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Properdin deficiency in a family with fulminant meningococcal infections

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SUMMARY

Three males in a large family showed a selective deficiency of properdin (P). One of the P deficient individuals died from a fulminant infection with *Neisseria meningitidis* group C. The family history revealed three previous cases of similar infections with a fatal outcome. The deficiency did not appear to be associated with repeated bacterial infections. The pattern of inheritance suggested an X-linked mode of transmittance. However, heterozygous carriers were not clearly distinguished in the family. P deficient serum supported immune haemolysis in a normal fashion. Alternative pathway functions, such as the activation of C3 by inulin or zymosan, lysis of guinea-pig erythrocytes in agarose gel and opsonization of endotoxin coated oil particles, were grossly impaired in P deficient serum while efficient C3 activation was produced by addition of cobra venom factor.

INTRODUCTION

Deficiencies inherited as autosomal recessive traits have been recognized for several of the proteins belonging to the complement system. Defects of the classical pathway components C1, C4 or C2 appear to predispose to development of immune complex diseases such as SLE, and recurrent infections with pyogenic bacteria, including meningococci, are encountered in C3 deficiency syndromes (Alper *et al.*, 1972; Thompson & Lachmann, 1977; Agnello, 1978). Deficiencies of the terminal components C5, C6, C7 or C8 are associated with recurrent bacteremia caused by *Neisseria meningitidis* or *N. gonorrhoeae* (Peterson *et al.*, 1979; Snyderman *et al.*, 1979).

The alternative pathway of complement activation is known as a mechanism for amplification of C3 activation through factor B, factor D and properdin (P) controlled by the factors I and H, and is considered to provide non-immune defence against microbial infection (Fearon & Austen, 1980). In the present study we describe a large family in which three maternally related males were P deficient. One of these died from a fulminant infection with *N. meningitidis* group C. Previously, fatal infections had occurred at different times in three male members of the family. The P deficiency was documented and studied in relationship to alternative pathway functions.

CASE REPORTS

Case 1 (III: 10). The index patient was a previously healthy 15 year old boy who was admitted to hospital with high fever and in shock 6 hr from the onset of disease. In the hospital he developed

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petechial bleedings and was treated with antibiotics and hydrocortisone. The patient died after 5 hr of hospitalization. Cultures from spinal fluid and blood showed *N. meningitidis* type C.

Case 2 (III:8). The previously healthy 6 year old brother of III:10 died in hospital before treatment was given, within 24 hr from the onset of headache, high fever and petechial bleedings. *N. meningitidis* was cultured from the blood. Necropsy revealed purulent meningitis, haemorrhagic pneumonia and bilateral haemorrhagic necrosis of the adrenals.

Case 3 (II:5). This patient was previously healthy with the exception of a pneumonia at the age of 33. At 36 years of age he fell ill with fever, confusion and a petechial rash and died after 28 hr, in spite of treatment with antibiotics and hydrocortisone. Necropsy showed purulent meningitis and generalized purpuric lesions.

Case 4 (II:1). In 1934, this previously healthy 6 year old boy fell ill with nausea, fever and headache. The next day he was unconscious and died before arrival at hospital. The cause of death was considered to be septicaemia.

Case 5 (III:6). This 25 year old male has been healthy with exception for two episodes of meningitis. Cultures were negative and the spinal fluid glucose normal in both instances. The clinical and laboratory examination carried out at the time of the investigation gave no indication of disease.

Case 6 (II:15). This individual is a 40 year old healthy male. Recurrent infections or other disease manifestations were not present in the case history. The physical examination, and laboratory tests were normal.

Further family data. The family history revealed no further cases with severe or recurrent infections, or with other diseases. I:1, I:3 and I:5 reached high age and died from apparently unrelated causes.

MATERIALS AND METHODS

Serum and plasma. Serum and EDTA plasma samples were stored in aliquots at −80°C. A single sample obtained during meningococcal septicaemia was available from the patient III:10. Three samples were drawn from III:6 and one from II:15.

