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Prevalence of hereditary properdin, C7 and C8 deficiencies in patients with meningococcal infections

M. SCHLESINGER, Z. NAVE, Y. LEVY*, P. E. SLATER† & Z. FISHELSON‡ *Barzilai Medical Centre, Ashkelon, *Ben-Gurion University of the Negev, Beer Sheba, †Department of Epidemiology, Ministry of Health, Jerusalem and ‡The Weizmann Institute of Science, Rehovot, Israel*

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SUMMARY

High incidence of hereditary complement (C) deficiencies was found among 101 patients who had a meningococcal disease. This study revealed 11 non-related patients with complete C deficiency: five deficient in C7, three in C8, two in properdin and one in C2. Additional C-deficient individuals, most of them with no history of severe bacterial infections, were detected in family studies. The C8-deficient patients were found to have a selective deficiency of the C8-beta subunit and a reduced expression of the alpha/gamma subunit. Only a few families with properdin deficiency have been described so far. However, it is likely that frequent analysis of the activity of the alternative C pathway in survivors of severe bacterial infections will disclose numerous properdin-deficient patients. All our C7-, C8- and properdin-deficient patients are Sephardic Jews whose families originated from Morocco, Yemen (C7 and C8 deficient) or Tunisia (properdin deficient). This and other findings indicate that the type of complement abnormality found in association with meningococcal infections varies with the ethnic origin of the patient.

Keywords meningococcal infections genetic deficiency properdin C7 C8

INTRODUCTION

The association of meningococcal infections with congenital deficiency of one of the late complement (C) components C5–C9 is well documented (reviewed by Ross & Densen, 1984; Rother, 1986; Hauptmann 1989; Nagata *et al.*, 1989). High incidence (10%) of C7 or C8 deficiency was recently reported in Israeli patients who survived a meningococcal disease (Zimran *et al.*, 1987). Similarly, meningococcal infections have been described in individuals with properdin deficiency (Sjöholm, Braconier & Söderström, 1982; Fijen *et al.*, 1989). However, the overall incidence of properdin deficiency in such patients has not yet been determined, and only few families with absence or dysfunction of properdin have been described so far (Sjöhlom *et al.*, 1982; Sjöholm & Nilsson, 1985; Späth *et al.*, 1985; Densen *et al.*, 1987; Gelfand *et al.*, 1987; Nielsen & Koch, 1987; Sjöholm *et al.*, 1988). In order to evaluate further the association between meningococcal disease and hereditary deficiency of complement components, we analysed the activity and components level of both alternative and classical pathway of complement in patients who had one or more episodes of meningococcal infection. Of 101 propositi examined, ten C-deficient patients were characterized as deficient in C7, C8 or properdin. These C abnormalities appeared to be prevalent among Jews of Sephardic origin.

Correspondence: M. Schlesinger, MD, Department of Paediatrics and Clinical Immunology, The Barzilai Medical Centre, Ashkelon 78306, Israel.

PATIENTS AND METHODS

The average annual incidence of meningococcal diseases in Israel for the years 1980–1987 was 1·46 per 100000 (Department of Epidemiology, 1989). About 70 new cases per year were recorded during this period. Hospital discharge summaries are sent routinely to each of 15 district Health Offices and are kept on file for the entire country at the Department of Epidemiology of the Ministry of Health. Surveillance is passive, lag-time in summary transmittal is often substantial, and the current study was performed based on those summaries available in March 1989. Summaries of all cases of meningococcal disease available were reviewed for the period 1980–1988. Patients who survived and had a valid address were invited to the Immunology Clinic at Barzilai Medical Centre for further evaluation. Of 280 patients invited, 101 (36·1%) appeared for evaluation. Although this number fell short of 100%, the sub-sample attending was similar to the entire sample invited with respect to age, sex and ethnic origin group. All the patients were tested at least 1 year after the infection. Blood (5 ml) was aspirated and left to clot at room temperature for 1 h and the serum was then separated after centrifugation at 4°C. The sera were kept in aliquots at −80°C until tested. All sera were examined for both CH_{50} and

AP_{50}. Normal serum pool composed of a mixture of sera from 15 healthy volunteers was also prepared and kept in aliquots at −80°C. Each sample of a propositus or a family member was tested together with a sample of the pooled normal serum. The results are expressed as percent of the normal serum. Patients with low CH_{50} or AP_{50} (less than 70% or 60% of normal, respectively) in repeated examinations were also tested for the concentration of several C components by radial immunodiffusion (RID). Serum samples taken from family members (siblings, parents and children) of the C-deficient propositi were also tested.

