J. Hyg., Camb. (1962), **60**, 501
Printed in Great Britain

Meningococcal 'aseptic' meningitis

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(*Received* 11 *July* 1962)

INTRODUCTION

Aseptic meningitis has now been associated with an increasingly large number of viral agents (Lennette, Magoffin, Schmidt & Hollister, 1959; Meyer, Johnson, Crawford, Dascomb & Rogers, 1960; Combined Scottish Study, 1961). However, there are still many cases of aseptic meningitis in which no virus aetiology can be demonstrated and from whose cerebrospinal fluids (C.S.F.) no bacteria can be isolated. It seemed possible that some of these might be in patients with bacterial meningitis in whom the infecting organism had been suppressed by antibiotic therapy before admission to hospital. In such cases, despite failure to isolate an infecting organism, evidence of infection might be detectable by serological examination. In our laboratory sera from cases of aseptic meningitis are routinely tested by complement fixation (CF) against a battery of viral antigens: it seemed, therefore, that some cases of meningococcal infection might be detected by the inclusion of meningococcal antigens in this battery.

The use of CF for demonstrating antibodies in the blood of patients with known meningococcal infection was reported by Bell (1920) and by Cruickshank (1941). Both found CF more reliable than agglutinin tests. Bell reported that complement was fixed more completely or in higher dilutions with antigen of the same type as the infecting meningococcus; this suggests that an antigen made from one meningococcal strain may be mainly strain-specific. However, as there is some degree of antigenic crossing among all groups of meningococci, it seemed possible that a CF antigen made from one strain would be sufficiently group-specific to detect antibodies to other infecting strains. Strains responsible for the majority of meningococcal infections have been placed in two broad serological groups on the basis of agglutination reactions: Group A strains which are responsible for most epidemics, and Group B strains for most interepidemic infections (Branham, 1953).

The purpose of the present study was to assess (*a*) the relative strain-specificity of the meningococcal CF test, by investigating sera from patients with meningococcal meningitis from whom meningococci had been isolated and (*b*) the value of the CF test in the diagnosis of meningococcal infection in sera from cases in which no meningococci had been isolated.

MATERIALS AND METHODS

The patients in this study were admitted to hospital during the 2 year period January 1960 to December 1961. From each case paired sera were obtained, i.e. one specimen taken during the acute stage of the illness and a second specimen taken in convalescence, generally 10–14 days after the first specimen. The patients comprised two main groups:

Group 1: proven meningococcal (bacteria isolated from C.S.F. and/or blood);

Group 2: aseptic meningitis (no bacteria seen in or isolated from C.S.F.). This group was further subdivided into (*a*) those with a predominantly lymphocytic cellular response in C.S.F., and (*b*) those with a predominantly polymorphonuclear exudate. As meningococcal meningitis mainly affects young children, only those under 10 years of age were included in Group 2.

Complement-fixation technique

Separate antigens were made from five strains of meningococci: four of these, namely Group A strains 'Jordan' and 'Ipswich' and Group B strains 'Holmes' and 'M2092', were obtained from the National Collection of Type Cultures; the remaining strain 'McKenna' (Group B) was isolated in January 1961 from the C.S.F. of a child with meningococcal meningitis.

The method of preparation of antigens was a modification of that described by Price (1932). Each strain was cultured on several plates of chocolate agar which were incubated at 37° C. for 48 hr. in an atmosphere containing approximately 5% of carbon dioxide. The growth was washed off with N sodium chloride using 5 ml. per plate, and filtered through sterile gauze to remove fragments of medium. 0·2 ml. N sodium hydroxide was added per 5 ml. suspension which was then placed in a water-bath at 37° C. for 2 hr. The pH was then adjusted to 7·4 with N hydrochloric acid, and sodium azide was added to a concentration of 0·08%. The antigen kept equally well at 4° C., −40° C., and freeze-dried: no loss of titre was detected after 18 months' storage by any of these methods. The optimal dilution of each batch of antigen was determined by chessboard titration against positive human meningococcal antiserum.