Plasma protein levels. C1q, C1r, C1s, C2, C3, C4, C5 factor B, P, and $C\bar{1}$ inactivator ($C\bar{1}$ IA) levels were determined by electroimmunoassay (Sjöholm, 1975; Laurell, Mårtensson & Sjöholm, 1978). The same technique was used for measurement of C6, C7, C8, factor I, factor H, the C4 binding protein and C-reactive protein. C9 was demonstrated by double diffusion using commercial anti-C9 (Behringwerke A.G., Frankfurt am Main, W. Germany). Factor D was determined by haemolysis in gel (Martin *et al.*, 1976). IgG, IgA and IgM were quantified by electroimmunoassay (Grubb, 1970, 1974) and IgE by PRIST (Pharmacia Diagnostics, AB, Uppsala, Sweden).

Complement functions and activation. Screening for complement deficiencies was performed by haemolysis in gel using sensitized sheep erythrocytes for the classical, and guinea-pig erythrocytes in magnesium ethylene glycol tetraacetic acid (MgEGTA) for the alternative pathway (Truedsson, Sjöholm & Laurell, 1981). Immune haemolysis was also examined by measurement of the CH_{50} (Rapp & Borsos, 1970). Granulocyte uptake of Oil Red O coloured di-isodecylphtalate emulsified in endotoxin was measured as described by Stossel, Alper & Rosen (1973) with some modifications (Olofsson, Odeberg & Olsson, 1976), Inulin (BDH Chemicals Ltd., England), highly purified cobra venom factor (CVF) (kindly provided by Dr G. Eggertsen, Uppsala, Sweden) and zymosan (Koch-Light laboratories Ltd, England) were used as activators of the alternative pathway. The zymosan was handled as described by Götze, Medicus & Müller-Eberhard (1977). The cleavage of C3 in the sera was studied by crossed immunoelectrophoresis (Ganrot, 1972). In some experiments the results were evaluated by planimetry, giving the proportion of cleaved C3, mainly C3c, as a percentage of the area covered by precipitate. Crossed immunoelectrophoresis of C1 subcomponent complexes (Laurell, Mårtensson & Sjöholm, 1977) was used for estimation of $C\bar{1}r$-$C\bar{1}s$-$C\bar{1}$-IA complexes in serum.

Purified P. P, in native form, was prepared as described by Götze *et al.* (1977), with omission of the final immune absorption step. The preparation was gel filtrated on Sepharose CL-6B and on Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden). The purified P contained small

amounts of IgG as the only known contaminant. The concentration of P was estimated immunochemically assuming that the pooled reference serum contained P at 27·5 mg/l (Davis & Forristal, 1980).

RESULTS

Complement components

In three individuals (II:15, III:6 and III:10) P was undetectable in serum and plasma by electroimmunoassay. With undiluted samples the finding implied values that were below 2% of the normal, or less than 0·55 mg/l assuming a normal mean for P of 27·5 mg/l (Davis & Forristal, 1980). III:10 showed concentrations of C1q, C4 and C5 in the low normal range and a moderately reduced C2. By crossed immunoelectrophoresis, the serum was found to contain C$\bar{1}$r-C$\bar{1}$s-C$\bar{1}$-IA complexes in clearly increased amounts. The concentrations of other complement components tested were normal or raised in the P deficient individuals (Table I).

The levels of P, C1q, C1s, C3 and C4 were determined in the four generations of the family studied. The pedigree, including the P values is given in Fig. 1. With the reference area used, clearly decreased P was found in three females (II:3, II:19 and III:17), and in the 5 month old son (IV:1) of III:6. The low values for P, and also for C1q, in this child were considered normal for his age (Davis, Vallota & Forristal, 1979). The P levels were normal in the parents of III:10 (II:11 and II:12) and in those of III:6 (II:9 and II:10), as well as in the father of II:15 (I:4). The values obtained for C1q (50–140%), C1s (94–154%), C3 (81–155%) and for C4 (62–200%) were largely normal in the family members.

