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Complement abnormalities during an epidemic of Group B meningococcal infection in children

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SUMMARY

During an epidemic of group B meningococcal infection mean values obtained in 96 consecutively affected children showed a reduction in classical pathway function (CH50), normal alternate pathway function (AP50), C4 and factor B levels, and raised C3 levels.

CH50, C3 and Factor B were however significantly lower in those children who had a rapid onset of illness, were in shock, had signs of septicaemia, had extensive skin purpura, or who died. The presence of detectable meningococcal antigen by Counter Immuno Electrophoresis (CIE) and laboratory evidence of Disseminated Intravascular Coagulation (DIC) also correlated with lower complement levels. The significant reduction in CH50 and Factor B in the more severely affected patients suggests that activation of both classical and alternate pathways occurs in group B meningococcal infection.

Keywords group B meningococcal infection children complement activation

INTRODUCTION

Complement levels are often abnormal in patients with acute meningococcal infection. Studies in Nigeria (Greenwood, Onyewotu & Whittle, 1976), Brazil (Ribeiro, Netto & Dos Santos (1981) and America (Hoffman & Edwards, 1972) have reported activation of the classical pathway of complement with low levels of total haemolytic complement (CH50) or C3 in patients with acute meningococcal infection. Alternative pathway activation has been demonstrated in patients with gram negative bacteraemia and shock (Fearon *et al.*, 1975) and activation of complement in patients with septicaemia may be a contributory factor to peripheral circulatory collapse, shock and death (Greenwood *et al*, 1976). Alternative pathway complement activation in patients with Dengue viral haemorrhagic fever was accompanied by signs of disseminated intravascular coagulation and shock (Bokisch *et al.*, 1973). In this study we have measured both classical and alternative pathway activity in children during an epidemic of group B meningococcal infection and correlated the levels with various clinical and laboratory measures of illness.

MATERIALS AND METHODS

Patients. Ninety-six consecutive children with acute meningococcal infection during an epidemic caused by group B *neisseria meningitidis* were studied on arrival at hospital. The patients were admitted to either the Red Cross War Memorial Children's Hospital or the City Hospital for

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Infectious Diseases. Informed consent was obtained from the childrens' parents or guardians and this study was approved by the Ethics and Research Committee of the University of Cape Town.

Bacteriological confirmation of the diagnosis was obtained in 89% of the children by means of positive blood or CSF culture; Gram stain of CSF, buffy coat or skin scraping; or positive Counter Immuno Electrophoresis (CIE) of serum or CSF. The 11 children in whom bacteriological studies were negative presented with the classical clinical picture of meningococcal infection and most of these children had already received antibiotic treatment outside the hospital. Thirty-seven of 41 cultures obtained from different patients that were suitable for typing were group B. Eighteen children were underweight, four had marasmus, one had kwashiorkor and 73 were well nourished. Three clinical groups of patients were identified: 32 children who had meningitis only, 13 who presented in a septicaemic state (purpura, ecchymosis and signs of shock) and 51 who had combined clinical signs of both meningitis and septicaemia. Seventeen children were in shock as defined by a systolic blood pressure of less than 75 mmHg in children under 4 years and less than 85 mmHg in older children. There were five deaths and four out of the five deaths were in very young children (under 7 months of age).

The childrens age ranged from 1 month to 11 years with a mean of 28 months. Fifty-three children were male and 43 female.

Serum specimens were collected on all patients prior to commencing treatment and processed immediately or stored in aliquots at $-80°C$ until used.

Classical Pathway (CH50) titration was performed by standard methods (Mayer, 1971) and alternative pathway activation (AP50) was measured by a haemolytic diffusion plate assay (Lachman & Hobart, 1978). The patients values were expressed as a percentage of normal control serum which had been aliquoted and frozen at $-80°C$.

C3, C4 and Factor B were measured by single radial immunodiffusion (Behringwerke AG Marburg, FRG).

Meningococcal antigen in serum and CSF was measured by Counter Immuno Electrophoresis (CIE) using an equine group B meningococcal antisera produced by Dr Carl Frash (Edwards 1971).

Dr Ann Orren kindly performed the measurements of functional C6 and C7 in selected sera using C6 and C7 deficient reagents as previously described (Orren, Lerch & Dowdle, 1983).

Statistical analysis using pooled variances and Boneferroni probabilities for multiple comparisons and analysis of variances were computed by the BMDP Suite of Statistical Programmes (Dixon *et al.*, 1981).

RESULTS

The results of complement studies are summarized in Table 1 and Fig. 1.

