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Simultaneous occurrence of hereditary C6 and C2 deficiency in a French-Canadian family

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Summary. The sera of four sisters were found to lack the sixth component of complement (C6) and the serum of one was also partially deficient in the second component (C2). Two other blood relatives were found to be heterozygous for both deficiencies, while only one sibling had normal values. The father of these eight siblings was heterozygous for C2D and C6D and in the third generation, six children were heterozygous for C6 deficiency was treated for chronic active brucel-transmitted; the C6 deficiency was not linked to the HLA system, while the C2 deficiency segregated with the haplotype A10,B18. The proband, homozygous for C6 deficiency was treated for chronic active Brucellosis and in another sibling with C6 deficiency, toxoplasmosis was diagnosed. Neither bleeding disorders nor a tendency to collagen diseases have been observed and the opsonic activity was normal in the sera of all family members.

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Abbreviations: APTT, activated partial thromboplastin time; Cl INA, Cl esterase inhibitor; C2D, C2 deficiency; C6D, C6 deficiency; CH50, haemolytic whole complement assay; DGVB^{++}, dextrose gelatin Veronal buffer; EA, sensitized erythrocytes; PT, prothrombin time; SLE, systemic lupus erythematosus; TT, thrombin time.

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INTRODUCTION

Since the discovery that the genetically determined absence of the Cl esterase inhibitor (Cl INA) results in a specific disease, hereditary angioneurotic oedema, there has been growing interest in the study of hereditary deficiencies of the other complement components. The development and standardization of functional and immunochemical methods and the availability of purified complement components and specific antisera have made possible investigations of genetic deficiency states which have been recently reviewed by Schur (1977). In contrast to the Cl-INA deficiency, the gene-absence of other complement components does not cause any specific disease. Increased liability to infections and immune complex diseases has been suggested by Agnello (1978). Until now the number of known sibships with deficiencies of the late acting complement components has been too small to draw conclusions concerning their clinical implications. To date homozygous C6D has been reported in four individuals belonging to three different, unrelated families (Leddy, Frank, Gaither, Baum & Klemperer, 1974; Lim, Gewurz, Lint, Ghaze, Spheri & Gewurz, 1976; Glass, Raum, Balavitch, Kagan, Rabson, Schur &

Alper, 1978). Three of them had disseminated neisseria infection (Leddy *et al.*, 1974; Glass *et al.*, 1978) and a fourth relapsing meningococcal meningitis (Lim *et al.*, 1976); C6D in its heterozygous form was not related to disease (Lachmann & Hobart, 1977; Glass *et al.*, 1978). No genetic relationship for C6D to the HLA system was demonstrated in the first family studied (Mittal, Wolski, Lim, Gewurz, Gewurz & Schmid, 1976), but an association with the haplotype AW24 was suggested in a brief report (Raum, Glass, Carpenter & Schur, 1976) dealing with four Caucasian individuals heterozygous for C6D. This paper describes the clinical, biological and genetic studies in a Caucasian kindred with C6D. The proband had latent (brucella infection. Furthermore, it is the first report of simultaneous C6D and C2D. Because of the implications concerning the genetic and clinical aspects of deficiencies of the late acting complement components (Alper & Rosen, 1976), it is important to collate all data of these rare conditions.

