HLA-linked C2 deficiency in a Dutch patient with systemic lupus erythematosus

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SUMMARY A patient with systemic lupus erythematosus was studied whose blood serum on repeated occasions showed undetectable levels of haemolytic complement (C). A detailed investigation of individual C components in the serum of the probanda and her family revealed the absence of functional C2 in the patient and half-normal values in the relatives. C4 levels in the family, but not in the patient, were above normal, whereas the levels of factor B were low in all cases. No abnormalities were noted in C3, C9, or C1INH. Tissue typing showed linkage of the C2-deficiency gene with the HLA-A10/B18 and A9/B18 haplotypes. No linkage with red cell antigens and no relationship with plasma kallikrein levels was found.

Many defects of complement components have been described in man (C1r, C2, C3, C4, C5, C6, C7, C8, C1INH, C3bINH). The clinical manifestations reported in association with these defects are strikingly diverse. The rare deficiency of C2 production appears to predispose to serious susceptibility to infection, vascular and mesenchymal disease, renal disease, and, particularly, lupus erythematosus (LE) (Day *et al.*, 1973). Most patients with C2 deficiency studied so far had lupus erythematosus, systemic (SLE), discoid (DLE), or both (Agnello *et al.*, 1972; Day *et al.*, 1975; Fu *et al.*, 1975; Wolski *et al.*, 1976; Ochs *et al.*, 1977), although cases with recurrent throat infections, urinary tract infections, anaphylactoid purpura, or rheumatic diseases have been described (Gelfand *et al.*, 1975; Gibson *et al.*, 1976). In addition to C2 deficiency, abnormal C1 has also been described in a patient with LE (Pondman *et al.*, 1968). LE, of course, occurs most frequently in patients with no apparent abnormalities of the complement system.

Linkage of the C2 gene with the major histocompatibility complex (MHC) has been well documented. The gene coding for C2 has been located on chromosome 6, close to the HLA-D locus and at less than 5 centimorgans from the HLA-B locus (Ochs *et al.*, 1977). The same chromosome carries the genes coding for the C components C4, C6, C8, and factor B, the latter (Bf) being closely linked to the C2 locus (Alper, 1976; Merrit *et al.*, 1976; Teisberg *et*

al., 1976). Analysis of HLA antigens in families carrying the C2 deficiency gene has shown an association between C2 deficiency and HLA-A10 and B18, particularly the latter. We report the case of a Dutch homozygous C2-deficient patient carrying the A10/B18 and A9/B18 haplotypes.

Material and methods

CASE REPORT

The probanda, a Caucasian woman, born on 5 November 1950, had been known to our department since 1968 when she was referred because of a generalised, symmetrical, erythematopapulosquamous eruption with characteristic butterfly distribution on the face. She had livid, ulcerating lesions on the hands and feet together with distinct Raynaud's phenomenon. The eruption had developed after accidental burning of the face. On admission the patient was severely ill with fever up to 40°C, and there were signs of polyarthritis. A diagnosis of SLE was made, which was confirmed by laboratory findings, notably characteristic immunofluorescence of a skin biopsy, positive ANA, positive LE cell test, leucopenia, and hypergammaglobulinaemia. Normal values were obtained for haemoglobin, platelets, serum urea, creatinine, electrolytes, bilirubin, alkaline phosphatase, transaminases, and lipids. The Coombs' test was negative with anti gammaglobulin and was positive with an anti complement (C3) serum (titre 1:16); there were no abnormalities of the clotting system. Haemolytic

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activity of the complement (C) system was not demonstrable.

Soon after admission a severe psychiatric syndrome developed, consisting of psychosis, bradyphrenia, depression, and amentia. The clinical picture was complicated by various infections, for example, of the respiratory and urinary tracts, and sepsis with *Pseudomonas aeruginosa* ensued. Up to now there have been exacerbations of the disease on numerous occasions; in fact, complete remission has not been observed so far. During exacerbations the skin condition often resembled the picture described above; in addition, a characteristic 'light-sensitive dermatitis' or discoid LE were also observed. Twice, a severe neuropsychiatric syndrome with psychosis and extrapyramidal symptoms dominated the picture. Other organ systems were not involved, and, especially, LE-nephritis, haemolysis, and bleeding tendency were never observed. Infections, for example, pyoderma, otitis media, sinusitis, bronchopneumonia, cystitis, pyelitis, and sepsis, often occurred. Despite repeated and urgent advice to practise contraception, the patient became pregnant in 1971. Remarkably, the course of the disease remained relatively benign, and she delivered a boy who was dysmature (birth weight 1890 g) but who did not develop signs of LE. The further development of the child has been uneventful.

