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A second case of human C3b inhibitor (KAF) deficiency

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SUMMARY

The second case of C3b inhibitor deficiency is described in an 11-year-old girl who presented with recurrent attacks of meningitis, in between which she was well. Her serum showed all of the complement component changes noted in the first described case, although showing only a relatively slight defect in its ability to opsonize bacteria for phagocytosis and killing by polymorphonuclear leucocytes. This correlated with the patient's freedom from other infections.

INTRODUCTION

The clinical syndromes associated with genetic defects of individual complement components in man fall into three groups: (i) an increased tendency to bacterial infections as a consequence of a deficiency of C3, either primary (Alper *et al.*, 1972; Ballow, Shira, Harden, Soo and Day, 1975) or secondary (Alper *et al.*, 1970; Alper, Block & Rosen, 1973); (ii) an increased tendency to immune complex diseases, possibly because of poor antigen clearance, and usually associated with defects of the early classical pathway components C1 (De Braco *et al.*, 1974; Pickering *et al.*, 1971), C4 (Hauptmann *et al.*, 1974) and C2 (Ruddy *et al.*, 1970; Agnello, De Braco & Kunkel, 1972) and (iii) angio-neurotic oedema due to C1 esterase deficiency (Donaldson & Evans, 1963; Alper & Rosen, 1971) in which there is also an increased incidence of immune complex disease related to the secondary decrease in serum C4 and C2. Individuals with defects of the later components C5, C6, C7 and C8 have been described, and generally show little evidence of ill-health although they may show a tendency to Neisserian infections (Lim *et al.*, 1976; Peterson, Graham & Brooks, 1976).

In the only previous case of C3b inhibitor deficiency (Alper *et al.*, 1970) the lack of the inhibitor resulted in a continuing activation of the C3 feedback mechanism of the alternative pathway of complement (Alper, Rosen & Lachmann, 1972) so as to lower the C3 and factor B levels, and to exhaust the mechanism for enhancing C3 deposition, which is necessary for bacterial phagocytosis and killing (Fig. 1). The patient, who also had Klinefelter's syndrome and was mentally subnormal, had experienced recurrent upper and lower respiratory infections from an early age.

The present case, an 11-year-old girl, came to light because of recurrent attacks of meningitis from which she made a full and rapid recovery on antibiotic treatment, and in between which she was perfectly healthy. Initial investigations had shown that her serum immunoglobulin levels were normal, but that the levels of C3 and Factor B in her serum and plasma were considerably reduced, with appreciable amounts in the altered or activated form.

CASE HISTORY

The patient was a full-term normal delivery, with normal childhood development. At 4 months of age, she developed bacterial meningitis (*Streptococcus pneumoniae*), with a recurrence shortly after discharge from hospital.

At 1½ years she developed an ear infection which responded to treatment. At 7 years—bacterial meningitis again (*Neisseria meningitidis*). At 11 years—bacterial meningitis again (no organism isolated—associated with a purpuric skin rash). At 11½ years—bacterial meningitis again (*N. meningitidis*). Recovery from the infections has always been rapid and complete.

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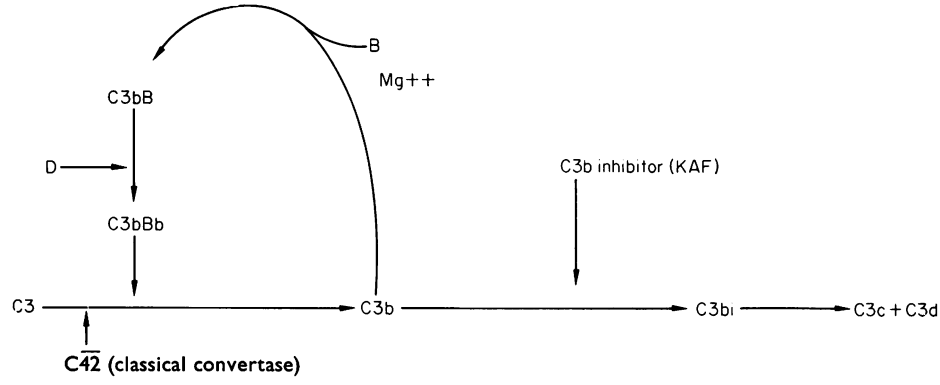

FIG. 1. C3 Feedback.

The patient and her mother deny a history of other infections (sore throats, pneumonia, etc.). There is also no history of the usual childhood exanthemata (measles, mumps or chicken pox) despite contact with cases in the neighbourhood, although the fact that she has serum antibodies to mumps and measles antigens suggests subclinical infections.