Table 1. Complement component levels in percentage of normal and the CH50 in the sera of three properdin deficient individuals. Complement components were measured by electroimmunoassay unless otherwise indicated

	III:10	III:6	II:15	Reference values*
C1q	74	129	183	78–130
C1r	—†	166	149	71–133
C1s	88	114	167	79–139
C4	56	113	83	53–207
C2	50	101	1·18	75–163
C3	91	128	180	70–136
C5	69	105	98	72–171
C6	110	97	100	55–148
C7	90	119	110	56–146
C8	58	92	50	30–175
C9‡	yes	yes	yes	yes
factor B	108	141	143	59–154
factor D§	160	100	145	36–160¶
properdin	<2	<2	<2	57–153
C$\bar{1}$ inactivator	82	136	150	72–153
factor I	105	105	140	54–142
factor H	80	110	142	61–150
C4 binding protein	98	97	141	58–102¶
CH_{50} (U/ml)	not done	50	30	30–40¶

* The 95% confidence interval as determined in 100 adults.
† Diffuse precipitate.
‡ Double diffusion.
§ Haemolysis in gel assay.
¶ Observed range in 25 adults.

Complement functions

In screening tests for the classical and alternative pathways by haemolysis in gel, all the sera from adult family members, except the sera of II:15, III:6 and III:10, produced normal patterns of lysis. Sera lacking P gave partial lysis in the assay for the alternative pathway, but full lysis in the classical pathway test. With addition of purified P, the P deficient sera gave full lysis in both assays. P deficient serum gave high or normal values in the CH_{50} test (Table I). Incubation at 37°C of sera from the healthy P deficients (II:15 & III:6) in the presence of MgEGTA and zymosan did not result in efficient C3 cleavage such as seen in the control sera. Also, the background C3 cleavage was markedly low in the P deficient sera. Addition of purified P at a physiological concentration normalized the background C3 conversion and the capacity to support C3 activation by zymosan. One experiment is shown in Fig. 2.

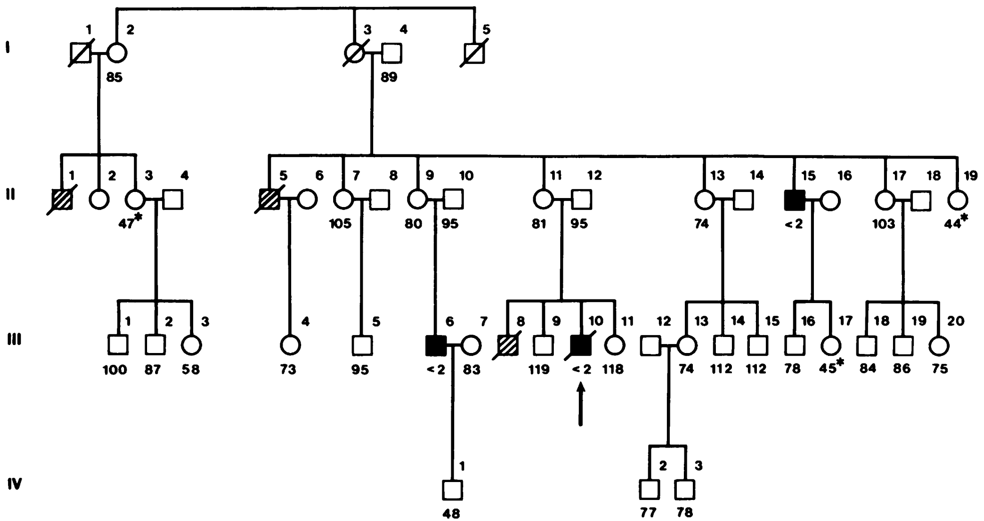

Fig. 1. Pedigree of family with properdin deficiency. Generations are indicated by Roman numerals, individuals in each generation by numbers. Males are represented by squares and females by circles. Decreased members of the family are indicated by an oblique line across the symbol. Closed symbols denote individuals with a complete deficiency of properdin, the index patient is marked with an arrow. Hatched symbols are used for individuals previously dead in fulminant infections. The properdin values in percentage of normal are given below the symbols. Values lower than 2 s.d. of the normal mean (adults) are marked with asterisks.

