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A case of hereditary combined deficiency of complement components C6 and C7 in man

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SUMMARY

Immunological investigation of a patient presenting with candidiasis and toxoplasmosis revealed a combined deficiency of C6 and C7. Deficiency of C6 was total, but small amounts (less than 1 μg/ml) of apparently normal C7 were present in the serum. All family members (three sibs and both parents) were heterozygous for the combined deficiency. This is only the second reported case of combined homozygous deficiency of the closely linked and immunochemically similar proteins C6 and C7, and only the third kindred in which this defect has been demonstrated.

Keywords complement deficiency C6 C7

INTRODUCTION

Inherited deficiencies of individual complement components in man have lately been described with increasing frequency, both in association with autoimmune or infectious diseases and in the apparent absence of disease. Recent reviews have provided comprehensive information on these deficiency states (Ross & Densen, 1984; Fries, O'Shea & Frank, 1986; Schur, 1986). Isolated deficiences of terminal complement components (C5–C9) have been demonstrated in individuals with recurrent neisserial infections (Matthews *et al.*, 1980; Orren *et al.*, 1987), or with autoimmune disease (Reinitz *et al.*, 1986; Trapp *et al.*, 1987). Up to 1984, 104 patients with homozygous deficiences of terminal complement components had been reported, of whom 33 were C6 deficient and 22 were C7 deficient. Co-existent deficiency of two complement components is, however, very rare. Here we describe a case of homozygous combined with persitent vaginal candidiasis and toxoplasmosis. Combined deficiences of C6 and C2 (Delage *et al.*, 1979) and C7 and the B allotype of C4 (Chapel *et al.*, 1987) have been reported, but neither of these pairs of proteins show any genetic linkage and family studies confirm that these associations have arisen by chance.

C6 and C7 are structurally related (Podack *et al.*, 1979), and have been shown by examination of genetic polymorphism to be closely linked (Hobart, Joysey & Lachmann, 1978; Tokunaga *et al.*, 1986). Two kindreds with combined deficiences of C6 and C7 have previously been reported. In the first of these (Lachmann, Hobart & Woo, 1978) the propositus presented at the age of 68 with non-specific symptoms; homozygous deficiency of C6 and C7 was transmitted as a single genetic characteristic in the family. The second kindred (Glass *et al.*, 1978), contained several individuals with a heterozygous combined deficiency of C6 and C7, but no homozygous individuals.

We describe here only the second reported occurrence of combined homozygous deficiency of the closely linked and physicochemically similar terminal complement components C6 and C7. Immunochemical studies demonstrated the presence of a very small amount (less than 1 μg/ml) of an apparently normal C7 molecule but no normal C6. Family studies revealed that all first degree family members (parents and three sibs) had half the normal levels of C6 and levels of C7 at the low end of the normal range. Examination of C6 polymorphic variants confirmed that all family members were heterozygous C6 deficient.

MATERIALS AND METHODS

Case history

The propositus, CJ, a 21-year-old female caucasian presented with a 6-week history of malaise, lethargy and cervical lymphadenopathy. Past medical history revealed persistent vaginal candidiasis since the age of 15. The presence of thyroid microsomal antibodies had previously been noted. The patient had three siblings (two female, one male) all of whom had detectable levels of thyroid microsomal antibodies, the brother being clinically hypothyroid and on replacement therapy at the age of 19 years. The parents were unrelated and in good health.

On examination the patient was apyrexial and clinically euthyroid. Non-tender lymphadenopathy was detected in the anterior cervical and inguinal regions. No other abnormalities were detected.

Initial investigations, including haematological and biochemical profiles, chest X-ray and electrocardiogram, were

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normal. Screening for infection with influenza A and B, parainfluenza, Epstein–Barr, adeno-, respiratory syncitial-, and cytomegalo-viruses and for mycoplasma and rickettsia, were all negative, but screening for toxoplasma infection was positive and active infection was confirmed using IgM-ELISA and Sabin–Feldman dye tests (positive at a titre of 2000 iu/ml). Treatment was commenced with Pyrimethamine 25 mg o.d. and sulphadiazine 3 g b.d. with folinic acid supplements. Because of the history of long-standng candidiasis and evidence of active toxoplasma infection, further immunological assessment was carried out.

