Inherited Deficiency of the Second Component of Complement

RHEUMATIC DISEASE ASSOCIATIONS

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ABSTRACT The prevalence of homozygous and heterozygous deficiency of the second component of complement (C2) was determined in patients with rheumatic disease including 137 with systemic lupus erythematosus (SLE), 274 with juvenile rheumatoid arthritis, and 134 with rheumatoid arthritis. 1 C2 homozygous deficient and 19 possible heterozygous deficient individuals were identified by using both immunochemical and functional assays to determine C2 levels. Of the 20, 8 had SLE (5.9%), 10 had juvenile rheumatoid arthritis (3.7%), and 2 had rheumatoid arthritis (1.4%), the homozygous deficient individual having SLE. The prevalence of C2 deficiency in the SLE and juvenile rheumatoid arthritis patients was significantly increased ($P = 0.0009$ and $P = 0.02$, respectively) when compared with controls, 6 (1.2%) of 509 blood donors having C2 levels consistent with heterozygous deficiency. 15 of the 20 C2 deficient patients were HLA typed and found to have antigens A10(Aw25), B18, or both.

The patients with C2 deficiency and SLE had earlier age of onset of disease and less antinuclear antibody when compared with the C2 normal SLE patients. 11 families of the propositi were studied and found to have one or more C2 heterozygous deficient individuals. The family members had an equal distribution of rheumatic disease and antinuclear antibody in the C2 deficient and C2 normal groups. C2 deficient individuals were found to have significantly lower levels of properdin Factor B (242 μg/ml±54) when compared with the non-C2 deficient family members (282 μg/ml±73).

These data support the concept that inherited deficiency of C2 is significantly associated with both SLE and juvenile rheumatoid arthritis.

INTRODUCTION

Inherited deficiencies of many of the proteins contributing to the classical and terminal complement pathways have been described in man. Homozygous and heterozygous deficiencies are found in healthy individuals. However, illness in the form of inflammatory rheumatic disorders and infections has been associated with homozygous deficiencies (1–3). The hereditary deficiency reported most frequently is that of the second component of complement (C2).[1] Homozygous or heterozygous deficiency of C2 has been found in both healthy individuals and in association with systemic lupus erythematosus (SLE), discoid lupus, dermatomyositis, vasculitis, and Henoch-Schönlein, or anaphylactoid purpura (4–16). The recent linkage of particular HLA-A, B, and D serotypes with C2 deficiency provides a useful adjunct to the complement assays in the identification of potentially C2 deficient subjects (11, 16–20).

The nature of the association reported between the genetically determined defects and the rheumatic diseases like SLE and discoid lupus is not established (16–20). To evaluate the extent of this relationship, we report a study of hereditary C2 deficiency, its incidence in healthy and rheumatic disease individuals, and characteristics of the associated diseases.

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[1] *Abbreviations used in this paper:* ANA, antinuclear antibody; BF, properdin Factor B; C2, second component of complement; JRA, juvenile rheumatoid arthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

METHODS

The subjects studied, all of whom attended the Robert B. Brigham Hospital, included 137 with SLE, 274 with juvenile rheumatoid arthritis (JRA), and 134 with rheumatoid arthritis (RA). None of these patients were referred here specifically for evaluation of complement abnormalities. The patient populations were evaluated in terms of the appropriate American Rheumatism Association criteria (21–23). The patients with SLE and JRA consisted of all those who have attended the respective clinics over a 5-yr period and about whom there was sufficient criteria known to make a positive diagnosis at least on clinical grounds. Among the 137 SLE patients there was one family with three sisters with SLE (none were C2 deficient) and among the 274 JRA patients there was one family with two sisters (one of whom is C2 deficient). For the purposes of this study they were all included. The RA patients were selected over a 3-mo period and formed a consecutive series attending the Hospital.

121 of the 137 SLE patients had four or more American Rheumatism Association criteria; the remaining 16 had three or less. The 274 JRA patients were only diagnosed as such after other diseases were excluded (22). Of 134 RA patients, 111 were diagnosed as definite, 16 as probable, and 7 as possible (23). The SLE population had a mean age of onset of disease of 29.8±13.9 yr; the JRA patients were less than 17 yr at onset, and the RA patients' mean age of onset was 47.4±15.1 yr.

Family members of C2 deficient patients were questioned as to rheumatic disease symptomatology. Sera from 509 blood bank donors were kindly provided by Dr. Zymanski, Massachusetts Red Cross, for C2 level determination.