Like zymosan, inulin did not produce efficient C3 activation in P deficient serum unless P was added. In contrast, incubation with CVF gave C3 cleavage to the same extent in P deficient sera as in the control sera. Unchelated sera were used in these experiments. The sera from II:15 and III:6 showed low background C3 conversion, which was corrected by addition of purified P. Serum from the patient with meningococcaemia III:10) contained cleaved C3 and showed more C3 conversion than the other sera on the addition of P, possibly indicating the presence of complement activating substances in the serum (Table 2). P deficient serum failed to opsonize endotoxin coated oil particles for granulocyte phagocytosis in a normal fashion (Table 3). Addition of P at 23 mg/l and also at 3 mg/l largely restored the uptake to that obtained in the presence of normal sera. Addition of P to the pooled normal serum used as reference did not influence opsonization.

Other plasma proteins

With exception for a high IgE value in III:6 the immunoglobulin levels were normal in the three P deficient individuals with IgG at 6·9–12·7 g/l, IgA at 0·5–1·6 g/l and IgM at 0·5–1·5 g/l and in several of the family members investigated. C-reactive protein was slightly raised in III:10.

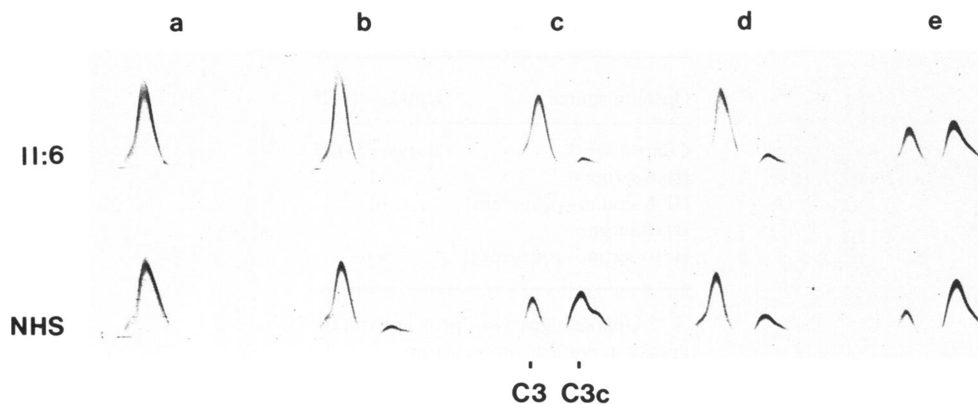

Fig. 2. Analysis by crossed immunoelectrophoresis of the C3 cleavage in properdin deficient serum (II:6) and in normal human serum (NHS) after incubation with zymosan at 37°C for 1 hr. Pelleted zymosan was suspended in the sera at 2 mg/ml. The incubation was carried out in the presence of Mg^{++} (2 mmol/l) and EGTA (10 mmol/l). (a) serum + buffer, 0°C; (b) serum + buffer, 37°C; (c) serum + buffer + zymosan, 37°C; (d) serum + properdin, 37°C and (e) serum + properdin + zymosan, 37°C. The properdin, in native form, was added at a final concentration of 29 mg/l with respect to serum. The final concentration of serum in the incubation mixtures was 75%.

Table 2. Cleavage of C3 in properdin deficient sera incubated with inulin or with cobra venom factor (CVF) at 37°C for 30 min. The values were given in percentage C3 conversion as assessed by crossed immunoelectrophoresis

Volumes in reaction mixtures				
20 μl 2 μl 5 μl	III:10	III:6	II:15	Controls
Serum + NaCl + Tris	26	5	6	19–37§
Serum + inulin* + Tris	28	14	34	51–79¶
Serum + NaCl + properdin†	54	22	16	33–38§
Serum + inulin + properdin	74	60	77	77–89§
Serum + CVF‡ + Tris	not done	66	81	63–86§
Serum + CVF + properdin	not done	76	90	not done

* Inulin at 100 mg/ml suspended in 0·15 M NaCl.