Sera

Sera from the propositus, family members and normal controls were freshly obtained, stored in small aliquots at −70°C and discarded after a single thaw.

Proteins and antisera

Purified human C6 and C7 were initially purhased from Cytotech (San Diego, CA), and later purified by the method of DiScipio & Gagon (1982). Antisera to human C6, C7, C8 and C9 were produced in rabbits by standard methods. Antisera to human C5, C6 and C7 were purchased from Cytotech. Peroxidase-labelled secondary antibody was purchased from ICN Biochemicals (High Wycombe, Bucks).

Complement assays

Classical and alternative pathway haemolytic activities were measured by a microplate modification of the methods of Brown & Hobart (1977). C3 and C4 were measured by rate nephelometry using commercial antisera. C5, C6, C7, C8 and C9 were measured by electroimmunoassay using in-house or commercial antisera. C1 inhibitor, C1q, C1r, C1s and C2 were measured by simple immunodiffusion or electroimmunoassay using commercial antisera. Absence of components in the patient serum was confirmed by examining the effects on haemolytic activity of adding back purified components. The capacity of the patient serum to produce the C56 complex was investigated after activation with zymosan as described by Lachmann *et al.* (1978). The ability of patient serum to support lysis by preformed C56 was examined utilizing purified C56 (Podack *et al.*, 1978) at a concentration which, in the presence of normal serum at 1:20 dilution, was just sufficient to cause 100% lysis of the target erythrocytes.

Gel electrophoresis and immunoblotting

Patient and control sera were run on 10% SDS polyacrylamide gels using the discontinous buffer system of Laemmli (1970), either untreated (1–5 μl serum/track) or after precipitation by 40% saturated ammonium sulphate (Lachmann *et al.*, 1978). The gels were then blotted onto nitrocellulose and probed first with antiserum to C6 or C7, and then with the appropriate peroxidase-labelled secondary antibody, essentially as described by Nurnberger *et al.* (1988). The blots were developed using HRP colour development reagent (Biorad, Watford, UK).

Isoelectric focusing and immunoblotting

Serum samples (1–5 μl), either untreated or after precipitation with 40% ammonium sulphate, were focused in thin agarose gels containing 8% ampholines (pH 5–8, Pharmacia) and then pressure blotted onto nitrocellulose (Walker *et al.*, 1983). The blots were then probed with antibody and stained as described above.

Other immunological investigations

Immunoglobulins G, A and M were quantified by rate nephelometry. IgG sublcasses and κ and λ light chains were detected by simple immunodiffusion with monospecific antisera. Antibody production to the commensals *E. coli* and *Candida albicans* was measured, respectively, by an ELISA modification of the method of Webster, Efter & Asherson (1974) and by immunofluoresence (Phillips & Matthews, 1987). T Cell subsets were measured by fluorescence-activated cell sorting using commercially available monoclonal antibodies.

Table. 1. Screening for immunodeficiency

Component	Result	
IgG	11·5*	(6–15)
IgA	1·73	(1–4·8)
IgM	1·04	(0·5–2·5)
IgG subclasses	All present	
κ, λ chains	Both present	
Zone electrophoresis	Normal Ig distribution	
Antibodies to commensals		
E. Coli	46%	(>25%)
C. albicans (titre)	32	(>8)
T cells		
$CD3^+$	2·2†	(>0·8)
$CD4^+$	0·9	(>0·6)
$CD8^+$	0·6	(>0·3)
Complement CH 50	<10 HU	(1000–1500)
Complement AH 50	50 HU	(80–200)
Immune adherence	1280	(>640)

Values in parentheses are normal ranges in our assays.
* Results expressed in g/l.
† Results expressed as number of cells $\times 10^9$/l.