In 1973, the patient was given a contraceptive (500 mg medroxyprogesterone acetate) intramuscularly. A flare-up of the disease resulted, again with involvement of the central nervous system. Therapy included the administration of prednisone, azathioprine, and chloroquine in varying doses and combinations. During the psychotic episodes, very high doses of prednisone were necessary to control the symptoms. Furthermore, antibiotic and antipsychotic treatment was given when indicated. Sterilisation was performed in 1974.

On various occasions during the last few years, laboratory evidence has been obtained of auto-immune activity, for example, false-positive treponemal serology, ANA, anti-double-stranded-DNA, positive LE cell tests, and cytotoxic anti-leucocyte antibodies. Immunofluorescence of the uninvolved skin revealed granular deposits at the dermoepidermal junction of IgA, IgG, IgM, C1q, C4, and C3 factors. C1q, C3, and IgM were also present in the superficial capillary walls.

No member of the family had a medical history suggestive of LE (Table 3).

HLA and blood group typing

HLA typing was done by the NIH standard microlymphocytotoxicity technique, using 140 well-defined antisera to 35 specificities. Blood group typing was

done by traditional agglutination techniques.

Complement components

Blood samples were collected by venepuncture. Citrated plasma and serum (after clotting in glass) were obtained from the samples and stored in small aliquots at -70°C until used. Total haemolytic C was determined with serial serum dilutions, using 5×10^8 sheep erythrocytes (E) optimally sensitised with rabbit amboceptor (A); CH_{50} values were read from van Krogh plots. Functional C2 was determined by molecular titration using EA1,4 cellular intermediates, as described by Borsos *et al.* (1961), with some slight modifications (Berrens *et al.*, 1976). C components C3, C5, and B were determined immunochemically by single radial immunodiffusion using commercially available antibody-containing agarose plates (Behringwerke, Marburg/Lahn, Germany). Component C9 and the inhibitor C1INH were assayed by electroimmunodiffusion, according to Laurell (1966), using commercial rabbit antisera as described by Berrens *et al.* (1976).

Plasma (pre)kallikrein was evaluated according to Ratnoff and Saito (1976) with the synthetic amide *N*- α -benzoyl-L-proline-L-phenylalanine-L-arginine-HCl (PPAN) (Pentapharm, Basel, Switzerland); 0.25 ml of plasma was mixed with 0.25 ml dextran sulphate (MW 500 000, Pharmacia, Sweden, 25 $\mu\text{g}/\text{ml}$) and kept at 0°C for 10 minutes. After another 10 minutes at room temperature, 0.25 ml of the mixture and 0.25 ml of substrate solution (0.18 μM) were brought into a cuvette with 2 ml 0.15 M Tris-imidazole buffer, pH 7.9, at 37°C . The reaction was followed by observing the increment of extinction at 405 nm with the rate analyser of the Unicam SP1800 spectrophotometer. The results are expressed in mU kallikrein/ml, as described in the manufacturer's manual, taking the extinction change in the third minute as the basis for calculation.

Results

The patient's serum was examined several times over a number of years but was never found to contain detectable haemolytic C. Analysis of the individual C components showed an absence of functionally active C2 in every sample (Table 1). The levels of the components B and C4 fluctuated between approximately half-normal and normal, possibly dependent on the activity of the disease. No striking abnormalities were observed in the serum levels of C3 or C1INH (Table 1), although the values in the probanda were higher than in other members of the family (Table 2). The C9 level, expressed in percent of a normal serum pool, was above the range of normal values observed in this laboratory, which

might reflect its nature as an acute-phase protein (Kawachi-Takahashi *et al.*, 1975).

Members of the patient's family available for study were investigated on two occasions, with an interval of about one year. As shown in Table 2, all subjects had half-normal values of functional C2 in the blood serum, causing low CH₅₀ values in only one member (subject 8). The levels of factor B were depressed below the normal range in each member of the family, confirming earlier findings of linkage between the genes coding for C2 and factor B (Alper, 1976). C4 and C₁INH levels were in the upper normal range; no apparent deviation was noted in the serum levels of the protease inhibitors α_1 -antitrypsin (α_1 AT) and α_2 -macroglobulin (α_2 M).