Total haemolytic activity of the classical C pathway (CH_{50}) was tested with haemolysin-coated sheep erythrocytes as described (Mayer, 1961), with small modifications. Haemolytic activity of the alternative C pathway (AP_{50}) was measured in a microtitration (100 μl) assay with rabbit erythrocytes (Platts-Mills & Ishizaka, 1974) and in the presence of magnesium chloride and EGTA (Sigma, St Louis, MO) (2·5 and 10 mM, respectively). RID (Mancini, Carbonara & Heremans, 1965) was performed in 1% agarose gels containing monospecific goat antibodies to human C components. Western blotting of C8 and properdin was performed as previously described (Towbin, Staehlin & Gordon, 1979). In principle, serum samples (20 μl of serum diluted 20-fold with saline) were exposed to SDS–PAGE (Laemmli, 1970) and then transferred to nitrocellulose paper. C8 and properdin were made visible by serial incubations with monospecific antibodies, followed by second antibodies conjugated with peroxidase (Sigma) and then with diaminobenzidine (DAB) (Sigma) as substrate.

RESULTS

Eleven of the 101 patients enrolled in the study were found to be complement deficient: five were deficient in C7, three in C8, two in properdin and one in C2. Two patients (P3 and P8) had a recurrent infection. Of 13 episodes, 10 were meningitis, two were meningitis with bacteraemia and one was bacteraemia. No acquired C deficiency was detected among the patients. The parents of two C8-deficient patients (P6 and P7) were first cousins, and those of one C7-deficient patient (P2) were second cousins. No consanguinity was found among the parents of the other propositi. The characterization and the complement values of these patients are summarized in Table 1. All patients with C7 or C8 deficiency had undetectable activity of both CH_{50} and AP_{50}. The two patients with properdin deficiency had normal CH_{50} but very low AP_{50}. One child had a C2 deficiency with undetectable CH_{50} and normal AP_{50}. No C7 could be detected by RID in the C7-deficient patients and no properdin in those with properdin deficiency. However, in patients with C8 deficiency the amount of C8 detected by RID was about 30% of normal. This is due to the fact that only one of the three C8 polypeptide chains (alpha, beta and gamma) (Kolb & Müller-Eberhard, 1976) is absent in these patients. Analysis by Western blotting revealed a selective deficiency of the C8-beta subunit (Fig. 1). The C8 alpha/gamma complex level was also reduced in these patients. This could be due to a linkage between the C8 alpha/gamma and C8 beta genes on chromosome 1 (Rodge *et al.*, 1986).

The eight C7/C8-deficient patients originated from Morocco (six) or from Yemen (two), and the two properdin-deficient patients from Tunisia. They were not related and came from different parts of their original countries. The only C deficiency found among the Ashkenazi Jews examined was a C2 deficiency. This is in contrast to the ethnic distribution in the 101 patients who originated from Morocco (18), Yemen (15), Tunisia (four), Ashkenazis (22) and from other countries (42). The ethnic distribution in Israel during the period of the study was: Moroccans 13·8%; Yemenites 4·8%; Tunisians and Algerians 3·5%; Ashkenazis 39%; Jews from other countries 22·2%; and native non-Jews (Moslems and Christian) 16·7%.

No C deficiency was found among 200 healthy volunteers of Sephardic and Ashkenazi origins who had no history of meningococcal disease. The C deficiency in the normal population was found in other studies to be quite rare (Ross & Densen, 1984; Inai *et al.*, 1989).

The average age at infection of the 11 C-deficient patients was 14·2 years (median 13·5, range 3–38) and that of the C-sufficient patients, 6·4 years (median 4, range 2 months–60 years). Thus, in agreement with previous reports (Ross & Densen, 1984; Orren *et al.*, 1987), meningococcal infection occurred at a later age in C-deficient than in C-sufficient patients ($P < 0·01$, Mann–Whitney U-test).

Table 1. Complement-deficient patients in this study

Propositus	Age (years)	Sex	Origin	C component (% normal level)	CH_{50}	AP_{50}
				C7		
P1	6	F	Morocco	0	0	0
P2	13	M	Morocco	0	0	0
P3	21	M	Morocco	0	0	0
P4	18	F	Yemen	0	0	0
P5	38	M	Morocco	0	0	0
				C8		
P6	12	M	Yemen	30*	0	0
P7	7	F	Morocco	30*	0	0
P8	8	M	Morocco	36*	0	0
				Properdin		
P9	16	M	Tunisia	0	100	21
P10	14	M	Tunisia	0	95	37
				C2		
P11	3	M	Europe	0	0	100

*Very weakly stained.