The C2 deficient individuals were defined as previously described (20) and summarized below. Serum was separated within 3 h of venipuncture and stored at −70°C until required for assay. All sera were assayed for total hemolytic complement CH50 and C1s, C1q, C2, C4, C3, C5, C6, and properdin Factor B proteins (24–27). The complement components were assayed initially by radial immunodiffusion (27). Inherited deficiency of C2 was suspected when the C2 protein was low (less than 2 SD below the mean) or low normal (less than 1 SD below the mean), particularly if other components of the classical pathway were within the normal range (Table I). Functional C2 activity was then measured in those individuals with this apparently isolated deficiency of C2 or a low normal value. The normal values for complement levels with mean±2 SD for 102 normal individuals are given in Table I. If the functional C2 level was less than 2 SD below the mean, multiple samples stored at intervals during routine clinical follow-up were analyzed retrospectively by both C2 protein and functional assays to exclude acquired changes in C2 levels.

HLA tissue typing was performed (28) on available individuals with consistent evidence of C2 deficiency. Serum complement levels and HLA typing were determined in family members to establish the inherited nature of the C2 deficiency.

Four groups of individuals were classified as follows: (*1*) 'definite' inherited C2 deficiency supported by occurrence of similar abnormalities in one or more of the other family members in association with HLA A10(Aw25) or B18, or both; (*2*) 'probable' inherited C2 deficiency characterized by isolated low C2 levels in association with HLA A10 (Aw25) or B18, or both; (*3*) 'possible' inherited C2 deficiency by occurrence of isolated low C2 levels where HLA or family studies were not available; and (*4*) C2 normal, when C2 values were within 2 SD of the normal mean.

Antinuclear antibodies and precipitins to nuclear antigens and nucleic acids were determined by immunofluorescence and by counterimmunoelectrophoresis, as previously described (29).

TABLE I

Complement Levels: Family Members and Controls

	Family members		
Number	C2 deficient* 30	Non-C2 deficient 32	Normal controls 100‡
	mean ±2 SD		
C2 protein, *μg/ml*	12.9±5.8§	23.3±9.4§	27±11.2
Observed range	(8.0–16.8)	(13.2–31.9)	
Functional C2, *U/ml*	10,890±5,806§	26,869±18,472§	26,625±11,048
Observed range	(5,440–14,951)	(17,658–50,000)	
CH50, *U/ml*	151±50§	195±78§	200±50
Clq, *μgN/ml*	425±146	448±174	448±122
C4, *μg/ml*	487±260	455±276	498±302
C3, *μg/ml*	1,721±686	1,621±646	1,612±488
C5, *μg/ml*	150±92	144±80	153±58
Factor B, *μg/ml*	242±108‖	282±146‖	275±110

* Excluding propositi.
‡ 509 for C2 protein.
§ $P < 0.001$.
‖ $P < 0.01$.

TABLE II
C2 Deficient Subjects

Population	Numbers	Homozygous deficient	Heterozygous deficient			Total deficient	
			Possible	Probable	Definite		
							%
RA	134	0	0	1	1	2	(1.4)
JRA	274	0	1	3	6	10*	(3.7)
SLE	137	1	3	1	3	8‡	(5.9)
Controls	509	0	6	0	0	6	(1.2)

* $P = 0.02$ with respect to controls.
‡ $P = 0.0009$ with respect to controls.

RESULTS

Of the 545 rheumatic disease individuals studied, 20 were C2 deficient: 1 homozygous and the remaining 19 heterozygous deficient. Of the 509 blood donor controls, 6 were thought to be C2 deficient. The difference in incidence between the rheumatic disease and blood donor populations with low C2 levels was statistically significant ($\chi^2 = 6.79$, $P = 0.009$). When the distribution of the C2 deficient individuals was evaluated by disease, as shown in Table II, differences between disease and control groups were significant for SLE (8 of 137), and for JRA (10 of 274) compared with the control group ($\chi^2 = 11.06$, $P = 0.0009$ and $\chi^2 = 5.43$, $P = 0.02$, respectively); but not for the RA group (2 of 134).

The complement and HLA data by which these C2 deficient individuals were defined are given in Tables III and IV. In 11 deficient individuals, confirmatory family studies were possible in one of the RA patients (P. L.), in six of the JRA patients (M. Mu, P. B., K. M., J. M. F., J. R., P. P.), and in four of the SLE patients (E. R., M. P., A. S., A. W.), including the homozygous deficient individual (A. W.). In 10 instances, 1 or more of the other family members were found to have C2 levels consistent with C2 heterozygous deficiency and to share a similar HLA haplotype with the propositus (A10[Aw25], B18, or both) (Tables III and IV). These were classified as definite C2 deficient. In one family study of a JRA patient (P. P.), several family members were C2 heterozygous deficient in association with A10,B18, and evidence of a crossover in the region of C2 in the propositus was obtained.[2]

[2] Raum, D., D. Glass, C. B. Carpenter, C. A. Alper, and P. H. Schur. The chromosomal order of genes controlling the major histocompatibility complex, properdin Factor B and deficiency of the second component of complement. Submitted for publication.