CASE REPORTS

Medical history of the three sisters homozygous for C6D

Case 1. The proband C.F. (no. II 7 in Fig. 1), a 40 year old female of French-Canadian descent, married to a farmer, was admitted to the hospital in October 1977 for hysterectomy. Routine laboratory studies including basic coagulation tests (bleeding time, PT, APTT and TT) were normal. The surgical intervention was without complication and the histologic diagnosis of leiomyoma was made. The patient had complained of some shoulder pains, chills without fever and weakness from time to time and was further investigated. Common childhood infections had been handled without complications. At age 15 she underwent tonsillectomy because of recurrent tonsillitis and otitis media. At age 19, a history of exposure to brucella was probable because she drank milk from an infected cow. At age 21 she had pneumonia and in January 1977 a cholecystectomy was performed because of gallstones. The patient had four children and one spontaneous abortion. Physical examination on admission in October 1977 was normal. Tests for anti-nuclear antibodies and rheumatoid factors were negative and the peripheral blood values for immunoglobulins G, M and A were normal. Serodiagnosis for brucella was positive 1/640. A control 1 month later was again positive 1/320. No prozone phenomenon was noted, and a diagnosis of chronic active brucellosis was made. The haemolytic complement titre (CH50) was 5 u/ml and persisted at this level after the patient had been treated for 8 weeks with tetracycline 2 gm i.d. and the agglutination titre for brucella had fallen to 1/40. When it became clear that this extremely low CH50 titre represented a specific deficiency of C6 (see below) the patient's family was investigated. The father, a 78 year old retired farmer, was in good health. His past was uneventful except that at age 75 he had pneumonia following prostatectomy. The patient's mother died from an accident and no important disease had been reported. There was no known consanguinity between the parents. The couple had fifteen children, eight of whom are living. Five died soon after birth; two from pneumonia, one was malformed and for the other two, no information could be obtained. A sixth sibling died at age of 6 months from pertussis and the seventh sibling died at age 20 in a car accident. Two brothers and five sisters of the proposita could be investigated. At the present time they all are without clinical disease symptoms.

Case 2. R.A. (no. II 6 in Fig. 1), is a 48 year old housewife. Common childhood diseases passed uneventfully. At age 14 she had infectious hepatitis. Later as an adult she had hysterectomy and cholecystectomy without sequelae. During the last 10 years she intermittently suffered from periods of fever the cause of which was unknown and at times she felt weak and tired easily. In October 1977 she was admitted to her local hospital for the investigation of a supraclavicular lymph node. At biopsy the histological diagnosis of granulomatous lymphadenitis of the type Piringer–Kuchinka was made. This pattern suggests toxoplasmosis and two positive Dye tests (University of Guelph, Ontaria, Canada) at 1:1024 (the highest dilution used) at three month intervals further indicated this. The clinical investigation was negative except for brucella agglutinins present at low levels, varying from 0 to 160 on eight successive measurements, performed in two different laboratories. Because of the vague symptomatology the patient was dismissed without treatment and at the present investigation she seems to be well.

Case 3. D.A. (no. II 8 in Fig. 1), is a 42 year old housewife, her childhood and adolescence have been uneventful medically except for tonsillectomy. At 19, she had contact with tuberculous people. Some years

ago she had hysterectomy and cholecystectomy for benign disease without any complications. At present time she is in good health.

Medical history of a sister homozygous for C6D and heterozygous for C2D

Case 4. M.A. (no. II 2 in Fig. 1), is a 43 year old housewife. At age 9 she had infectious hepatitis. At age 33 she had cholecystectomy, which was complicated by postoperatory peritonitis. Since then she has had no important disease.

Medical history of a sister heterozygous for C6D and C2D

Case 5. M.S. (no. II 1 in Fig. 1), is a 35 year old married woman and she is working as a secretary. Common childhood infectious diseases were handled without complication. Besides the ablation of an ovarian cyst she has always been in good health.

METHODS

Sera

Freshly obtained human and guinea-pig sera were stored at $-70°$ in small aliquots and discarded after one thaw.