Table 2. Complement profile of CJ

Component	Result	
C1	95%	
C4	170	(110–340)
C2	105%	
C3	990	(900–1920)
C5	72	(55–85)
C6	<1	(48–72)
C7	<1	(40–70)
C8	68	(45–75)
C9	75	(45–90)
C1 Inh.	110%	
C56 generation	<5%	
C56 utilization	<5%	

Values in parentheses are normal ranges in our assays. Percentages are given as those of a normal serum pool. Other results are expressed in mg/l

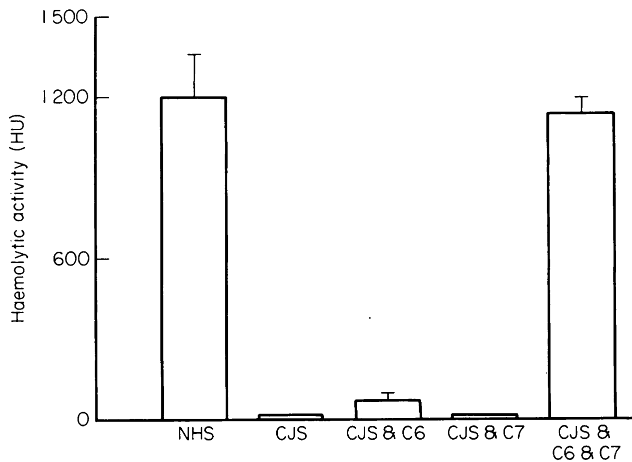

Fig. 1 Addition of C6 and/or C7 to CJ serum. The lytic capacity of CJ serum was assessed in a classical pathway haemolysis assay before and after the addition of physiological amounts of pure C6 and/or C7 (60 mg/l of each). Results are expressed as units of haemolytic activity (HU). The first column represents the average lytic activity of 20 normal sera (NHS) and the error bar represents the standard deviations of this population. The other columns represent the means of triplicate determinations on CJ serum (CJS), and error bars represent the standard deviations of these measurements.

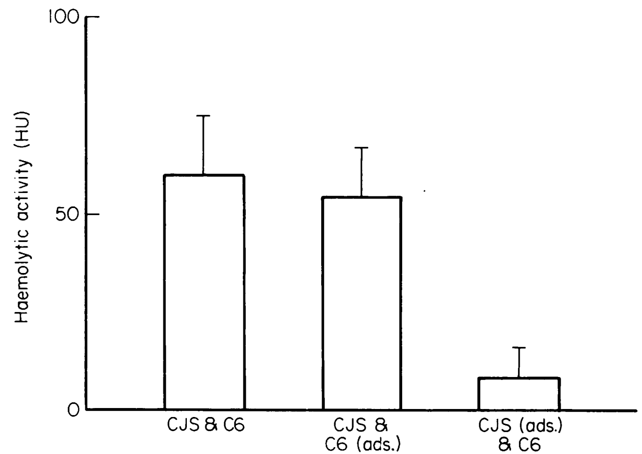

Fig. 2. Effects of adsorption with anti-C7 on lytic activity of CJ serum and C6. Polyclonal anti-C7 IgG was coupled to Sepharose and used to adsorb out any traces of C7 present in CJ serum (CJS) or the pure C6. The effects of these pretreatments on the lytic activity of CJS plus C6 are shown in the figure. The first column represents lysis by CJS plus C6 at physiological concentrations wtihout preadsorption. The second column represents lysis by CHS plus C6 preadsorbed with anti-C7. The third column represents lysis by preadsorbed CHS plus C6. Results are expressed as units of haemolytic activity, and the error bars represent the standard deviations of triplicate determinations.

RESULTS

Screening tests for immunodeficiency

No abnormalities in T cell numbers or immunoglobulin concentrations were found (Table 1). The patient's serum contained all four IgG subclasses and had normal titres of antibody to commensal organisms, indicating normal ability to produce specific antibody. However, classical pathway haemolytic activity was undetectable and this prompted further studies on complement function.