† Purified properdin (160 mg/l) in 0·1 M Tris, 0·4 M NaCl, pH 7·4.

‡ Purified CVF (160 mg/l).

§ Range in five healthy controls.

¶ Range in 18 healthy controls.

DISCUSSION

In II:15 and III:6 the P deficiency could not be attributed to disease. We found no other immunological defect. Alternative pathway dysfunctions in the sera could be corrected with purified P. CVF gave efficient C3 cleavage in the sera, suggesting that additional defects of the alternative pathway were not present.

The P deficient patient III:10 showed evidence of classical pathway activation in the course of a fatal meningococcal infection. The P deficiency might have been present also in II:1, II:5 and III:8, who died of fulminant infections.

Table 3. Uptake of endotoxin coated oil by control granulocytes in the presence of properdin deficient sera

Opsonin source	Uptake of oil*
Control sera†	Range 75–135
III:6 serum	11
III:6 serum+properdin‡	61
II:10 serum	5
II:10 serum+properdin‡	43

* Uptake is given as a percentage of the uptake in pooled control serum.

† $n=7$.

‡ Purified properdin added to serum at a physiological concentration (23 mg/l).

On the assumption that the deficiency was inherited, the most likely mode of transmission was X-linked. Only males were affected, and to our knowledge, the fathers of II:15, III:6 and III:10 were unrelated. The half normal P levels in three females on the maternal side (II:3, II:19 and III:17) and perhaps also in III:3 would fit this model. The half normal value in IV:1, a 5 month old boy, could be explained by his low age (Davis *et al.*, 1979). However, the normal P levels in the parents of the deficient males made the genetic analysis uncertain. In families with a partial P deficiency, the defect appeared to be inherited as an autosomal recessive trait (Davis & Forristal, 1980; Wyatt, Julian & Galla, 1981).

According to current concepts, the only action of P is to stabilize the amplification C3 convertase C3bBb (Fearon & Austen, 1980). Thus, in P deficiency a number of biological functions dependent on efficient C3 activation may be impaired. The observation that incubation at 37°C gave less C3 cleavage in P deficient than in normal sera may imply a role of P in the fluid phase initiation of the alternative pathway.

The fairly well established disease associations of complement deficiency states have suggested a role of the alternative pathway in resistance to bacterial infections (Fearon & Austen, 1980). The present observations indicate that P deficiency may have predisposed to the fulminant infections in the family studied. None of the family members gave evidence of a general susceptibility to bacterial disease with recurrent infections.

The fulminant infections were caused by meningococci in at least two of the patients (III:8 & III:10). It is not known if protective antibodies (Goldschneider, Gotschlich & Artenstein, 1969; Robbins, 1978) were present in the patients, and the definite role of P in this situation has yet to be decided. Abnormal immune responses in members of the P deficient family cannot be excluded and would complicate the interpretation of the association between P deficiency and the infections seen.

Deficiencies of P are probably not common. On the other hand, studies of P levels in health and disease have hardly been extensive. In screening for complement deficiencies, defects of P will be overlooked, unless suitable tests for alternative pathway function are employed.

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REFERENCES

Alper, C.A., Colten, H.R., Rosen, F.S., Rabson, A.R., Macnab, G.M. & Gear, J.S.S. (1972) Homozygous deficiency of C3 in a patient with repeated infections. *Lancet*, **ii**, 1179.

Agnello, V. (1978) Complement deficiency states. *Medicine*, **57**, 1.

Davis, C.A. & Forristal, J. (1980) Partial properdin deficiency. *J. lab. clin. Med.* **96**, 633.

DAVIS, C.A., VALLOTA, E.H. & FORRISTAL, J. (1979) Serum complement levels in infancy: age related changes. *Pediat. Res.* **13,** 1043.

FEARON, D.T. & AUSTEN, K.F. (1980) The alternative pathway of complement—a system for host resistance to microbial infection. *N. Engl. J. Med.* **303,** 259.