Complement reagents and haemolytic assays

Titrations of whole complement (CH50) and of each of the nine complement components together with methods for establishing normal ranges and standard deviations were performed as previously reported (Delâge, Bergeron, Simard, Lehner-Netsch & Prochazka, 1977). Anti-complement activity was assayed by the method of Dausset (1956). Functionally pure complement components from C1 to C9 were purchased from Cordis Laboratories, Miami, Florida, U.S.A. All haemolytic assays were performed in tubes and two controls of known normal sera were run in each experiment. Normal mean values $\pm$ 2SD and normal ranges are shown in Table 1. Two methods were used for C6 titration. First, a screening test was performed in the following manner: serial dilutions of the test serum in $DGVB^{++}$ (0·2 ml) were reacted with 0·2 ml of the proposita's serum (diluted: 1:25) having a CH50 titre of 5 u/ml, 0·2 ml of EA cells (1×10^8/ml) and 0·4 ml of $DGVB^{++}$ were added to give a final volume of 1·0 ml. After incubation at 37° for 60 min, the reaction was terminated by adding 1·5 ml of cold saline and centrifuged. Lysis was determined by read-

Table 1. Measurement of individual complement components in the patient's serum

Component	Proband C.F.	Normal range or mean $\pm$ 2SD*
Haemolytic assay	u/ml	
C1	30,000	32,000 $\pm$ 8,000
C2	2,000	1,430 $\pm$ 370
C3	4,250	4,000 $\pm$ 1,000
C4	16,000	15,000 $\pm$ 3,000
C5	6,250	6,000 $\pm$ 2,000
C6	22	45,000 $\pm$ 15,000
C7	28,000	32,000 $\pm$ 5,200
C8	128,000	160,000 $\pm$ 96,000
C9	32,000	24,000 $\pm$ 8,000
Radial immunoprecipitation	mg/100 ml	
C3	94	65–120
C4	46·8	20–50
Factor B	21·5	15–30
C3 activator	27	12–30
C1 INA	38	15–35
Ouchterlony's method	Serum dilution	
C1q	8	4–8
C1s	8	8–16
C6	0	6–8

* Representing normal values for this laboratory (see Methods).

ing the optical density at 415 nm. The normal range for C6, as established by the titration of ten normal donors' sera was 2300–3200 u/ml. The functional activity of C6 was further determined by the method of Nelson, Jensen, Gigli & Tamura (1966) with modifications suggested by Cordis Lab. To serial dilutions of the serum in DGVB^{++}, one volume of a mixture containing 100 u/ml of C3, C5, C7 each and one volume of C2 (100 u/ml) and one volume of EAC1g-p4hu (1×10^8/ml) were added. After incubation at 37° for 30 min, one volume of a mixture containing C8 and C9 75 u/ml each was added and the tube were further incubated at 37° for 60 min. The reaction was blocked and haemolysis was determined in the usual manner. The normal range for C6 was 30,000–60,000 as established by titration of fifteen individual donors' sera and two pools of five known control sera each. Functional C6 was expressed as a mean of three determinations. These C6 titrations were performed on all members of the family and two controls were run in each experiment.

Immunoassay for complement components

C4, C3, Factor B, C3 activator and C1 1NA were quantified by single radial diffusion on immunoplates purchased from Behring-Werke AG, Marburg/Lahn, West Germany. Ouchterlony's double diffusion method was used for semi-quantitative determination of Clq, Cls and C6. Specific antisera to Clq and Cls were obtained from Behring-Werke; the mono-specific goat anti-human C6 was a generous gift of Dr H. J. Müller-Eberhard, Scripps Clinic and Research Foundation, La Jolla, California. The results of the Ouchterlony analysis were expressed as the reciprocal of the highest dilution of the test serum giving a visible precipitin line and were read by four independent observers. The normal range was established by assays of ten individual donors' sera and two pools of five donors' sera each.

HLA studies

Seventeen members of this family have been typed for HLA antigens in order to evaluate the relationship between C6 deficiency and the major histocompatibility complex. Eleven specificities of the 'A' series and fifteen of the 'B' series were investigated: HLA-A1,2,3, W23, W24, W25, 10,11, 28,29. W30 and HLA-B5,7,8,12,13,14,15,16,17,18,22,27,35,37,40. Each antigen was tested by several mono- and multi-specific sera, with the exception of A28, B18 and BW35, which were tested with duo-specific sera. The typing was done by the lymphocyte microcytotoxic technique using antisera kindly giving by the National Institute of Health, Bethedsa, Md.