TABLE III
RA and JRA Patients

Name	Serum complement levels*								HLA A/B	Confirmatory‡ family studies	C2 Status
	CH50	C1q	C4	C3	C5	Factor B	C2 Protein	C2 Function			
RA											
A. M.	129	373	254	1,242	129	146	13.5	6,129	A10, B18	ND§	Probable heterozygous, deficien
P. L.	162	493	656	1,863	172	221	12.7	13,425	*A10, B18*,‖ AW24, BW40	+	Definite heterozygous, deficien
JRA											
M. Mu.	133	362	320	1,728	118	205	12.9	5,440	*A2, B18*,‖ A11, BW22	+	Definite heterozygous, deficien
P. B.	145	414	251	1,150	120	267	8.0	9,240	A2, B15, *A10, BW40*‖	+	Definite heterozygous, deficien
K. M.	214	343	521	2,247	143	346	11.5	8,207	*A10, B18*‖ A2, B12	+	Definite heterozygous, deficien
J. M. F.	187	567	651	2,343	185	393	15.1	9,204	*A10, B18*‖ A28, B12	+	Definite heterozygous, deficien
J. R.	143	414	431	1,653	113	197	8.0	1,202	A2, B5, *A2, B18*‖		Definite heterozygous, deficien
M. M. A.	184	350	404	1,840	131	395	13.5	12,628	A2, A24, B27, B18	ND	Probable heterozygous, deficien
B. F.	164	352	461	1,550	240	237	15.8	11,907	A9, A29, BW35, B18	ND	Probable heterozygous, deficien
H. S.	186	380	1,000	2,670	222	325	13.3	8,778	A2, A10, B5, B18	ND	Probable heterozygous, deficien
D. P.	131	390	462	1,520	117	203	10.0	8,523	ND	ND	Possible heterozygous, deficien
P. P.	179	453	469	1,850	163	186	15.0	13,861	A2, B12, AW28, B12	+	Definite heterozygous, deficien

* For units and normal values, see Table II.
‡ One or more family members C2 deficient.
§ ND, not done.
‖ Haplotype inherited with C2 deficiency.

TABLE IV
SLE Patients and Controls

Name	Serum complement levels* CH50	C1q	C4	C3	C5	Factor B	C2 Protein	C2 Function	HLA A/B	Confirmatory‡ family studies	C2 Status
SLE Patients											
A. W.	0	453	387	1,880	142	320	0	585	*A10, B18*‖ *A1, B8*‖	+	Definite heterozygous, deficient
E. R.	115	468	387	1,880	142	115	12.8	14,400	*A10, B18*,‖ A3, B8	+	Definite heterozygous, deficient
M. P.	162	420	355	1,170	170	299	15.8	13,552	*A10, B18*,‖ A1, B8	+	Definite heterozygous, deficient
A. S.	118	415	297	1,640	147	238	10.0	8,747	*A10, B18*‖ A11, BW17	+	Definite heterozygous, deficient
S. S.	152	409	409	1,640	207	210	14.0	11,704	A2, AW25, , B18	ND	Probable heterozygous, deficient
J. Ca.	132	370	370	2,016	165	356	14.0	9,109	Deceased	ND	Possible heterozygous, deficient
H. P.	129	470	358	1,816	214	315	12.0	4,268	Deceased	ND	Possible heterozygous, deficient
C. B.	121	420	417	1,624	155	273	13.0	14,760	ND	ND	Possible heterozygous, deficient
Controls											
1	125	473	450	1,787	164	238	14.9	8,287	ND	ND	Possible heterozygous, deficient
2	146	523	414	1,879	146	190	12.0	10,917	ND	ND	Possible heterozygous, deficient
3	118	354	939	1,625	164	198	13.7	3,885	ND	ND	Possible heterozygous, deficient
4	140	316	232	1,354	143	ND	12.6	13,597	ND	ND	Possible heterozygous, deficient
5	150	458	654	1,680	151	ND	13.5	10,656	ND	ND	Possible heterozygous, deficient
6	147	316	379	1,341	180	178	13.9	11,393	ND	ND	Possible heterozygous, deficient

* For units and normal values, see Table II.
‡ One or more family members C2 deficient.
§ ND, not done.
‖ Haplotype inherited with C2 deficiency.

This patient (P. P.) is classified as definite C2 deficient, even though she is not A10(Aw25),B18. Five other C2 deficient individuals were tissue typed (A. M., M. Mu., B. F., H. S., Table III, and S. S., Table IV) and found to have antigen B18, previously demonstrated to be associated with C2 deficiency (20). Family studies were not possible in these instances and the individuals are classified as probable C2 deficient. The remaining four patients (D. P., Table III, J. Ca, H. P., C. B., Table IV) were not available for tissue typing and are classified as possible C2 deficient based on the observation of isolated C2 deficiency in multiple blood specimens.