GANROT, P.O. (1972) Crossed immunoelectrophoresis. *Scand. J. clin. lab Invest.* **29,** Suppl 124, 39.

GOLDSCHNEIDER, I., GOTSCHLICH, E.C. & ARTENSTEIN, M.S. (1969) Human immunity to the meningococcus, I. The role of humoral antibodies. *J. exp. Med.* **129,** 1307.

GRUBB, A. (1970) Quantitation of immunoglobulin G by electrophoresis in agarose gel containing antibodies. *Scand. J. clin. lab. Invest.* **26,** 249.

GRUBB, A. (1974) Crossed immunoelectrophoresis and electroimmunoassay of IgM. *J. Immunol.* **112,** 1420.

GÖTZE, O., MEDICUS, R.G. & MÜLLER-EBERHARD, H.J. (1977) Alternative pathway of complement: nonenzymatic, reversible transition of precursor to active properdin. *J. Immunol.* **118,** 525.

LAURELL, A.-B., MÄRTENSSON, U. & SJÖHOLM, A.G. (1977) Studies of C1 subcomponents in chronic urticaria and angioedema. *Int. Arch. Allergy appl. Immunol.* **54,** 434.

LAURELL, A.-B., MÄRTENSSON, U. & SJÖHOLM, A.G. (1978) The development of simple tests for C1q, C1r, C1s, and C2 and the determination of properdin. In *Clinical aspects of the complement system* (ed. by W. Opferkuch. K. Rother & D. R. Schulz) p. 12. George Thieme Publishers, Stuttgart.

MARTIN, A., LACHMANN, P.J., HALBWACHS, L. & HOBART, M.J. (1976) Hemolytic diffusion plate assays for factors B and D of the alternative pathway of complement activation. *Immunochemistry*, **13,** 317.

OLOFSSON, T., ODEBERG, H. & OLSSON, I. (1976) Granulocyte function in chronic granulocytic leukemia. II. Bactericidal capacity, phagocytic rate, oxygen consumption and granule protein composition in isolated granulocytes. *Blood*, **48,** 581.

PETERSEN, B.H., LEE, T.J., SNYDERMAN, R. & BROOKS, G.F. (1979) *Neisseria meningitidis* and *Neisseria gonorrhoeae* bacteremia associated with C6, C7 and C8 deficiency. *Ann. Intern. Med.* **90,** 917.

RAPP, H.J. & BORSOS, T. (1970) *Molecular basis of complement action.* Appleton-Century-Crofts, New York.

ROBBINS, J.B. (1978) Vaccines for the prevention of encapsulated bacterial diseases: current status, problems and prospects for the future. *Immunochemistry*, **15,** 839.

SJÖHOLM, A.G. (1975) Complement components in normal serum and plasma quantitated by electroimmunoassay. *Scand. J. Immunol.* **4,** 25.

SNYDERMAN, R., DURACK, D.T., MCCARTHY, G.A., WARD, F.E. & MEADOWS, L. (1979) Deficiency of the fifth component of complement in human subjects. Clinical, genetic and immunologic studies in a large kindred. *Am. J. Med.* **67,** 638.

STOSSEL, T.P., ALPER, C.A. & ROSEN, F.S. (1973) Serum dependent phagocytosis of paraffin oil emulsified with bacterial lipopolysaccharide. *J. exp. Med.* **137,** 690.

THOMPSON, R.A. & LACHMANN, P.J. (1977) A second case of human C3b inhibitor (KAF) deficiency. *Clin. exp. Immunol.* **27,** 23.

TRUEDSSON, L., SJÖHOLM, A.G. & LAURELL, A.-B. (1981) Screening of deficiencies in the classical and alternative pathways of complement by hemolysis in gel. *Acta path. microbiol. scand. Sect C.* **89,** 161.

WYATT, R.J., JULIAN, B.A. & GALLA, J.H. (1981) Properdin deficiency with IgA nephropathy. *N. Engl. J. Med.* **305,** 1097.