When the results for all the 96 patients studied were analysed the mean CH50 level was reduced (78% of normal); the mean AP50, C4 and Factor B levels were within normal limits, and the mean C3 level above the normal range.

Division of the patients into various clinical subgroups based on the presence or absence of shock; survival or death; a duration of illness of less or of more than 24 hours; and minimal or extensive purpura (more than 20 purpura spots or ecchymotic skin lesions) showed significantly lower complement levels particularly for CH50, C3 and Factor B in the more severely affected groups. The CH50, C3 and Factor B levels were also significantly lower in patients who presented with a septicaemic or combined septicaemic and meningitic illness. The presence of disseminated intravascular coagulation (determined by prolonged prothrombin and partial thromboplastin time, increased fibrin split products, thrombocytopaenia ($< 100{,}000$ platelets/mm^{-3}) and leukopenia ($< 5{,}000$ WCC/mm^3) was accompanied by significant decreases in all except C4 complement levels. The presence of detectable circulating serum antigen by CIE was significantly associated with reduced levels of CH50, C3 and factor B.

There were no significant differences in the levels of CH50, AP50, C3, C4 and Factor B in children of different age, sex, race or nutritional status.

Table 1. Mean values and standard deviations

	No.	CH 50 (% of normal control)		AP 50 (% of normal control)		C3 (mg/100 ml		C4 (mg/100 ml)		B (mg/100 ml)	
Reference range		(80–120)		(90–105)		(55–120)		(20–50)		(10–45)	
Total patients	96	78·3	(36·2)	101·95	(21·6)	123·9	(49·7)	34·1	(14·5)	29·7	(9·5)
In shock	17	42·9	(22·5)	85·2	(39·3)	67·3	(21·3)	23·4	(8·6)	18·7	(5·7)
Not in shock	79	86·0*	(34·0)	105·6*	(13·3)	136·0*	(45·4)	36·6*	(14·5)	32·0*	(8·4)
Duration 24 h	29	49·8	29·3	(89·5)	(26·2)	85·9	(39·1)	29·9	(12·4)	21·9	(8·1
Duration 24 h	67	90·6*	(31·7)	107·3*	(16·8)	140·3*	(44·7)	35·9	(15·0)	33·0*	(8·0)
Died	5	30·00	(0)	79·8	(29·4)	62·4	(10·2)	18·6	(4·7)	14·8	(3·5)
Survived	91	81·0†	(35·3)	103·2‡	(20·6)	127·2†	(48·8)	35·00‡	(14·4)	30·5*	(9·0)
Purpura > 20	45	62·8	(33·3)	94·5	(28·4)	99·1	(46·0)	30·6	(12·0)	25·3	(8·9)
Purpura < 20	51	92·0*	(33·1)	108·5†	(9·0)	145·7*	(42·2)	37·4‡	(15·9)	33·6*	(8·3)
Ecchymosis – present	21	53·1	(32·5)	96·3	(34·3)	90·3	(48·4)	28·8	(12·7)	22·5	(9·0)
Ecchymosis – absent	75	85·4*	(34·1)	103·5	(16·4)	133·3*	(46·1)	35·7‡	(14·7)	31·7*	(8·7)
Meningeal	32	94·4	(31·5)	108·2	(9·1)	148·2	(41·2)	37·4	(14·9)	33·7	(7·6)
Combined	51	73·6†	(36·3)	99·9	(26·1)	114·6†	(51·8)	32·6	(14·6)	28·7†	(10·1)
Septicaemic	13	57·2†	(32·1)	94·4	(21·4)	100†	(37·0)	32·3	(12·5)	23·3*	(6·7)
DIC – present	8	30·0	(0)	73·1	(29·4)	59·5	(20·5)	24·7	(11·2)	16·6	(2·9)
DIC – absent	26	94·7*	(33·3)	108·3*	(13·7)	149*	(45·1)	41·4†	(18·3)	34·5*	(7·7)
Platelets decreased	13	54·2	(33·1)	84·9	(31·5)	87·8	(51·9)	27·6	(11·8)	21·4	(9·4)
Platelets normal	77	82·7†	(35·2)	103·3†	(15·7)	129·9†	(46·8)	34·8	(14·5)	31·4*	(8·7)
WCC decreased	4	30	(0)	76·5	(32·8)	72·0	(20·8)	26·0	(14·4)	14·5	(4·2)
WCC normal	88	80·6†	(35·6)	101·9†	(18·2)	126·7‡	(49·8)	34·3	(14·4)	30·5*	(9·1)
CIE positive	39	61·4	(31·0)	97·2	(28·1)	99·9	(45·3)	30·9	(14·3)	25·1	(9·4)
CIE negative	55	90·9*	(34·7)	105·9	(13·3)	141·4*	(45·5)	36·4	(14·6)	33·1†	(8·2)

* $P<0·001$
† $P<0·01$
‡ $P<0·05$

separate variance t-test for comparisons in each subgroup (e.g., died v survived).