The patterns of disease in the C2 deficient individuals are given in Tables V and VI. Female preponderance was a consistent feature in the three patient groups, there being only 2 males among the 20 C2 deficient individuals (Tables V and VI). The two C2 deficient

TABLE V
Clinical Data: RA and JRA

Name	Sex	Age at onset	Type of onset	Diagnostic category	RA functional grade	Serology
RA						
P. L.	F	34	—	Definite	I	Negative
A. M.	F	44	—	Definite	I	Negative
JRA						
M. Mu.	F	12	Oligoarticular	—	I	ND
P. B.	F	6	Oligoarticular	—	I	Negative
K. M.	F	7	Oligoarticular	—	I	Negative
J. M. F.	F	6½	Polyarticular	—	II	Negative
J. R.	F	13	Polyarticular	—	I	Negative
M. Ma.	F	16	Polyarticular	—	II	Negative
B. F.	M	2	Sudden onset	—	I	Negative
H. S.	F	16	Polyarticular and SS*	—	III	Negative
D. P.	M	2½	Oligoarticular	—	I	Negative
P. P.	F	17	Polyarticular	—	I	Negative

* SS, Sjögren's disease.

TABLE VI
Clinical Data: SLE

Name	Sex	Age at onset	Number of ARA* criteria	Arthritis arthralgia	Rashes	Alopecia	Photosensitivity	Raynaud's	Pleurisy pericarditis	Renal	CNS*	Hematologic	Highest ANA titer	Presence or absence of DNA antibodies
A. W.	F	9	4	+	+		+	+					1:10	+
E. R.	F	15	7	+	+	+		+		+	+		1:5120	+
M. P.	F	22	7	+	+		+	+				+	1:320	+
A. S.	F	30	4	+	+			+				+	1:80	−
S. S.	F	15	4	+	+		+				+		1:160	+
J. Ca.‡	F	25	9	+	+	+	+		+	+	+		1:20	
H. P.‡	F	52	4		+				+			+	1:10	−
C. B.	F	18	3	+				+					1:10	−

* ARA, American Rheumatism Association; CNS, central nervous system.
‡ Deceased.

patients with RA had seronegative, nonerosive disease; in addition, A. M. had features suggestive of SLE (Table V). The JRA patients (Table V) included individuals with all of the three types of onset to their symptoms that have been defined in this disease: sudden, oligoarticular, and polyarticular (30). The patterns of severity conformed to that found in the JRA population as a whole, 3 of the 10 JRA subjects having degrees of disability such as to impair their level of function in daily activities.

The C2 deficient SLE patients were not readily distinguishable from the non-C2 deficient SLE group. The mean age of onset of the C2 deficient SLE patients, 23.2 yr, compared with 29.8 for the non-C2 deficient SLE group ($P = 0.1$). The disease ranged widely in its severity from a mild nondisabling type to the severe forms, two of the eight C2 deficient patients dying from the complications of SLE (J. Ca., H. P.). The homozygous deficient individual (A. W.) has mild SLE with minimal disability after 15 yr of follow-up, but had the earliest age of onset of the whole group of 137 SLE patients with disease beginning at the age of 9 yr. 4 of the 8 patients have had low titers of ANA (1/20) throughout their period of follow-up, compared with 13 of the remaining 129 SLE patients, a significant difference with $P = 0.0009$ ($\chi^2 = 11.05$). Low titers of ANA could be associated with severe disease; the two patients dying of the disease (J. Ca., H. P.) were among the four C2 deficient patients with low ANA. None of the 8 C2 deficient SLE patients had vasculitis, a complication seen in 6 of the other 129 patients with SLE ($\chi^2 = 0.40$). There were no other apparent differences between the C2 deficient and non-C2 deficient patients.

62 individuals from the families of these C2 deficient propositi were available for study; 30 were identified as C2 heterozygous deficient, of whom 19 were female and 11 male. The total group of 62 relatives also had a female: male preponderance, the ratio being 39: 23. All 30 C2 deficient family members were heterozygous. No additional homozygous deficient individuals were identified. The C2 values, both immunochemical and functional determinations, are given in Table I; the C2 protein levels in 3 of the C2 deficient relatives were within the normal range (i.e., mean±2 SD) with values up to 16.8 μg/ml. The functional C2 values of these three relatives were all below 2 SD below the mean for that assay. The C2 levels in those family members thought not to have an inherited deficiency were within the normal range (mean±2 SD) with one exception. This exception, the mother of a C2 deficient propositus with SLE (M. P.—Table IV), had a low C2 protein of 13.2 μg/ml and a normal C2 functional value of 17,658 U/ml. Two other relatives of M. P., including the father, had C2 protein and functional levels which were below normal and HLA haplotypes A10,B18 as did the propositus; the mother did not. The low C2 protein in the mother was not, therefore, established as an inherited change; it has not been possible to obtain a further blood sample.