Complement-fixation tests were carried out by the method previously described (Ross, 1961). Titres were expressed as the reciprocal of the highest dilution of serum giving complete or almost complete fixation: a test was considered positive if there was either a fourfold or greater rise between acute and convalescent specimens or if the titres although not rising were high (16 or over).

RESULTS

Group 1: proven meningococcal

As a preliminary test paired sera from 15 patients who became ill during 1960 were tested against separate antigens from only two of the strains, namely, 'Jordan' (Group A) and 'Holmes' (Group B). Only 7 of the 15 were positive to one or both antigens; 6 showed rising titres and one had high titres in both

specimens. This high proportion of negative results suggested that the antibody response might be relatively strain specific. For this reason sera collected from cases during 1961 were also tested against antigens from three additional strains, namely 'Ipswich' (Group A), 'M2092' (Group B) and one local strain 'McKenna' (Group B).

Table 1. *Meningococcal CF results in sera from 26 cases from which meningococci were isolated*

Case	Age (years) Sex	Month illness commenced (1961)	Days of illness of sera (acute/convalescent)	Serum titres (acute/convalescent) Group A Jordan	Group A Ipswich	Group B Holmes	Group B M 2092	Group B McKenna
1	1 (M)	Jan.	4/14	<4/8	<4/32	0*	4/16	<4/8
2	1 (M)	Jan.	3/13	0	0	<2/4	<2/4	Not done
3	5 (F)	Jan.	2/12	8/16	8/8	<4/16	4/16	<4/32
4	10/12 (F)	Jan.	3/12	0	0	Not done	0	16/8
5	12 (F)	Jan.	16/27	8/8	8/4	8/8	16/8	256/64
6	11/12 (F)	Jan.	8/18	8/2	16/8	0	32/16	32/8
7	1 (M)	Feb.	2/10	0	0	0	0	0
8	10/12 (M)	Feb.	4/12	0	0	0	0	<4/512
9	6/12 (M)	Feb.	2/15	0	0	0	0	<4/512
10	4 (F)	Feb.	2/10	0	0	<8/16	0	0
11	3/12 (F)	March	Not known	<16/<16	<4/32	4/16	<16/<16	4/64
12	2 (F)	March	4/13	0	<4/32	0	4/64	<4/16
13	4 (M)	March	6/14	0	0	0	0	16/16
14	3 (M)	March	2/14	0	0	0	0	<4/16
15	1 (M)	April	3/12	0	0	0	<4/16	0
16	1 (M)	April	2/12	<8/32	<8/32	<8/32	<8/64	<8/16
17	10/12 (F)	April	8/19	<8/8	<4/16	0	<4/16	0
18	1 (M)	April	Not known	16/8	32/16	0	<4/16	16/8
19	11/12 (M)	May	3/13	0	<4/8	0	<4/8	<4/8
20	25 (F)	May	2/10	4/32	<4/64	0	0	0
21	3 (M)	May	3/11	0	0	0	0	<4/16
22	1 (F)	June	2/12	4/8	16/32	4/16	16/32	16/16
23	70 (F)	June	3/19	0	0	0	0	<4/8
24	7/12 (M)	Sept.	3/13	0	0	0	0	0
25	8/12 (F)	Oct.	9/18	0	0	0	0	0
26	1 (F)	Nov.	6/34	0	0	0	0	0
No. of cases with rising titres				3	7	6	9	11
No. of cases with high titres				2	3	0	3	6
No. of cases with rising or high titres				5	10	6	12	17

*0 <4/<4.