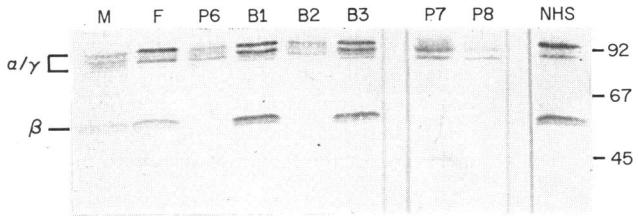

Fig. 1. Pattern of C8 alpha/gamma and beta subunit of patients and their relatives. Serum samples of three C8-deficient patients P6, P7, P8, family members of P6 (M, mother; F, father; B, brother) and normal human serum (NHS), were analysed by Western blotting. First antibody goat anti-human C8; second antibody peroxidase-conjugated rabbit anti-goat IgG; substrate DAB.

Table 2. Complement levels in sera of patients' families

Subject	Sex	Protein* (% normal level)	CH_{50}	AP_{50}
Family of P2 (C7 deficiency)				
P2	M	0	0	0
F	M	34	91	100
M	F	35	98	100
S1	M	57	78	91
S2	M	41	91	100
S3	F	44	89	76
Family of P6 (C8 deficiency)				
P6	M	30†	0	0
F	M	79	84	98
M	F	100	100	100
S1	M	100	100	100
S2	M	100	100	100
S3	M	30†	0	0
Family of P10 (properdin deficiency)				
P	M	0	95	37
F	M	100	ND	100
M	F	96	ND	100
S1	M	100	ND	81
S2	F	100	ND	100
S3	F	48	ND	100
S4	F	92	ND	100
S5	M	0	ND	29

P, propositus; F, father; M, mother; S, sibling.

*Protein, C7 in family A, C8 in family B and properdin in family C.

†Very weakly stained.

ND, not determined.

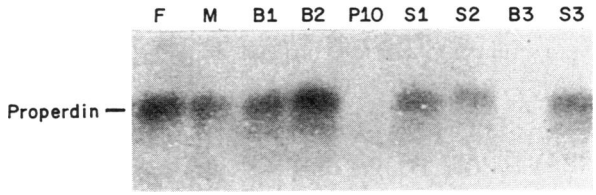

Fig. 2. Pattern of properdin of patient P10 and his family members. Serum samples of P10 and his mother (M), father (F), brothers (B1, B2, B3) and sisters (S1, S2, S3) were analysed by Western blotting. First antibody goat anti-human properdin; second antibody biotinylated-rabbit (IgG) anti-goat IgG; enzyme peroxidase–avidin, substrate DAB.

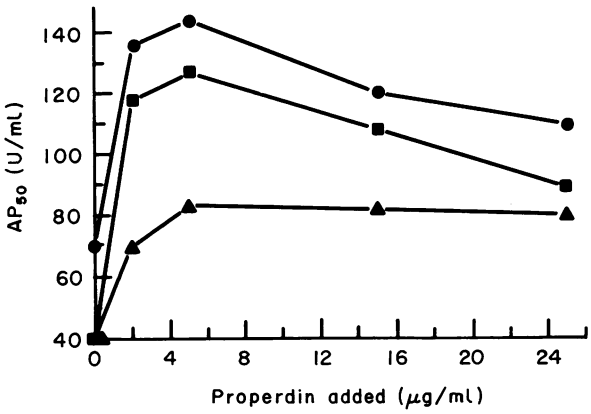

Fig. 3. Restoration of AP_{50} in properdin-deficient sera. Purified properdin was added to serum samples of P10 (●), his brother S5 (■) or P9 (▲) at the final concentration indicated. Each sample was then analysed for total haemolytic activity of the alternative pathway of complement (AP_{50}).

Family studies of five propositi led to the identification of three C7-deficient, two C8-deficient and one properdin-deficient individuals, four of them with no history of meningococcal infection. Complement values of three families are shown in Table 2. The family members of the C7 propositus P2 are probably all heterozygotes, and have 34–57% of normal serum concentration of C7 (55 μg/ml). However, their CH_{50} and AP_{50} are normal or only slightly reduced. Patient P6 and his brother S3 are C8-deficient and have no detectable CH_{50} or AP_{50} (Table 2). The mother appeared to have normal levels of C8 antigen, CH_{50} and AP_{50}. Since she is expected to be partially deficient in C8, it is concluded that RID, CH_{50} and AP_{50} are not efficient in diagnosing C8-deficient heterozygotes. Analysis of the C8 protein by Western blotting (Fig. 1) demonstrated that the mother of P6 has a low serum concentration of C8 alpha/gamma and beta and the father has only reduced C8-beta subunit level. In agreement with previous reports (Rittner *et al.*, 1986; Nürnberger *et al.*, 1989), the pattern of C8 (Fig. 1) indicates a polymorphism in the alpha/gamma subunit. P6 and B2 appear to lack the alpha/gamma 2 form whereas P7 and P8 lack the alpha/gamma form 1 (Fig. 1). A selective deficiency of the alpha/gamma form 1 has recently been described in 12 C8-deficient patients (Nürnberger *et al.*, 1989).