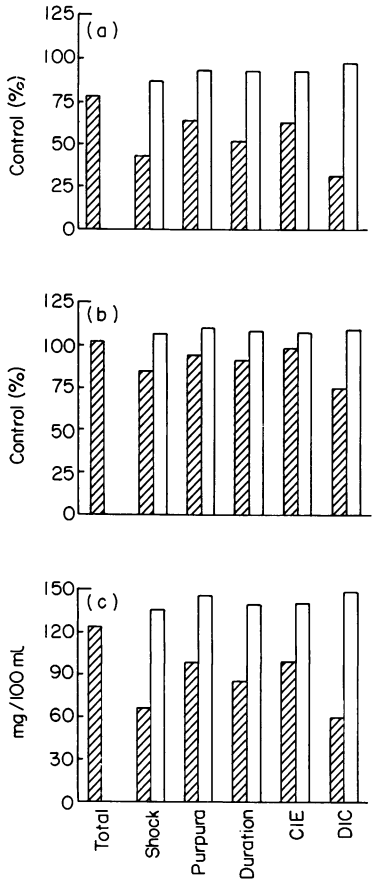

Fig. 1. The mean levels of CH50 (a), AP 50 (b) C3 (c) are shown for all the patients and for those with shock, extensive purpura, short duration, positive serum CIE and DIC (■). The mean values for those patients without these features are shown alongside (□).

One patient who had total absence of detectable CH50 and AP50 activity was C6 deficient. C3, C4, Factor B and C7 were normal in this patient. C6 and C7 levels were normal in a further 12 patients who had very low (but not absent) CH50 and AP50 activity.

DISCUSSION

Our results show that during an epidemic of group B meningococcal infection, complement levels were significantly lower in children in whom the disease had a rapid onset; who were in shock; who had extensive skin lesions and evidence of DIC: in those that died; and in those who had positive CIE results. The release of the biologically active cleavage fragments C3a and C5a may have contributed to the development of shock as these patients had significantly lower levels of C3. Children who presented with only meningitis had normal or elevated levels of complement especially C3, suggesting a normal acute phase protein response.

Low levels of complement may be age related (Fireman, Zuchowski & Taylor 1969) but this was not a factor in our patients because no correlation was found, between age and complement levels. Low levels may also be found in severe malnutrition due at least in part to decreased protein synthesis (Haller, Zubier & Lambert 1978). This also was not a factor in our study because of the lack of correlation between nutritional status and complement levels.

In sporadic and recurrent cases of meningococcal infection, congenital or acquired deficiencies of the terminal complement proteins (C5, C6, C7 & C8) are often found (Ellison *et al.*, 1983). In this study of 96 patients during an epidemic the frequency of complement deficiency as expected was much less and one patient had C6 deficiency.

The functional activity of the classical pathway of complement activation (CH50) was more depressed than alternative pathway activity (AP50). However, severely ill children had markedly reduced levels of the alternative pathway protein factor B, suggesting extensive alternative pathway activation. This apparent difference between factor B and AP 50 may be due to the fact that the haemolytic plate diffusion assay is less sensitive than either the CH50 assay or direct measurement of factor B. In our study the patients whose complement levels were most depressed were those with a rapid onset of symptoms. This and the fact that children have lower antimeningococcal antibodies than adults (Goldschnider, Gotschlich & Artenstein 1969), does not suggest that classical pathway activation by antigen/antibody complexes was a major cause of complement depletion. Bacterial endotoxins are activators of the alternative pathway but endotoxin levels in meningococcal infection have not been shown to correlate with complement levels (Tubbs, 1980) although some workers feel that shock and morbidity follow endotoxin mediated complement activation (Bjorvatn *et al.*, 1984). We are unable to confirm this because we did not measure endotoxin or antibody levels in this study but have shown that the presence of meningococcal antigen detected by CIE is strongly associated with reduced complement levels.

It seems likely that decreased complement levels are due to increased activation and consumption of complement by both classical and alternative pathways as has been proposed previously (Greenwood *et al.*, 1976; Riberio *et al.* 1981; Hoffman & Edwards, 1972). Our findings in a large series of children with group B meningococcal infection show that both complement pathways are activated and the degree of complement depletion correlates closely with the severity of illness and the presence of circulating meningococcal antigen.

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