Complement studies

Propositus. The complement profile of the propositus is shown in Table 2. All components measured, except C6 and C7, were present in normal amounts. C6 and C7 were undetectable by electroimmunoassay (detection limit 1 μg/ml). Classical pathway haemolytic activity was undetectable, but haemolysis via the alternative pathway, though low, remained at measurable levels. C56 activity was absent from the patient's serum and could not be produced on activation of the serum with zymosan. Further, the patients serum was not able to complete the reactive lysis pathway in the presence of purified C56 at dilutions causing 100% haemolysis of target cells in the presence of normal serum. However, higher concentrations of serum did cause detectable lysis, not detectable using C8-deficient serum, suggesting the presence of low levels of C7 (data not included). The effects of adding back purified C6 and/or C7 on the lytic capacity of the patients serum are shown in Fig. 1. Addition of physiological concentrations of C7 did not restore haemolytic activity, whereas addition of C6 at physiological concentrations restored haemolytic activity by about 5%. Repletion with both C6 and C7 at physiological levels resulted in complete restortion of lytic activity. The possibility that the partial restoration of serum lytic activity by addition of C6 alone was due to trace contamination of the C6 preparation with C7 was investigated by preadsorption with anti-C7 antibody immobilized on Sepharose. This treatment did not diminish the restoration of lysis on adding back C6, suggesting that a small amount of lytically active C7 was present in CJ serum (Fig. 2). Preadsorption of CJ serum with solid-phase anti-C7 abolished the lytic activity on restoring C6 alone, confirming the presence of trace amounts of active C7 in this serum (Fig. 2).

Table 3. Complement profile of family

Case	Component				
	C5	C6	C7	C8	C9
CJ (Propositus)	72	1	1	68	75
TJ (Mother)	69	31	42	71	70
AJ (Father	65	28	45	60	59
ALJ (Sib)	70	35	38	64	65
AnJ (Sib)	59	26	34	60	65
NJ (Sib)	63	24	40	65	60

All values are in mg/l and are the means of triplicate determinations. Normal ranges all in mg/l are: C5 55–85; C6 48–72; C7 40–70; C8 45–75; :C9 45–90.

Family studies. Complement studies were performed on all three siblings and on both parents. The results are summarized in Table 3. All family members had normal serum haemolytic activity. Measurement of individual components revealed levels of C6 of about 50% of normal in all family members, and C7 levels at the low end of the normal range. All other components were present at normal levels.

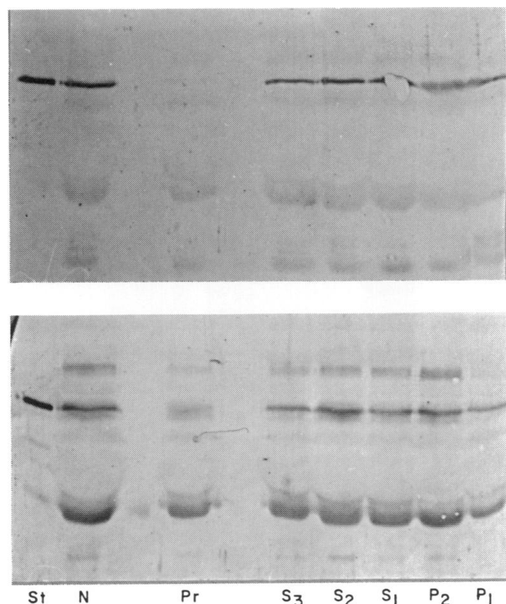

Fig. 3. SDS-PAGE immunoblotting for C6 (top) and C7 (bottom) Each serum was fractionated by precipitation with 40% ammonium sulphate to concentrate the C6 and C7 and then separated on a 10% SDS polyacrylamide gel under non-reducing conditions. Half a microlitre of the solubilized amonium sulphate cut (equivalent to approximately 2 μl unfractionated serum) was loaded in lanes P_1 and P_2 (parents), S_1–S_3 (siblings) and N (normal control). For the propositus, 5 μl of solublized ammonium sulphate cut (equivalent to approximately 20 μl unfractionated serum) was loaded (lane Pr). A pure protein standard (0·1 μg protein) was loaded in lane St. After separation on the gel the proteins were electroblotted onto nitrocellulose and probed with the appropriate antibody, as detailed in Materials and Methods.