The levels of complement components in the family members other than the C2 deficiency and low or low normal CH50 levels were essentially normal, with the exception of Factor B levels. The serum levels of Factor B were lower among the C2 deficient family members (242±108) when compared to their nondeficient relatives (282±146) (Table I); this difference was statistically significant with a P value of < 0.01).

The distribution of rheumatic disease among the relatives of propositi appeared to be independent of the inheritance of C2 deficiency: 6 of 26 C2 deficient relatives, compared with 7 of 32 C2 normal relatives had symptoms suggestive of rheumatic disease. Of the C2 deficient relatives, three had Raynaud's phenomenon, one had JRA, and one had RA. Of the normal C2 relatives, three had RA and three had JRA. ANA were found to a similar extent in the sera of C2 deficient

relatives and the C2 normal group: two and three subjects in each group.

Among the control group, four of the possible C2 deficient individuals had isolated deficiencies of C2 (1–3, 5) (Table IV). The other two blood donors (4, 6) had borderline levels of C1q.

DISCUSSION

The identification of heterozygous deficiency, especially when homozygous deficient individuals are not found in the families, may present difficulties. The availability of both protein and functional assays for C2 has made the identification of heterozygous deficiency easier, as is the knowledge of linkage between genes determining the low levels of C2 in serum and the HLA system, in the Caucasian population studied.

The presence of changes from acquired disease can be a further difficulty, particularly in the instances in which family studies could not be carried out. Inflammation can elevate and immune complex disease depress C2 levels, although C2 is one of the complement proteins least likely to fall in this latter situation. The 129 SLE patients without apparent inherited C2 deficiency in this study had 50 clinical exacerbations of lupus nephritis; C4 and(or) C3 levels fell on all occasions. In contrast, a fall in C2 protein to below 2 SD below the normal mean was detected in only three instances.

We have found when individuals have both low serum C2 protein and C2 functional values and other components are normal, that study of more family members revealed others with similar isolated low C2 values. In our experience, these individuals and their relatives with low C2 levels (generally less than 2 SD below the normal mean) also had HLA antigens A10(Aw25) and(or) B18 which have been shown to be strongly linked to C2 deficiency (20). By these means individuals were defined as C2 heterozygous deficient in the absence of homozygous deficiency within their family.

In patients in whom the supportive evidence from HLA typing and(or) family studies was not available, every serum drawn from that person and stored at − 70°C in the Robert B. Brigham Hospital serum bank was checked to ensure that the C2 levels were consistently in the heterozygous range and that levels of other complement components were normal. In these instances, the inherited nature of the low C2 blood levels could not be established and the qualification 'possible' was used to describe the C2 deficiency. 4 of the 20 patients in this series were in this category, as were the six controls. 1 patient in the SLE group of 137 had low C2 levels on every occasion; but as C3 and C4 levels were also low when C2 was measured, separation of these changes from those of acquired disease was not possible and, therefore, the individual has not been included in the C2 heterozygous deficient group.

Given these qualifications about the identification of the deficient individuals, this survey of C2 deficiency includes all of the available patients in the JRA and SLE clinics at the Robert B. Brigham Hospital. All of the patients studied, including the homozygous individual (A. W.—Table IV), presented themselves to the Robert B. Brigham Hospital with disease and were not referred because of complement abnormalities. The controls, the blood donor population, do not constitute the ideal control group since HLA, clinical and family studies were not possible. Two of the six individuals in the control group with low C2 levels also had borderline levels of one or more of the other complement components. For the purpose of this study, all of the six blood donors have been assumed to have an inherited deficiency of C2.

This represents a 1.2% incidence of C2 heterozygous deficiency among the 509 blood donors. Stratton et al. found one homozygous C2 deficient individual among 10,000 blood bank donors (31). By using the Hardy-Weinberg law (32), one would have therefore expected a 2% incidence of C2 heterozygous deficiency among normal controls. Conversely, the expected incidence of homozygous C2 deficiency based on our observed gene frequency of 0.01 for C2 heterozygous deficiency would be 1 : 40,000.

The most significant association in this study between C2 deficiency and disease was with SLE. Females predominated in both the C2 deficient and C2 normal SLE population. This suggests that heterozygous C2 deficiency is not a sufficiently strong factor to disturb the disproportionate female : male ratio found in SLE.