Similar results were observed during the second investigation of the relatives. This time, low levels of total haemolytic C were found in subject 1 but not in subject 8. As shown in Table 3, C2 and B levels were again depressed to approximately half-normal values, while C4 was in the upper range or slightly above normal. The plasma prekallikrein levels in all subjects, including the *proposita*, were not signi-

fically different from those observed in normal volunteers (Table 3).

The results shown in Tables 1, 2, and 3 are suggestive of hereditary transmission of C2 deficiency and indicate that the *proposita* is homozygous for the trait and the (clinically unaffected) members of the family are all heterozygous for this defect. As shown by the results of HLA-typing (Figure), all members of the family are also heterozygous for the antigen B18, while the *proposita* is homozygous. These results are in full accord with an association between C2 deficiency and B18 phenotype, as described in the literature (Fu *et al.*, 1975; Gibson *et al.*, 1976; Opelz and Glovsky, 1976).

As expected, no genetic linkage was found between C2 deficiency and the red cell blood group antigens (Table 4).

Discussion

The results of this study are suggestive of the hereditary transmission of C2 deficiency as an autosomal recessive trait, the *proposita* being

Table 1 Longitudinal analysis of serum samples in the *proposita*

Date	CH ₅₀ /ml (% of normal)	C2H ₅₀ /ml	Factor B (mg/100 ml)	C4 (mg/100 ml)	C3(B1A/C) (mg/100 ml)	C9 (% of normal)	C ₁ INH (mg/100 ml)	Clinical picture
25 May '73	0	0	ND	ND	ND	ND	ND	Skin active; sepsis
26 Oct '73	0	0	ND	ND	ND	ND	ND	Recurrence due to im contraceptive
3 Oct '74	0	0	ND	9.8	98	ND	25.0	Remission
15 Sept '75	0	0	11.2	6.4	134	148	40.8	Flare-up due to sunlight
13 Feb '76	0	0	19.2	9.2	114	165	43.9	Skin active; arthritis
20 Apr '77	0	0	20.8	20.4	146	ND	ND	Mild activity; arthralgia
23 Jun '77	0	0	18.8	15.0	130	161	ND	Mild activity; arthralgia
14 Jul '77	0	0	20.2	15.6	127	177	ND	Remission
Normal range	75-125	1200-2000	20-30	20-45	60-120	75-125	20-35	

ND = not detected

Table 2 Complement components and enzyme inhibitors in the family, 1st series (1975)

Subject*	Sex	Born	CH ₅₀ /ml (% of normal)	C2H ₅₀ /ml	Factor B (mg/100 ml)	C4 (mg/100 ml)	C3(B1A/C) (mg/100 ml)	C9 (% of normal)	C ₁ INH (mg/100 ml)	α_1 AT (mg/100 ml)	α_2 M (mg/100 ml)
1	M	1909	81	600	14.0	42.8	80	95	33.4	180	290
2	F	1918	90	760	15.2	39.2	88	84	31.5	145	368
3	F	1939	94	760	13.6	46.0	132	98	32.5	180	408
4	F	1944	62	650	11.8	31.2	128	83	30.8	200	244
6	F	1950	0	0	11.2	6.4	134	148	40.8	375	308
8	F	1960	39	680	13.6	28.0	86	103	25.8	380	268
9	M	1971	76	540	8.8	21.2	140	94	42.0	ND	ND
Normal range			75-125	1200-2000	20-30	20-45	60-120	75-125	20-35	200-400	150-400

*Subjects numbered as indicated in the Figure. Subject 6 is the *proposita*.