Other studies

Opsonic activity was measured as described previously (Delâge *et al.*, 1977). Agglutinating antibodies to *Brucella abortus* antigen (Lederle) were titrated according to the standard method (FAO/WHO) by multiple dilutions in tubes (1/20–1/2560). *Brucella abortus* and *Brucella melitensis* antigens were used in the outside hospital for case no. 2 (R.A.), the latter antigen being a little more sensitive. (There is complete cross-reactivity between the three main brucella antigens). Determination of immunoglobulins G, A, M and antinuclear antibodies were performed according to standard methods.

RESULTS

Establishment of the C6D in the proposita

Routine complement studies revealed that the CH50 titre in C.F.'s serum was 5 u/ml while the levels of C3 and C4 as measured by the immunoprecipitation method were normal. Haemolytic titration of C1 gave a normal result, while C2 was found at higher levels than in the normal controls. The patient's serum had no lytic effect on EAC1, EAC14 and EAC142 intermediates and no anticomplement activity was found. The haemolytic activity of the serum could only be restored by adding functionally pure C6 (100 u/ml), whereas the addition of each of the other complement components failed to increase the CH50 titre. The C6 activity as measured by Nelson's method was 22 u/ml and persisted at this extremely low level after the patient had been treated by tetracycline for 8 weeks and the agglutination titre against brucella had fallen from 1:640 to 1:40. The C6 protein could not be detected in the Ouchterlony analysis. Haemolytic and immunochemical assays of all other complement components were normal and all values are shown in Table 1.

Genetic studies of the C6D and detection of simultaneous C2D

The proband's father, her two brothers and six sisters, her husband and her four children were investigated. The husband and the two children of case 4 were also studied. The family pedigree is shown in Fig. 1 and the

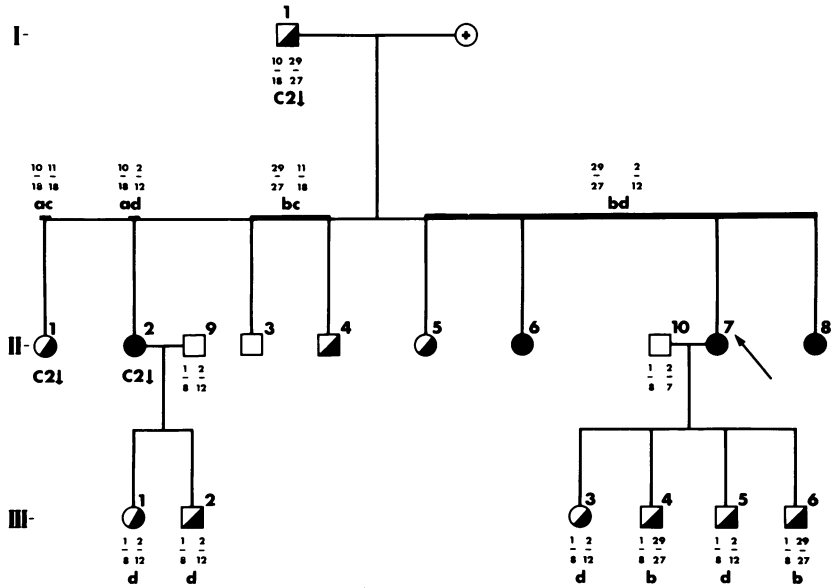

Figure 1. Pedigree of the studied family. Males are indicated by squares, females by circles. Each studied member is identified by a number. C6D in the homozygous state is shown by fully blackened symbols, C6D in the heterozygous state is shown as half-blackened symbols. The proposita is indicated by an arrow and a cross marks a deceased member. Heterozygous C2D is indicated by C2↓.