The SLE patients with C2 deficiency differed from the other SLE patients by the relative low titers of ANA, 4 of 5 having low titers compared with 11 of the remaining 129 patients. This feature of patients with other genetically determined complement deficiencies and SLE has been observed by others (11, 33–35). Low titer ANA did not indicate mild disease in that two of the patients (J. Ca., H. P.—Table VI) with ANA of 1 : 10 died of the disease. A range of protein deficiencies in addition to C2, including other complement components and immunoglobulins, have been associated with SLE generally as individual case reports (33–37); population studies have not as yet been carried out with the exception of the survey of IgA deficiency which has been found in 4 of 87 (4.1%) patients with SLE (38). This increased frequency of isolated IgA deficiency in SLE is similar to that for C2, although family studies were not reported for the IgA deficient subjects.

The JRA population, in which the incidence of C2

deficiency is marginally significant, has been studied over a mean period of 5 yr. This population may contain individuals who, if followed longer, may turn out to have had other forms of arthritis. The possibility exists that there is a more marked association of C2 deficiency with a subpopulation of the JRA patients; the other seronegative arthritides cannot be separated readily with the exception of the Bw27 positive individuals.

The RA population, a group with the onset of disease later in life than either the JRA or SLE patients, did not have an increased incidence of C2 deficiency. In these patients, the age of related failure of immunosurveillance described in man and in experimental animals may be more relevant to pathogenesis than inherited immunodeficiencies (39, 40).

The extent to which serum levels of other complement components are affected by the C2 heterozygous deficiency can be seen in Table I. In addition to CH50, the Factor B levels were significantly lower in deficient than in the C2 normal family members. This tendency for Factor B levels to be lower has been reported by others in patients with homozygous C2 deficiency (9–11, 14) but not hitherto in the apparently healthy C2 deficient relatives. Further study will be required to ascertain the inherited or acquired nature of the low Factor B protein determinations.

The mechanism by which C2 deficiency is associated with rheumatic disease, and SLE in particular, is not known. Genes determining C2 deficiency could be linked to those predisposing to SLE, although the existence of such genes, as with other immune response genes in man, is not yet established. There is linkage disequilibrium between gene(s) controlling C2 levels with HLA-A10,B18 (18–22), for the D locus antigen LD7a (19) and for the slow allotype of properdin Factor B (BfS) (41). A single mutation which occurred some generations ago, but yet not sufficiently recently for equilibrium (42) to have developed, could explain the observed association between these five factors (A10, B18, LD7a, BfS, and C2d) and rheumatic disease. A recombinant frequency of 3–4% has been observed between C2 deficiency and HLA-A and B loci (19, 20). With this rate of recombination, linkage disequilibrium would be lost over approximately 150 generations (43). If such a mutant did occur so relatively recently, our Caucasian population would have had a common ancestry. There is no evidence for this. The alternative explanation of selective advantage in this linkage group is difficult to evaluate.

Alternatively, some failure of a biological role of the early components of the classical pathway of complement could be involved. The functions specific to the early components of the classical pathway include fixation by immune complexes, in viral neutralization, at least in fluid phase as distinct from cell surfaces (44, 45). A defect of opsonizing activity in C2d serum has been reported, although this has not been found consistently (46).

The dearth of ANA in these C2 deficient SLE patients could represent another type of defect in a function of complement. The T-cell dependent antibody response has been demonstrated to be dependent on C3 in experimental animals (47). Classical pathway activation may be involved, a conclusion reinforced by the poor antibody response found in C4 deficient guinea pigs (48). A relative failure of complement dependent, B-cell function need not exclude other possible causes of the C2 deficiency and disease associations reported in this paper. In particular, linkage with specific immune response genes, discussed above, could also be present.

The importance of environmental and inherited factors like the complement component deficiencies in the etiology of the inflammatory rheumatic diseases and SLE in particular is not known. Families in which several members have SLE have been reported (49) and homozygous twins have a concordance rate for SLE of 57%, including one pair separated in infancy and raised apart (50). Such examples are rare, suggesting heredibility in the disease may be important but may not alone be sufficient to give disease. Recent studies have shown an increased frequency of lymphocytotoxic antibodies in sera from household contacts of patients with SLE, be they consanguineous or not, suggesting the relevance of both genetic and environmental factors in etiology (51, 52).

Rheumatic disease and ANA in those C2 deficient families are equally distributed in the deficient as in the C2 normal individuals. These findings also suggest both hereditary and environmental factors may be important in etiology.

C2 heterozygous deficiency does appear to predispose an individual to rheumatic disease, particularly SLE. The elucidation of mechanisms of this predisposition may give insights into the environmental interaction that seems critical in the development of disease in the genetically susceptible individual.

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REFERENCES

1. Ruddy, S., I. Gigli, and K. F. Austen. 1972. The complement system in man. *N. Engl. J. Med.* **287**: 489–495, 545–549, 592–596, 642–646.
2. Polley, M. J., and A. G. Bearn. 1975. Genetic aspects of diseases of complement: an explosion. *Am. J. Med.* **58**: 105–111.