Gel electrophoresis and immunoblotting

SDS polyacrylamide gel electrophoresis and immunoblotting of sera from family members confirmed the presence of intact C6 and C7. No C6 was detectable in CJ serum by immunoblotting even when a 40% ammonium sulphate cut was used at ten times the amounts giving strong C6 reactivity in the family sera (Fig. 3). By overloading the gel in this way it was possible to demonstrate small amounts of apparently normal C7 in CJ serum (Fig. 3), confirming the results obtained in functional studies.

Isoelectric focusing and immunoblotting

Isoelectric focusing of sera followed by immunoblotting for C6 enabled the detection of polymorphic variants of C6. The two common alleles, C6*A (gene frequency 0·68) and C6*B (gene frequency 0·31) (Whitehouse & Putt, 1983), were easily distinguishable by this technique (Fig. 4). The parents had the phenotypes C6A (Mother, T in Fig. 4) and C6B (Father, Ant in Fig. 4). Two of the siblings had the B phenotype (Al and An in Fig. 4), and the third the A phenotype (N in Fig. 4). The presence of A or B phenotypes in the siblings is only possible if the genotypes of the parents are A— and B— respectively, and all the siblings must also be heterozygous C6-deficient as suspected from the immunochemical investigations.

No polymorphisms at the C7 locus were detected in the family, so confirmation of heterozygous deficiency of C7 was not possible.

DISCUSSION

We describe here the second reported case of homozygous combined deficiency of the closely linked and physicochemically similar terminal complement components C6 and C7. The case, though similar in many respects to that previously reported by Lachmann *et al.* (1978), does display some important differences. The previously reported case had low levels of apparently normal C7, and of an abnormal form of C6. Our case also had very low levels (less than 2%) of apparently normal C7, but we were unable to detect any C6 in her serum. Although classical pathway haemolytic activity was undetectable, alternative pathway activity was persistently present at about 30% of the normal mean value. Two C8-deficient sera tested gave zero activity in both assays, suggesting that the finding was not an assay artefact. Further, several cases of C6 deficiency have been reported in which C6 and classical pathway haemolytic activity have been undetectable, but alternative pathway activity has been present at close to normal levels (Vogler *et al.*, 1979; Tedesco *et al.*, 1981). The mechanisms of alternative pathway lysis in our patient are under investigation at present.

Deficiencies of single terminal complement components are known to be associated with increased susceptiblity to meningococcal or gonococcal infections (Petersen *et al.*, 1979; Orren *et al.*, 1987) and immune complex diseases (Trapp *et al.*, 1980; Reinitz *et al.*, 1986). Our patient has not had meningitis, and presented with persistent vaginal candidiasis and toxoplasmosis. Association of toxoplasma with deficiency of C6 has been noted previously (Delage *et al.*, 1979), and toxoplasma infection has been reported to be more severe in the presence of terminal complement deficiency (Feldman & Schreiber, 1977). Whether the persistent candidal infection is related to complement deficiency in this case is uncertain, but C5-deficient mice have been shown to be more susceptible to cutaneous candidiasis (Wilson & Sohnle, 1988), suggesting that complement membrane attack may play a role in killing of *Candida albicans*. It is entirely possible that neither the toxoplasmosis nor candidiasis in this patient are causally related to her complement deficiency. In support of this alternative, the previously reported case of deficiency of C6 and C7 presented in his sixties with no history of infective problems (Lachmann *et al.*, 1978).