CH50 titre, C6 and C2 levels are listed in Table 2. The father E.B. (no. I 1 in Fig. 1) was found to have a CH50 titre of 55 u/ml; C6 was at 1575 u/ml in the screening test and 11,500 u/ml when measured by Nelson's method. In the Ouchterlony analysis the C6 protein content was decreased to 2. Because of the presence of the haplotype A10,B18, C2 was titrated and found to be 825 u/ml, indicating a partial deficiency of this component. Three of the proband's four children (nos. III 3, III 4 and III 5 in Fig. 1) had CH50 titres above 80 u/ml and the fourth (no. III 6 in Fig. 1) a 6 year old boy had 68 u/ml, a normal level when compared with the individual sera of ten healthy children of the same age. Estimation of C6 by the screening test revealed values about half normal in the sera of all of the proband's children. Subsequent specific C6 titrations confirmed this, giving values between 9000 and 13,500 u/ml. The C6 protein was diminished to 1·5 to 2 in the Ouchterlony assay. From these results it could be concluded that the C6D was hereditary in this family. Among the proband's siblings only one brother (no. II 3, in Fig. 1) was found to have normal C6 values. Two sisters (nos. II 6 and II 8, in Fig. 1) had a complement profile similar to the proposita. The serum of a third sister (no. II 2) was not only deficient in C6, having 22 u/ml C6 in the functional assay and being absent in the immuno-assay, but was also partially deficient in C2 with a functional activity of 800 u/ml. All other complement components were at the normal level. Her two children (nos. III 1 and III 2), age 11 and 7 had CH50 titres of 63 u/ml, a normal range for this age; C6 was at 600 and 700 u/ml respectively in the screening test and 7000 and 7200 u/ml when measured by Nelson's method. In the Ouchterlony analysis the C6 protein content was 1·5. The C2 activity in the sera of these two children was normal. Sibling no. II 1 (Fig. 1) had a CH50 titre of 58 u/ml, her C6 was 17,000 u/ml in the functional assay and C6 protein was low at 1·5. Her haemolytic C2 level was also low at 850 u/ml. Sibling no. II 5 had a normal C6 value in the screening test, but in the specific C6 titration this component was found to be lower at 25 000 u/ml and gave an abnormally low titre in the Ouchterlony analysis. Sibling no. II 4 had a borderline CH50 titre of 67 u/ml, his C6 was about half normal in all specific titrations whereas the C2 titre with 1650 u/ml was normal.

The data on the complement studies in this family strongly suggests homozygous C6D for the proband

Table 2. HLA genotypes, whole complement, C6 and C2 levels in the studied family

Subject (nos as in Fig. 1)	Genotypes	Family haplotypes §	CH50	C6			Deficiency state for		
				Screening	Nelson's method	Protein	C2	C6	C2
			u/ml		u/ml	Serum dilution	u/ml		
Normal*			96 ± 25	2300–3200	45,600 ± 15,000	6–8	1,430 ± 370		
1st generation (I)									
(1)	A10,B18/A29,B27	ab	55	1575	11,500	2	825	heterozygous	heterozygous
2nd generation (II)									
(7) proposita	A2,B12/A29,B27	bd	5	ND‡	22	0	2000	homozygous	normal
(1)	A10,B18/A11,B18	ac	58	1050	17,000	1·5	850	heterozygous	heterozygous
(2)	A2,B12/A10,B18	ad	6	14	22	0	800	homozygous	heterozygous
(3)	A29,B27/A11,B18	bc	100	2850	54,000	8	1850	normal	normal
(4)	A29,B27/A11,B18	bc	67	1100	18,000	2	1600	heterozygous	normal
(5)	A29,B27/A2,B12	bd	73	2520	25,000	2	1650	heterozygous	normal
(6)	A2,B12/A29,B27	bd	6	< 10	16	0	1850	homozygous	normal
(8) husband of (2)	A2,B12/A29,B27	bd	5	12	20	0	1200	homozygous	normal
(9) husband of (7)	A1,B8/A2,B12	–	98	3000	39,000	ND‡	1700	normal	normal
(10)	A1,B8/A2,B7	–	89	2400	34,000	6	1600	normal	normal
3rd generation (III)									
Children of (2)									
(1)	A1,B8/A2,B12	d	63	600	7,000	1·5	1300	heterozygous	normal
(2)	A1,B8/A2,B12	d	63	700	7,200	1·5	1200	heterozygous	normal
Children of (7)									
(3)	A1,B8/A2,B12	d	85	1200	13,500	1·5	1350	heterozygous	normal
(4)	A1,B8/A29,B27	b	82	1900	12,000	2	1400	heterozygous	normal
(5)	A1,B8/A2,B12	d	83	1200	12,500	1·5	1250	heterozygous	normal
(6)	A1,B8/A29,B27	b	68	1325	9,000	2	1100	heterozygous	normal