3. Schur, P. H. 1975. Complement in lupus. *In* Clinics in Rheumatic Disease, Systemic Lupus Erythematosus, ed. N. F. Rothfield, vol. 1, W. B. Saunders Co., Ltd., London, p. 519–543.
4. Klemperer, M. R., H. C. Woodworth, F. S. Rosen, and K. F. Austen. 1966. Hereditary deficiency of the second component of complement (C2) in man. *J. Clin. Invest.* **45**: 880–890.
5. Klemperer, M. R., K. F. Austen, and F. S. Rosen. 1967. Hereditary deficiency of the second component of complement (C2) in man: further observations on a second kindred. *J. Immunol.* **98**: 72–78.
6. Cooper, N. R., R. Ten Bensel, and P. F. Kohler. 1968. Studies of an additional kindred with hereditary deficiency of the second component of human complement (C2) and description of a new method for the quantification of C2. *J. Immunol.* **101**: 1176–1182.
7. Ruddy, S., M. R. Klemperer, F. S. Rosen, K. F. Austen, and J. Kumate. 1970. Hereditary deficiency of the second component (C2) in man: correlation of C2 haemolytic activity with immunochemical measurements of C2 protein. *Immunology.* **18**: 943–954.
8. Agnello, V., M. M. E. de-Bracco, and H. G. Kunkel. 1972. Hereditary C2 deficiency with some manifestations of systemic lupus erythematosus. *J. Immunol.* **108**: 837–840.
9. Day, N. K., H. Geiger, R. McLean, A. Michael, and R. A. Good. 1973. C2 deficiency. Development of lupus erythematosus. *J. Clin. Invest.* **52**: 1601–1607.
10. Sussman, M., J. H. Jones, J. D. Almeida, and P. J. Lachmann. 1973. Deficiency of the second component of complement associated with anaphylactoid purpura and presence of mycoplasma in the serum. *Clin. Exp. Immunol.* **14**: 531–539.
11. Osterland, C. K., L. Espinoza, L. P. Parker, and P. H. Schur. 1975. Inherited C2 deficiency and systemic lupus erythematosus: studies on a family. *Ann. Int. Med.* **82**: 323–328.
12. Leddy, J. P., R. C. Griggs, M. R. Klemperer, and M. M. Frank. 1975. Hereditary complement (C2) deficiency with dermatomyositis. *Am. J. Med.* **58**: 83–91.
13. Gelfand, E. W., J. E. Clarkson, and J. O. Minta. 1975. Selective deficiency of the second component of complement in a patient with anaphylactoid purpura. *Clin. Immunol. Immunopathol.* **4**: 269–276.
14. Friend, P., J. E. Repine, Y. Kim, C. C. Clawson, and A. F. Michael. 1975. Deficiency of the second component of complement (C2) with chronic vasculitis. *Ann. Int. Med.* **83**: 813–816.
15. Wild, J., N. Zvaifler, H. Müller-Eberhard, and C. Wilson. 1974. Deficiency of the second component of complement (C2) in a patient with discoid lupus erythematosus (DLE). *J. Clin. Invest.* **53**: 84a. (Abstr.)
16. Fu, S. M., R. Stern, H. G. Kunkel, B. DuPont, J. A. Hansen, N. K. Day, R. A. Good, C. Jersild, and M. Fotino. 1975. Mixed lymphocyte culture determinants and C2 deficiency: LD-7a associated with C2 deficiency in four families. *J. Exp. Med.* **142**: 495–506.
17. Fu, S. M., H. G. Kunkel, H. P. Brusman, F. H. Allen, Jr., and M. Fotino. 1974. Evidence for linkage between HL-A histocompatibility genes and those involved in the synthesis of the second component of complement. *J. Exp. Med.* **140**: 1108–1111.
18. Day, N. K., P. L'Esperance, R. A. Good, A. F. Michael, J. A. Hansen, B. DuPont, and C. Jersild. 1975. Hereditary C2 deficiency: genetic studies and association with the HL-A system. *J. Exp. Med.* **141**: 1464–1469.
19. Wolski, K. P., F. R. Schmid, and K. K. Mittal. 1975. Genetic linkage between the HL-A system and a deficit of the second component (C2) of complement. *Science (Wash. D. C.).* **188**: 1020–1022.
20. Gibson, D. J., D. Glass, C. B. Carpenter, and P. H. Schur. 1976. Hereditary C2 deficiency. Diagnosis and HLA gene complex associations. *J. Immunol.* **116**: 1065–1070.
21. Cohen, A. S., W. E. Reynolds, E. C. Franklin, J. P. Kulka, M. W. Ropes, L. E. Shulman, and S. L. Wallace. 1971. Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21**: 643–648.
22. Brewer, E. J., J. C. Bass, J. T. Cassidy, B. S. Duran, C. W. Fink, J. C. Jacobs, M. Markowitz, W. E. Reynolds, J. Schäller, J. S. Stillman, and S. L. Wallace. 1973. Criteria for the classification of juvenile rheumatoid arthritis. *Bull. Rheum. Dis.* **23**: 712–719.
23. Ropes, M. W., G. A. Bennett, S. Cobb, R. Jacox, and R. A. Jessar. 1958. Revision of diagnostic criteria for rheumatoid arthritis. *Bull. Rheum. Dis.* **9**: 175–176.
24. Kent, J. F., and E. H. Fife, Jr. 1963. Precise standardization of reagent for complement fixation. *Am. J. Trop. Med. Hyg.* **12**: 103–116.
25. Schur, P. H., A. Connelly, and T. C. Jones. 1975. Phylogeny of complement components in non-human primates. *J. Immunol.* **114**: 270–273.
26. Fearon, D. T., S. Ruddy, P. H. Schur, and W. R. McCabe. 1975. Activation of the properdin pathway of complement in patients with gram-negative bacteremia. *N. Engl. J. Med.* **292**: 937–940.
27. Alpert, E., M. Monroe, and P. H. Schur. 1970. A method for increasing the sensitivity of radial-immunodiffusion assay. *Lancet.* **1**: 1120.
28. Mittal, K. K., M. R. Mickey, D. P. Singal, and P. I. Terasaki. 1968. Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation. (Baltimore).* **6**: 913–927.
29. Schur, P. H., D. DeAngelis, and J. M. Jackson. 1974. Immunological detection of nucleic acids and antibodies to nucleic acids and nuclear antigens by counterimmunoelectrophoresis. *Clin. Exp. Immunol.* **17**: 209–218.
30. Barry, P. E., and J. S. Stillman. 1975. Characteristics of juvenile rheumatoid arthritis. Its medical and orthopedic management. *Orthoped. Clin. No. Amer.* **6**: 641–651.
31. Stratton, F., P. J. Lachmann. 1974. Genetic deficiencies of the complement system. *Boll. 1st Sieroter Milanese.* **53** (Suppl.): 195–207.
32. Hardy, G. H. 1908. Mendelian proportions in mixed populations. *Science (Wash. D. C.).* **28**: 49–50.
33. Pondman, K. W., J. W. Stoop, R. H. Cormane, and A. J. Hannema. 1968. Abnormal C1 in a patient with systemic lupus erythematosus. *J. Immunol.* **101**: 811. (Abstr.)
34. Day, N. K., H. Geiger, R. Stroud, M. deBracco, B. Mancado, D. Windhorst, and R. A. Good. 1972. Clr deficiency: an inborn error associated with cutaneous and renal disease. *J. Clin. Invest.* **51**: 1102–1108.
35. Hauptmann, G., E. Grosshans, and E. Reid. 1974. Lupus erythemateux aigus et deficits hereditaires en complement. A propos d'un cas par deficit complet en C4. *Ann. Dermatol. Syphiligr.* **101**: 479–496.
36. Kohler, P. F., J. Percy, W. M. Campion, and C. J.