Analysis of the family of the properdin-deficient patient P10 revealed that his brother S5 is properdin-deficient and has reduced AP_{50}. The father and the mother appear to have normal values of properdin and AP_{50}. However, a sister of the mother has half the normal amount of properdin and her son has undetectable AP_{50} and properdin (data not shown), indicating that the mother is a heterozygote carrier of this properdin abnormality. The level of properdin in sera of P10's family members was further examined using the western blotting technique (Fig. 2). No properdin could be detected by this technique in sera of the propositus and his brother S5 (B3 in Fig. 2). In contrast to the results of the RID (Table 2), the Western blotting technique (Fig. 2) demonstrated reduced level of properdin in the mother of P10.

Restoration of the haemolytic activity (AP_{50}) in sera of properdin-deficient patients was attempted using human properdin purified to homogeneity by the method of Medicus *et al.* (1980). As can be seen in Fig. 3, purified properdin efficiently restored in a dose-dependent manner the AP_{50} of three properdin-deficient sera. Maximal AP_{50} values were obtained at an input of 5 μg/ml properdin, a concentration which is below the normal serum properdin level (20 μg/ml). It is likely that the purified properdin contained some activated properdin (Medicus *et al.*, 1980), which may have led, at concentrations higher than 5 μg/ml, to complement consumption in the fluid phase and

to reduced AP_{50}. For an unknown reason, the restoration of AP_{50} to serum of P9 was less efficient than to the other two sera.

DISCUSSION

A high incidence (11%) of complete deficiency of complement components among patients who survived a meningococcal disease was found in this study. The complement components found missing in these patients are C7, C8, properdin or C2. Similar incidence of C7 and C8 deficiency associated with meningococcal infections in Israel was reported by Zimran *et al.* (1987). However, the present study also provides an assessment of the incidence of properdin deficiency in patients who had a meningococcal disease. Moreover, so far only a few patients (Sjöholm *et al.*, 1982; Sjöholm & Nilsson, 1985; Späth *et al.*, 1985; Densen *et al.*, 1987; Nielsen & Koch, 1987; Fijen *et al.*, 1989) with isolated properdin deficiency and one with combined C2 and properdin deficiency (Gelfand *et al.*, 1987) have been described worldwide. This is probably due to the incomplete analysis of complement in the clinics that usually neglect to examine the alternative pathway. Our results suggest that properdin deficiency occurs more frequently than suspected. An early detection of properdin deficiency in patients and their relatives is important, since these patients tend to have fulminant and fatal infections (Editorial, 1988; Densen *et al.*, 1987). Furthermore, as recently suggested, vaccination may increase resistance of properdin-deficient patients to infection (Densen *et al.*, 1987; Söderström *et al.*, 1989).

Schreiber & Müller-Eberhard (1978); Schreiber *et al.* (1979) have clearly demonstrated that properdin, although not a crucial component of complement activation, has an important enhancing regulatory activity. Thus, depletion of properdin results in two-fold or 3–4-fold diminution of alternative pathway lytic activity against rabbit erythrocytes (Schreiber & Müller-Eberhard, 1978) or *Escherichia coli* (Schreiber *et al.*, 1979). This can explain the reduced AP_{50} level observed in our properdin-deficient patients and relatives. Inheritance of properdin deficiency is sex-linked (Goonewardena *et al.*, 1988), and the properdin gene has been mapped recently to chromosome X (Goundis *et al.*, 1989). Accordingly, all our properdin-deficient patients (two) and family members (two) are male, whereas the females in the two families examined are either heterozygotes or normal.

The two patients with properdin deficiency described here are of Tunisian origin, yet the number is too small to conclude about the ethnic distribution of properdin deficiency. C7 and C8 deficiencies appear to be associated in Moroccan and Yemenite Jews with susceptibility to meningococcal infection (Zimran *et al.*, 1987, and our results). C9 deficiency is most common in Japan (Inaba *et al.*, 1987; Inai *et al.*, 1989) and is imposing increased risk of meningococcal meningitis on this population (Nagata *et al.*, 1989). C6 deficiency is associated with meningococcal disease in Coloured and Black Africans in Cape Town, South Africa (Orren *et al.*, 1987). All our patients with C8 deficiency lack the C8-beta subunit. Unlike black Americans with C8 deficiency who lack the alpha/gamma subunit, white American and European Caucasians are deficient in the beta subunit (Tedesco, 1986). Association between hereditary deficiency of one of the late acting C components (C5–C9) or of properdin with susceptibility to Neisserial infections is now well established. However, the latter findings indicate that certain populations or ethnic groups have typical complement deficiencies which can be risk factors for a meningococcal disease.

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