* Normal range or mean ± 2SD.
‡ Not determined.
§ Represented by letters: a = A10,B18; b = A29,B27; c = A11,B18; d = A2,B12.

and three other siblings (nos II 2, II 6 and II 8) and heterozygous C6D for the proband's father (no. I 1), three other siblings (nos. II 1, II 4 and II 5), the proband's four children (nos. III 3, III 4, III 5 and III 6) and the two children (nos. III 1 and III 2) of sibling II 2. Heterozygous for C2D was established in the father (no. I 1) and in two siblings (nos. II 1, II 2), all these individuals bearing the haplotype A10,B18.

It is noteworthy that the CH50 titre of the sera partially deficient in C6 were in the normal range, whereas this titre in the sera of the two members heterozygous for C6 D and C2D (nos. I 1 and II 1) was significantly lower (55 and 58 u/ml). Heterozygous C6D cannot be detected by measuring whole complement activity alone, but the screening test gave a good indication and in two dimensional gel diffusion, heterozygous sera exhibited clearcut results for the C6D. For one subject (no. II 5) there was no correlation between the screening test and the specific haemolytic C6 titration but the low protein value indicated heterozygosity for C6 in this particular sibling.

HLA typing (Table 2 and Fig. 1)

HLA typing was performed on the proposita and sixteen members of her family. In the first generation, the father was living and available for typing. In the second generation, all eight siblings, the spouse of the proposita and the spouse of the sibling no. II 2 were typed. In the third generation, the proposita's four children and the two children of no. II 2 were typed. Genotypes were then determined and subjects of the second generation were distributed in the following four sib groups: ac: A10,B18/A11,B18; ad: A10,B18/A2,B12; bc: A29,B27/A11,B18; and bd: A29,B27/A2,B12. The proposita (no. II 7), two sisters homozygous for C6D (nos. II 6 and II 8) and another one (no. II 5) with an heterozygous C6D shared the same sib group bd. The fourth subject homozygous for C6D was a sister (no. II 2), the only one to belong in the sib group ad. In the sib group ac there was a single sister heterozygous for this deficiency, and in the sib group bc, two brothers one of them with a normal C6 level and the other heterozygous for C6D. In the third generation, all children of the proposita and of sibling no. II 2 are heterozygous for C6D. It must be pointed out that the proposita's four children inherited from the proposita different haplotypes, two of them having b and the other two having d. Let it be stated that in the overall study of the family we found that only the three members bearing the haplotype A10/B18 (I 1, II 1, II 2) presented a confirmed C2 deficiency.

Other studies

The opsonic activity of serum was measured in all family members and no deficiency could be detected. All members were studied for brucellosis. In the proposita's serum, the agglutinin titre on the first investigation was 1 : 640, and went down 1 : 40 after treatment. Sibling R.A. (no. II 6 in Fig. 1) had on a first investigation a titre of 1 : 80 and three months later 1 : 40. All other sera were negative for brucellosis agglutinins. Determination of immunoglobulins G, A and M, and determination of anti-nuclear factors gave results within normal limits for every subject in the family.