The CF titres of sera from 26 patients against the five different antigens are shown in Table 1. Positive results were obtained with one or more of the antigens in 22 of the 26: 18 with rising titres, 4 with high titres. Of the five antigens 'McKenna' gave the highest number of positive results: 11 rising and 6 high titres (2 cases with high titres had rising titres to 1 of the other antigens). Although many of the sera showed antibody responses to several strains the highest titre

was generally to 'McKenna' which would again indicate that the most common infecting meningococci during this period were closely related to this strain; sera from case 3, from whom the 'McKenna' strain was isolated, showed higher titres to 'McKenna' than to the other antigens. Cross-reacting antibody responses with the antigens from the other strains showed no regular pattern. It was of interest that even in young infants good titres could be obtained: the highest titres were in two infants aged 10 months and 6 months respectively (cases 8 and 9).

Consideration of the seasonal distribution of these 26 patients showed that 23 became ill between January and June, 1961: only one of these 23 had a negative meningococcal CF test. In contrast, all three who became ill between June and December gave negative results with our meningococcal antigens. This is in keeping with strain specificity, and would suggest that the 'McKenna' strain was responsible for the majority of infections during the first half of 1961 but disappeared from the community thereafter.

Group 2: aseptic meningitis

In assessing results in this group we have excluded all cases which showed evidence of recent virological infection, i.e. isolation of a potentially pathogenic enterovirus from faeces or C.S.F. or serological evidence of recent infection with mumps, herpes, or ECHO 9, of which there was an epidemic during 1960. Of the remaining 59 cases of 'lymphocytic' meningitis, sera collected from 38 during 1960 were tested with two antigens, and sera from 21 during 1961 with five antigens (Table 2). Of these 59 cases, 3 (5 %) gave positive meningococcal CF tests. During the same period 10 cases of 'polymorphonuclear' meningitis were examined; 5 (50 %) gave positive results.

Table 2. *Positive meningococcal CF test in cases of aseptic meningitis*

Aseptic meningitis	No. positive/no. tested: 1960: with 2 antigens	1961: with 5 antigens	Total cases 1960–1961
Lymphocytic	3/38	0/21	3/59
Polymorphonuclear	2/4	3/6	5/10

The highest C.S.F. cell counts of the 8 cases of aseptic meningitis with positive meningococcal CF tests are shown in Table 3. The counts were low (below 30 per mm.^{3}) in 'lymphocytic' meningitis, whereas in 'polymorphonuclear' meningitis the cell counts were generally much higher. The finding of high CF titres in the first specimens of sera from cases 1 and 2 suggests that their illness was of longer duration than indicated by the clinical history, and perhaps a predominance of lymphocytes in the C.S.F. may indicate a process of recovery and be associated with a late stage of illness.

The usefulness of the test is illustrated by the clinical history of case 2. This child was ill for 5 days before admission to hospital on 13 May 1960, the main symptoms being refusal to feed, progressive irritability and cough. She was given

penicillin and tetracycline by her practitioner. On admission there was nuchal rigidity, Kernigism and strabismus. Penicillin was started because of the clinical findings, but the following day when the C.S.F. results were known (Table 3) penicillin was discontinued as it was considered that her illness was probably of viral aetiology, particularly as there were many cases of viral meningitis in the hospital at this time. However, her clinical course was unlike most of the other cases of viral meningitis in that she continued to be very irritable with general spasticity and inability to support her head. A further lumbar puncture done 4 days after admission still showed a slightly raised cell count (20 lymphocytes per mm.^{3}) with normal biochemical findings. When the meningococcal CF results were known on 14 June 1960, she was given a full course of sulphonamides with quite dramatic clinical improvement; the irritability disappeared and at the same time there was a return to full power as evidenced by an ability to lift her head and stand up unassisted.