Table 3 Complement components and prekallikrein levels (in plasma) in the family, 2nd series (1976)

Subject*	Sex	Born	CH ₅₀ /ml (% of normal)	C2H ₅₀ /ml	Factor B (mg/100 ml)	C4 (mg/100 ml)	C3(β1A/C) (mg/100 ml)	C9 (% of normal)	C ₁ INH (mg/100 ml)	Prekallikrein (mU/ml)
1	M	1909	23	800	17.0	50.8	110	119	35.2	60
2	F	1918	80	600	15.0	41.4	118	87	31.7	80
3	F	1939	70	820	15.8	52.8	110	117	29.0	76
4	F	1944	68	840	13.8	41.4	104	101	31.3	52
5	M	1949	58	620	12.4	34.0	90	83	32.3	44
6	F	1950	0	0	19.2	9.2	114	165	43.9	68
7	M	1952	78	580	16.8	51.6	110	86	34.9	56
8	F	1960	60	540	10.4	31.2	104	90	27.9	68
9	M	1971	76	540	8.8	21.2	140	94	42.0	ND
Normal range			75-125	1200-2000	20-30	20-45	60-120	(100)	20-35	60-100

*Subjects numbered as shown in the Figure. Subject 6 is the probanda.

Table 4 Red cell typing of the family

Subject	ABO	Rhesus	MNS	P ₁	Kell	Fy ^a	Lu ^a	C ^w
1	O	CcDEe	MNS—	P ₁ —	Kell—	Fy ^a +	Lu ^a —	C ^w —
2	A ₂	CcDee	MNS—	P ₁ +	Kell—	Fy ^a +	Lu ^a —	C ^w —
3	O	CcDee	MNS—	P ₁ —	Kell—	Fy ^a —	Lu ^a —	C ^w —
4	A ₂	ccDEe	MNS—	P ₁ +	Kell—	Fy ^a +	Lu ^a —	C ^w —
5	A ₂	CCDee	MNS—	P ₁ +	Kell—	Fy ^a —	Lu ^a —	C ^w —
6	O	CcDEe	MNS—	P ₁ —	Kell—	Fy ^a +	Lu ^a —	C ^w —
7	A ₂	CCDee	MNS—	P ₁ —	Kell—	Fy ^a —	Lu ^a —	C ^w —
8	O	CCDee	MNS—	P ₁ +	Kell—	Fy ^a +	Lu ^a —	C ^w —

homozygous and all members of the family heterozygous. This investigation further confirms the close linkage between the C2 gene and the HLA genes, because the C2 deficiency traits in the family segregate with the HLA haplotypes. The HLA-association would have been even more firmly established had one of the siblings carried the haplotypes A10/B7 and A2/B13 and shown a normal level of C2.

The depressed levels of factor B in the relatives also confirm the close linkage between the Bf and C2 loci, as has been reported before (Alper, 1976). The fact that factor B in the probanda on occasions reached normal levels may be due to its reported behaviour as an acute-phase protein (Schutte *et al.*, 1974), as has also been described for C9 (Kawachi-Takahashi *et al.*, 1975).

The association between C2 deficiency and the

HLA-B18 antigen is also confirmed in our study. In most families described in the literature, C2 deficiency was associated with the haplotypes A10/B18, for example, in 8 out of 10 families described by Gibson *et al.* (1976). In our patient, this haplotype is present on the maternal chromosome. The paternal chromosome also carries the B18, but in this case it is part of the A9/B18 haplotype. Opelz and Glovsky (1976) have suggested that C2 deficiency may have originated in an individual with the A10/B18 haplotype, but that the association with A10 may subsequently have become lost due to crossing over.

Although C2 deficiency is rare in groups of LE patients, it is nevertheless evident that individuals homozygous for the abnormality have a striking predisposition to LE. In a series of 38 homozygous, C2-deficient subjects described by Agnello (1976), 14 had LE (6 SLE, 5 DLE, 3 both). Nine patients had other diseases, and 15 were apparently healthy. The clinical characteristics of the C2-deficient LE patients appear to consist of a preponderance of discoid lesions, a low incidence of renal disease, a low incidence and titres of anti DNA antibodies, and a low frequency of immunoglobulin and C deposits in the skin.

The clinical picture of our proposita consisted of skin lesions much more extensive than discoid LE, together with arthritis and recurrent involvement of the central nervous system. LE nephritis did not occur in our patient during the period of observation. Deposits of immunoglobulin and C factors were observed in the skin, low titres of anti-double-stranded-DNA were occasionally demonstrable, there were circulating immune complexes, and cytotoxic antibodies against leucocytes were detectable. It seems remarkable that, despite these autoallergic phenomena, LE nephritis has not developed. It is tempting to speculate that C2 deficiency in this respect may offer some protection, because immune complexes in such patients might fail to generate the C3 and C5 fragments essential for the recruitment of cellular and humoral mediators of inflammation.

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