DISCUSSION

Evidence has been presented for the first known simultaneous occurrence of C6 and C2 deficiency in three members of a kindred. This family contained four siblings homozygous for C6D, one of whom was heterozygous for C2D. Ten were heterozygous for C6D and two of these were also heterozygous for C2D. One sibling had a normal C6 level. We have gathered data showing conclusively that the C6 and C2 deficiencies which occurred together in three members of this kindred constitute two distinct and unrelated hereditary deficiencies.

Absence of C6 was readily demonstrated by a CH50 titre near zero in the proposita and her three homozygous siblings and by its restoration to normal values upon addition of purified C6. The techniques recommended for the quantification of individual complement components, such as haemolytic titrations and immunochemical assays are suitable to detect most heterozygous C6 deficiencies. Partial deficiences cannot be demonstrated by the measurement of whole complement, the latter being normal or near normal in partial C6D. The screening test we employed, using a C6D serum having less than 5% of the normal activity, constituted a rapid method for separating normal from deficient sera in most cases (nine out of ten). The titre of CH50 was significantly lower than normal only when heterozygous C6D occurred together with C2D. This fact must be taken into account whenever a low CH50 is found in a case of partial deficiency of a late acting complement component. The low CH50 in par-

tial C6D led us to the discovery of the other deficiency (C2) and the presence of the haplotype A10,B18 in the proposita's father was a clear indication of such a deficiency. All other complement components were normal in the deficient individuals. The deficiency in C2 was not linked to C6D since several members of the family were deficient in C6 with a normal level of C2 in their sera.

Genetic analysis revealed a codominant Mendelian pattern for the C6 deficiency (Fig. 1) and independent segregation from the HLA system (Fig. 2). Three of four sisters, homozygous for C6D, belonged to the sib group bd together with a sister heterozygous for C6D. The fourth homozygous sister however belonged to a sib group ad. Of the two siblings sharing the group bc one had normal C6 level and the other one was heterozygous for C6D. Also, the two different haplotypes of the proposita were present in her four children, all heterozygous for C6 deficiency. An association between the gene for the C6 level and the HLA system is precluded by these data shown in Fig. 2. The C2 deficiency was found to be associated with the HLA system, particularly to haplotype A10,B18 as has been previously reported (Fu, Kunkel, Brusman, Allen & Fotino, 1974; Day, L'Espérance, Good, Michael, Hansen, Dupont & Jersild, 1975). The proband's father, having deficiencies of C2 and C6, transmitted this C2 deficiency to his children with haplotype A10,B18 independently from the transmission of the other deficiency. This finding again suggests that these two distinct hereditary defects are independent.

Simultaneous deficiencies of two complement components have been reported for C6 and C7 (Glass *et al.*, 1978; Lachman *et al.*, 1977), the complement components segregating together. The latter components are very close in their physico-chemical properties and it may be that the genetic error occurred prior to the splitting of C6 from C7 in the ontogenetic development. Partial C2D and C7D have been reported in an individual (Lehner-Netsch, Prochazka, Simard & Delâge, 1977) who had inherited the C2 deficiency from his father while the C7 deficiency was transmitted by his mother.

The association of C6 deficiency with susceptibility to disease remains a crucial question. In our observations, the proposita was considered to have chronic brucellosis because of her known exposure to a source of infection, the clinical presentation and the highly significant agglutinin titre which returned to normal after 8 weeks of tetracycline treatment. This return to a normal serology did not affect the low complement titres which remained unchanged after treatment. A homozygous sister of our patient had toxoplasmosis. While these data suggest sensitivity to disease in C6D individuals, two siblings homozygous for C6D had no signs of brucellosis and had negative serological tests despite the fact that they were exposed to the same environment as the proposita. It has been reported that in the absence of one of the late reacting components, there is an imperfect antibody response to toxoplasmosis (Feldman & Schreiber, 1977) resulting in a severe form of the infection in the host. This does not seem to be the case either for brucellosis and C6D in the proposita or for toxoplasmosis and C6D in her sister. Antibodies to meningococcus have been found at a high titre in a reported case of C6D (Lim *et al.*, 1976).

