CSF. The five samples from patients with meningitis symptoms were positive in the RB test, and all but one developed precipitation lines in less than 1 h when examined by CIEP using P antigen. The CSF that was positive in CIEP was also positive in the CF-P test. None of the control CSF samples from patients with brucellosis without meningitis symptoms were positive in any of the serological tests employed.

The results of adsorption show that *B. melitensis* 16M cells, but not P antigen or the rough

TABLE 1. Results of serological and bacteriological tests and concentration of IgG in CSF from patients with clinical symptoms of *Brucella meningitis*

Patient no.	Reciprocal of titers					CIEP ^a (no. of lines)	Culture	IgG concentration (mg/100 ml)
	RB	SAT ^b	Coombs	CF-B ^c	CF-P ^d			
1	2	20	1,280	128	16	2	<i>B. melitensis</i> biotype 2	27.2
2	1	10	320	32	16	2	ND ^e	23.2
3	4	40	160	32	<2	0	Negative ^f	13.2
4	4	40	320	ND ^e	ND ^e	2	ND ^e	ND ^e
5	8	80	2,560	256	128	5	Negative ^f	50

^a CIEP with P antigen.

^b SAT, Seroagglutination test.

^c CF test with whole cell smooth *B. abortus* 99.

^d CF test with P antigen.

^e ND, Not done.

^f Patients were under antibiotic treatment when the sample for the culture was obtained.

^g Not done because the sample was lost.

culture of *B. ovis*, were capable of removing antibodies active in the RB, seroagglutination, Coombs, and CF-B tests. On the other hand, antibodies active in CIEP and CF-P were only removed by P antigen. Our previous work (3, 4) demonstrated that *B. ovis* does not remove antibodies to LPS specific for the smooth phase of the genus *Brucella* and also that LPS is the antigenic component that plays the most important role in the RB, seroagglutination, and Coombs tests. Those results and our present findings show that in the CSF of patients with *Brucella meningitis* there are antibodies against two different structural antigenic components, which are detectable by RB and CIEP. On the other hand, the CF test can be used to detect antibodies against LPS and P antigens.

To confirm the existence of antibodies against the LPS antigen in CSF, the CSF of patients no. 1, 2, and 5 was concentrated five times by lyophilization. Double immunodiffusion using the LPS antigen against the concentrated CSF gave rise to the characteristic precipitin line of the LPS antigen (4).

The results of the test to determine the IgG concentrations in the CSF showed that in patients with brucellosis, but without symptoms of meningitis, the range of IgG values was less or equal to 3 mg/100 ml. However, in patients with *Brucella meningitis* the levels of IgG were significantly higher, these values varying between 13.2 and 50 mg/100 ml. These results indicated that the determination of IgG concentration in CSF could be of value in the diagnosis of *Brucella meningitis* in patients suspected of having brucellosis.

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