Table 3. *Cases of aseptic meningitis with positive meningococcal CF tests*

Case	Age (years)	Sex	Type of aseptic meningitis	Highest C.S.F. cell count (per mm.^{3})	Sera: Day of illness (acute/ convalescent)	Sera: CF titre (optimal) (acute/ convalescent)
1	9/12	M	Lymphocytic	15	4/16	64/64
2	1	F	Lymphocytic	29	5/15	64/128
3	1	F	Lymphocytic	10	4/13	< 8/512
4	1	F	Polymorphonuclear	1,000	7/18	< 8/32
5	1	M	Polymorphonuclear	1,000	2/15	< 8/16
6	2	M	Polymorphonuclear	1,800	3/12	< 4/16
7	3	F	Polymorphonuclear	15	4/14	< 4/16
8	8	F	Polymorphonuclear	750	3/11	< 4/8

DISCUSSION AND CONCLUSIONS

The present study has shown that the meningococcal CF test has certain limitations and certain advantages. The limitations of the test were shown in the preliminary tests on bacteriologically proven cases of meningococcal meningitis. In such cases it was found that an antigen made from a local strain detected both the greatest number of positive results and the highest antibody levels. Thus there would seem little doubt that meningococcal antibodies are relatively strain-specific. As cross-reacting antibodies to heterologous strains were irregular both qualitatively and quantitatively, sera would require to be tested against antigens from several strains, preferably from those prevalent during the period of testing.

The main advantage of the test was demonstrated in the positive results obtained from 3 cases of 'lymphocytic' meningitis. Although no positive virological findings had been obtained in these cases, a viral aetiology would have been assumed and they would not have received antibiotic therapy. As a result of the positive CF tests they received full courses of antibiotic therapy, and in this way probably

avoided the consequences of more chronic infection. Heycock (1959) has reported that even when diagnosis is not made until late in the illness total recovery usually occurs when adequate chemotherapy is given. In the present series the number of cases of 'lymphocytic' meningitis diagnosed as meningococcal was disappointingly small; this would indicate that undiagnosable bacterial infections do not account for a high proportion of those cases of meningitis in which a virus aetiology is assumed but not proven. Thus, the routine use of the meningococcal CF test would not appear necessary in all cases of aseptic meningitis. However, if the child's clinical illness is prolonged and no routine positive virological findings have been found, the meningococcal CF test is worth consideration. It would seem that even in infants the CF antibody response to meningococcal infection is good; this contrasts with the absence or slow development in young children of CF antibodies in response to many viral infections (Anderson, Donnelly, French, Kalra & White, 1953; Grist, 1957).

Quite apart from its application to aseptic meningitis the usefulness of the test was also demonstrated in cases presenting as septicaemia from whose blood no organisms had been detected. During the period of this study positive meningococcal CF tests were obtained in three cases of septicaemia from which no meningococci had been isolated. One of these, a child aged 1 year, was of particular interest in that he showed myocarditis and pericarditis during his acute illness. When treated with penicillin and sulphonamides as a result of the positive meningococcal CF findings his cardiac symptoms gradually disappeared.

SUMMARY

The usefulness of the meningococcal CF test was assessed by investigating sera from (*a*) patients with meningococcal meningitis from whom meningococci had been isolated, and (*b*) patients with aseptic meningitis in whom no virus aetiology had been demonstrated and from whose C.S.F. no bacteria had been isolated.

Meningococcal CF antibodies were relatively strain-specific; cross-reacting antibodies to heterologous strains were irregular both qualitatively and quantitatively.

Positive meningococcal CF tests were obtained in 8 cases of aseptic meningitis, 3 showing a predominance of lymphocytes and 5 a predominance of polymorphonuclears in C.S.F. As a result of these positive tests, the cases of lymphocytic aseptic meningitis were given energetic antibiotic therapy to avoid the possibility of more chronic infection.

We wish to thank many clinical and bacteriological colleagues for their co-operation, and Prof. S. Graham for details of one case. We are grateful to Dr Patricia Bradstreet, Standards Laboratory for Serological Reagents, London, N.W. 9, for meningococcal agglutination results, and to Miss C. Gourlay for valuable technical assistance.

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