In the serum of patients deficient in one of the late acting complement components, the activation of C1 to C5 proceeds normally, giving rise to the generation of normal amounts of opsonically active complement derivatives. Rabbits with C6D had no undue suscepti-

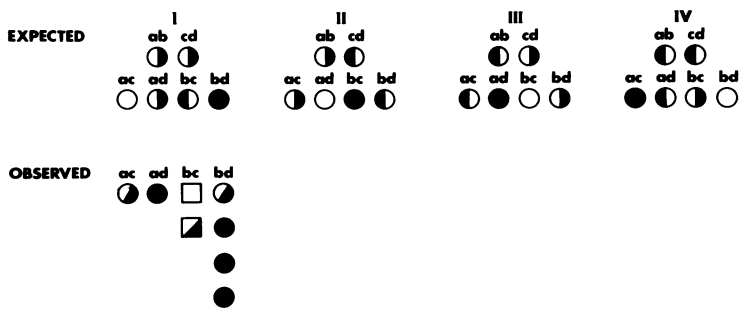

Figure 2. The four possible patterns of inheritance of a single normal C6 allele assuming close linkage between the HLA and C6 loci as compared to the observed situation. The HLA family haplotypes are represented by letters: a = A10,B18; b = A29,B27; c = A11,B18; d = A2,B12.

bility to common infections but the sensitivity to endotoxin was higher in C6 deficient animals than in normals (Johnson & Ward, 1971), a finding that is not unexpected. In our studies individuals heterozygous both for C6D and C2D as in the previously reported case of heterozygous C7D with heterozygous C2D (Lehner-Netsch *et al.*, 1977), show no sensitivity to a particular disease and appear to have normal health. At first glance, one could be prejudiced by the apparently high prevalence of infection in the family and also by the high neonatal mortality. The latter is undoubtedly due to the high neonatal mortality normal at the time in the remote areas where the family was living. Regarding infections, brucella was endemic in this particular rural area, and the other infections such as toxoplasmosis belong to the group of still common diseases. It should not be concluded therefore that there is an excess in occurrence of them in this family. It might be remarked that only one member of this family was found to be free of any complement deficiency.

Although haemostatic abnormalities of C6D have been reported in rabbits (Zimmerman, Arroyave & Müller-Eberhard, 1971), basic coagulation studies were normal in the proposita of our report. All four sisters with homozygous C6D underwent several surgical interventions without any bleeding complication, so that a deficient haemostasis can be ruled out. In an extensive study of the coagulation system of a C6D individual, no defect was found and it was concluded that the difference between the two species, rabbit and man, is related to a different platelet response (Heusinkveld, Leddy, Klemperer & Breckenridge, 1974). It may be speculated that complement deficiencies may carry some advantages. In C6D rabbits, the integrity of the immune system is affected in such a way as to weaken the Schwartzmann reaction (Fong, Rother & Good, 1974). In C2D humans the lupus-like syndrome has features different from the classical disease (Agnello, 1978), e.g. anti-nuclear antibodies are of a lower titre, and glomerulonephritis rarely if ever leads to irreversible renal failure (Rynes, Urizar & Pickering, 1977). The association of homozygous C2D to rheumatic and collagen diseases is well established and a highly statistical association of heterozygous C2D and SLE has been suggested (Glass, Raum, Gibson, Stillman & Schur, 1976).

Although simultaneous deficiency of one of the early acting with one of the late acting complement components has been reported in only one instance (Lehner-Netsch *et al.*, 1977), its occurrence may be higher than is presently thought. It has been suggested that the frequency of the gene for C2D approximates 1% (Fu *et al.*, 1975). Deficiency of C6 at least in its heterozygous form may not be as uncommon as once thought, but may escape detection because such heterozygous complement deficiencies are compatible with a normal CH50 level. HLA studies are mandatory in the investigation of complement deficiencies and the finding of the haplotypes known for their linkage to C2D may lead to the discovery of an additional complement deficiency.

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