Family history. The patient's parents are unrelated (aged 48 and 44), and she has three siblings (sister 23, and brothers 22 and 17), all of whom are healthy. Her father's family had no history of infections, but her mother is one of five surviving members of a family of ten, four of whom died during their childhood, two from diphtheria; as far as she could remember none had a tendency to recurrent infections. An aunt (mother's sister) is mentally subnormal following a single attack of meningitis in childhood.

Clinical examination. This showed a healthy chubby mentally normal girl in the early stages of puberty. No abnormal findings were detected. Her referring paediatrician had sought ENT and neurological advice as to a local cause for the recurrent meningitis, but none could be found, and tomography of the skull and paranasal sinuses was negative.

Intradermal injections of PPD (Evans Medical) and *Candida albicans* extract (Bencard) showed positive induration at 48 hr.

Laboratory investigations. A large number of biochemical and haematological investigations were carried out and were within normal limits. She was blood group O Rhesus negative (rr), and normal α and β isoagglutinins were present. Her red cells were positive in the direct antiglobulin (Coombs) test, using anti-C3, but were negative for IgG and C1q. Despite this there was no evidence of increased red cell destruction *in vivo*, and her haemoglobin was normal. A similar finding had been reported in the previous case. Culture of blood, faeces, urine, nose and throat swabs revealed no pathogens. Reversed passive agglutination for Hepatitis B antigen was negative. Cytogenetic analysis of urine smears were positive for the X chromosome and negative for the Y chromosome. A summary of the main immunological tests is given in Table 1, of the complement component analysis in Tables 2 and 3, and of chemotaxis and opsonization studies in Tables 4 and 5 respectively.

Methods for Complement analysis. CH_{50} titres were measured by Mayer's method (1961). Functional measurements of C1, C4, C2, C5, C6 and C7, and the C3b inhibitor, Factor B, Factor D and 'total alternative pathway', were made in agarose gel plates using appropriate reagents as described by Lachmann, Hobart & Aston (1973). Functional C4 and C2 were also measured in test tubes (R. A. Thompson, in preparation).

Immunochemical measurements of C1q, C4, C3, C6, C8, the C3b inhibitor, Factor B and properdin were done by single radial diffusion using specific antisera. Immunoelectrophoresis and two-dimensional electrophoresis were carried out by standard methods, using antisera to Factor B (Behringwerke) native C3 (B antigen), C3c and C3d (Netherlands Red Cross Blood Transfusion Service).

RESULTS

Serum immunoglobulin levels and parameters of peripheral blood lymphocytes and polymorph function were normal. There was evidence of normal antibody production although none could be detected against group specific meningococcal antigen.

The main abnormalities lay in the complement system (Tables 2 and 3). The early classical pathway components C1, C1 esterase inhibitor, C4 and C2 were normal. The C3 level was considerably reduced, and two dimensional electrophoresis of freshly obtained plasma and serum (using an anti-C3c antiserum) showed that approximately 50% was in the C3b position (Fig. 2). An antiserum to native C3 (B antigen) reacted in immunoelectrophoresis only with the cathodal portion of the C3. Using a specific antiserum, no free C3d could be detected in a more anodal α-2 position, although this is a common finding in

TABLE 1. Summary of immunological investigations*

Serum immunoglobulins (g/l):	IgG – 14·0 (6·0–16·0):	IgA – 2·2 (0·9–5·4):	IgM – 1·1 (0·4–1·8)
Serum IgE (IU/ml):	160 (50–500)		
Parotid saliva IgA (mg/l):	36 (30–68)		
Complement:	Tables 2 and 3		
Phagocytic (polymorph) function tests:			
Nitroblue tetrazolium reduction:		spontaneous 6% (up to 10) stimulated 20% (20–50)	
Chemotaxis:		see Table 4	
Bactericidal Index (by the method of Quie *et al.*, 1967):		0·04 (< 0·2)	
Peripheral Blood lymphocytes:			
Spontaneous sheep cell (E) rosettes:	58% (55–70)		
Complement sheep cell (EAC) rosettes:	25% (20–35)		
Surface immunofluorescence (anti-Fab):	27% (16–30)		
Lymphocyte transformation (Ratio of H3-thymidine uptake in test to control)			
Phytohaemagglutinin	2·0 μg/ml—116 (93–918)		
	0·2 μg/ml— 47 (30–700)		
Antibody activity: isoagglutinins present			
E. coli antibodies† positive 1/64 (> 1/32)			
Viral complement fixation tests	—positive for Mycoplasma pneumoniae, measles, mumps		
	—negative herpes simplex, influenza A, B and C		
Meningococcal complement fixation test—negative			

* Normal ranges in brackets.
† By the method of Webster, Efter & Asherson (1974).