All members of the immediate family of the propositus are heterozygous C6-deficient, both by immunochemical measurements and by phenotypic analysis. Serum concentrations of C7, though low in all family members, are consistenly higher than the 50% level expected in heterozygotes. The absence of polymorphic variants for C7 in this family made confirmation of heterozygous C7 deficiency impossible. The propositus and all three siblings had thyroid microsomal antibodies present in their serum, and one sibling was clinically hypothyroid and on replacement therapy at the age of nineteen. Though it is not possible to conclude from this that complement deficiency and autoimmune thyroid disease are causally related, it is noteworthy that C6 deficiency has previously been described in association with hyperthyroidism (Trapp *et al.*, 1987).

The nature of the genetic mutation resulting in combined deficiency of C6 and C7 is unclear. As noted earlier, C6 and C7 are closely linked and this led Lachmann *et al.* (1978) to hypothesize that the primary transcript for the C6-C7 region of the chromosome contained messages for both these proteins, allowing a single structural mutation to interfere with transcrip-

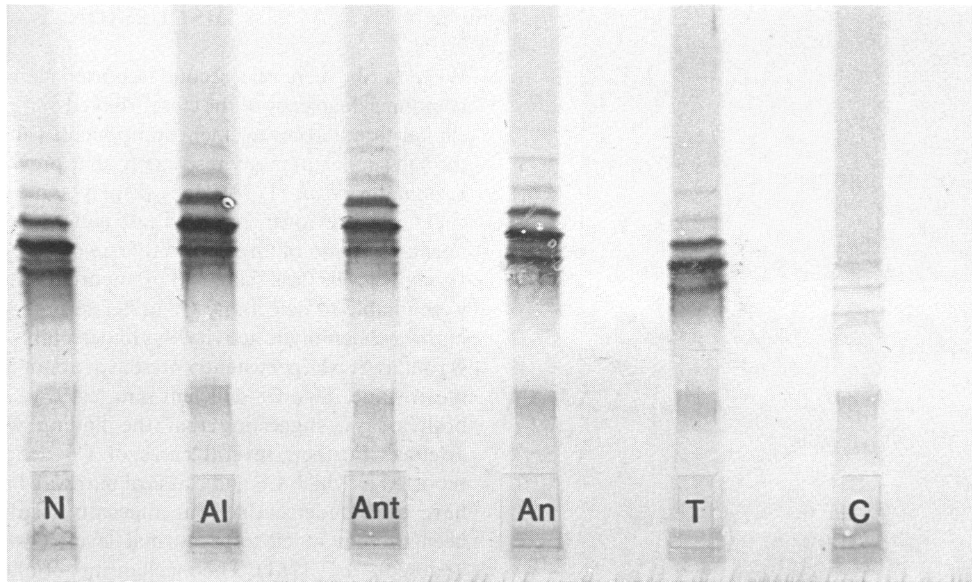

Fig. 4. Isoelectric focusing and immunoblotting C6. Sera were separated by isoelectric focusing in an agarose gel, then blotted onto nitrocellulose and probed with antibody as detailed in Materials and Methods. Two distinct banding patterns are evident: a more anodal pattern corresponding to the C6A phenotype (lanes T and N) and a more cathodal pattern corresponding to the C6B pehontoype (lanes A1, Ant amd An).

tion and translation of both proteins. They suggested that in their patient, who had small amounts of abnormal C6 and normal C7, a single mutation in the C6 structural gene could, by decreasing production of the C6-C7 transcript, account for the observed deficiencies. Though we do not find any C6 in the case reported here, this hypothesis is not incompatible with our findings as the C6 gene product may be unstable. DiScipio *et al.* (1988) have recently reported the isolation of a clone encoding the entire C7 sequence from a human liver cDNA library. The availability of probes to the C6 and C7 genes should enable the defect, or defects, in our patient to be fully elucidated.

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