TABLE 2. Complement (normal ranges in brackets)

	Functional	Immunochemical (% NHS standard)
CH_{50} (u/ml)*	10; 14 (28–45)	
C1q		93 (75–130)
C1	100% NHS†	
C1 INH		96 (70–140)
C4	* 16,000 u/ml ($10–30 \times 10^3$)	110 (55–180)
	92% NHS†	
C2	* 3500 u/ml ($1–3 \times 10^3$)	
	100% NHS†	
C3		30; iu35 (60–150)
C5 (% NHS)†	32: 38	
C6 (% NHS)	80†	100 (80–180)
C7 (% NHS)†	13: 25 (75–140)	
C8		95 (90–140)

NHS = Normal human serum. Repeat values given in some cases

* Tube assay.
† Agarose-gel cell red assay.

patients with low C3 levels. C5 was reduced, as was C7, but C6 and C8 were normal and C9 was not tested.

Of the alternative pathway components, Factor B was much reduced, and electrophoresis of fresh plasma or serum showed that it was all in the $\bar{B}b$ state (Fig. 3). There was no functional Factor B activity as determined by the ability to combine with purified cobra venom factor to form a C3 splitting enzyme, or to lyse guinea-pig red cells in the presence of human EDTA serum. The properdin level was also

TABLE 3. Alternate pathway (% standard serum)

Factor B	*10 (Functional test*—nil)
Factor P (proderdin)	*25 (70–120)
Factor D	†72
'Total' alternate pathway functional activity† (lysis)—nil	

* Immunochemical quantitation by single radial diffusion.
† Agarose-gel red cell test.

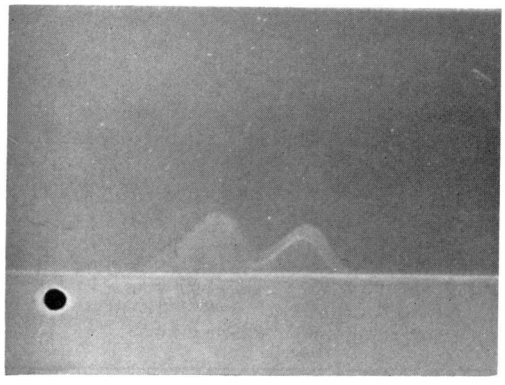

FIG. 2. Two-dimensional electrophoresis in agarose of fresh EDTA plasma from the patient. Anti-C3 antiserum was present in the agarose for the second dimension. Normal plasma gave a single peak.

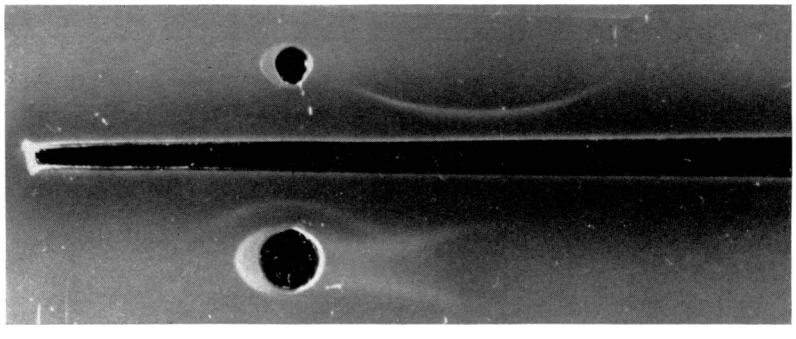

FIG. 3. Immunoelectrophoresis in agarose of fresh normal serum (top) and fresh serum from the patient (bottom). The trough contained anti-factor B, showing all in the Bb position in the patient. Anode to the right.

low although no assessment was made of its functional capacity. Factor D was probably normal. When purified Factor B was incubated with fresh patient's serum for 20 min at 37°C, there was complete conversion to Bb, while similar incubation with normal sera produced negligible conversion (Fig. 4). This had been reported in the original case and shows the presence in her serum of active C3b together with factor D. Tests for total alternative pathway activity, as detected by the lysis of unsensitized guinea-pig erythrocytes in agarose-gel containing MgEGTA, and by B antigen consumption on incubation of serum with zymosan, were negative.

The C3b inhibitor was completely absent on immunochemical testing of several specimens of serum, obtained over a 6 month period (Fig. 5). It was also not detected by more sensitive functional tests.

The serum showed a reduced ability to generate chemotactic factors for the patient's own and normal polymorphs, although the patient's cells responded normally to chemotactic stimuli (Table 4). There

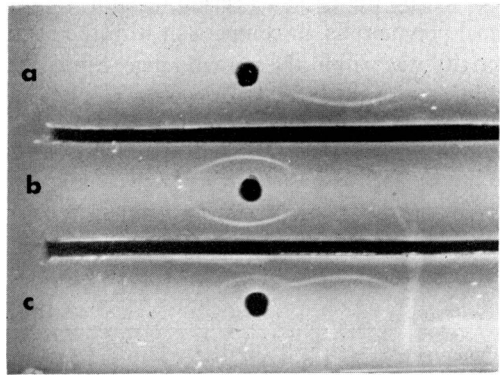

FIG. 4. Electrophoresis in agarose of a preparation of purified factor B, after incubation at 37°C with a fifth of its volume of (a) saline, (b) serum from the patient, (c) normal serum. Anti-factor B was in the troughs. Anode to the right.

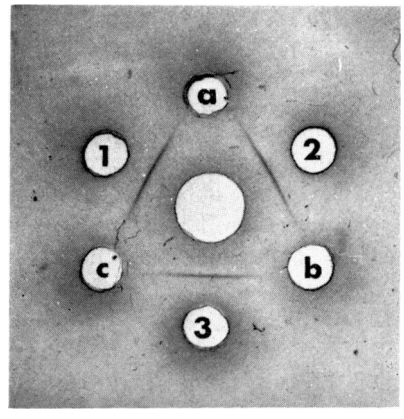

FIG. 5. Double diffusion in agarose-gel of three sera obtained on different occasions from the patient (a, b and c separated by intervals of 2 months and 3½ months respectively), and three sera from normal subjects (1, 2, and 3). The central well contained anti C3b inhibitor (KAF).

TABLE 4. Polymorph chemotaxis

	Responding cells		
	From patient	From normal subjects	
Chemotactic stimulus		A	B
Patients serum	80	85	42
Patients serum+zymosan			23
Normal serum	368	495	297
Normal serum+zymosan			324

* Method modified from Kay (1970) in which 10^6 purified peripheral blood polymorphonuclear leucocytes are suspended in balanced salt solution in a chamber on one side of an8·0 μm Millipore filter with serum or activated serum on the other side, the whole being incubated at 37°C for 90 min, after which the filters are stained and the cells which have migrated through are counted (total cells in eight random high power fields). Mean normal cell count in response to chemotactic factors from normal sera is 243 ± 58.

was slightly impaired opsonization by the patient's serum of both *Staphylococcus aureus* and *Candida albicans* for ingestion by normal polymorphs, in comparison with the control serum tested at the same time, although the level of activity was within the normal range. Similarly, the killing by normal polymorphs of *S. aureus* ingested in the presence of the patient's serum was slightly less than when normal serum cofactors were present, but the results were within the normal ranges for this test (Table 5).

TABLE 5. Bacterial killing and opsonization (with normal polymorphs)

			Normal serum	
		Patient's serum	(1)	(2)
S. aureus ($\times 10^3$) ingested after 20 min		22	43	35
Proportion (%) killed after 140 min		89	96	98
Candida albicans ingestion (mean organisms/cell)	Normal cells (1)	4·0	4·6	5·8
	Normal cells (2)	2·1	2·9	3·1 (No serum 0·1)

DISCUSSION

This patient is the second case of C3b inhibitor (KAF) deficiency to be reported. The defect is likely to be genetically transmitted since other members of her immediate family show reduced levels, although in none is the inhibitor completely absent. Full family studies of this and other components of the complement system, as well as other genetic markers are in hand, in an attempt to identify the mode of inheritance and linkage, if any, of this protein; these will be the subjects of further communications.

The serum shows all of the essential complement changes demonstrated in the previous case, and in the *in vitro* model proposed to explain the consequences of the defect (Nicol & Lachmann, 1973). However, while immune haemolysis was reduced, due to low levels of C3 and later factors, the overall functional activities of bacterial opsonization and killing in the presence of polymorphs were not grossly disturbed. The reason for the relative normality of these serum functions, in contrast to their defectiveness in the first reported case, is uncertain. The degree of alternative pathway 'exhaustion' may be less complete, or serum antibody and the classical pathway may be more effective in this patient. Whatever the reason the findings are in accord with her relative freedom from infections apart from meningitis.

There were no group specific anti-meningococcal antibodies demonstrable in her serum, and this may be why the recurrent infections took the particular form that they did. It is uncertain whether the risk from other infections will increase during adolescence and early adulthood, and her progress will be followed with much interest.

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