Smyth. 1974. Hereditary angioedema and "familial" lupus erythematosus in identical twin boys. *Am. J. Med.* **56**: 406–411.
37. Rosenfeld, S. I., and J. P. Leddy. 1974. Hereditary deficiency of the Fifth component of complement (C5) in man. *J. Clin. Invest.* **53**: 67a. (Abstr.)
38. Cassidy, J. T., A. Burt, R. Petty, and D. Sullivan. 1969. Selective IgA deficiency in connective tissue disease. *N. Engl. J. Med.* **280**: 275.
39. Walford, R. L. 1969. The Immunologic Theory of Aging. Munksgaard, Copenhagen. 248 pp.
40. Price, G. B., and T. Makinodan. 1972. Immunologic deficiencies in senescence. I. Characterization of intrinsic deficiencies. *J. Immunol.* **108**: 403–412.
41. Raum, D., D. Glass, C. B. Carpenter, C. A. Alper, and P. H. Schur. Linkage if Factor B, C2 deficiency and HLA, and of genes on chromosome 6. *Clin. Res.* In press.
42. McDevitt, H. O., and W. F. Bodmer. 1974. HL-A, immune-response genes, and disease. *Lancet.* **I**: 1269–1275.
43. Bodmer, W. F. 1973. Histocompatibility antigens. Part 2. Genetics of the HL-A and H-2 major histocompatibility systems. *In* Defense and Recognition. MTP International Review of Science. University Park Press, Baltimore. R. R. Porter, editor. **10**: 295–328.
44. Daniels, C. A., T. Borsos, H. J. Rapp, R. Snyderman, and A. L. Notkins. 1970. Neutralization of sensitized virus by purified components of complement. *Proc. Natl. Acad. Sci. U. S. A.* **65**: 528–535.
45. Joseph, B. S., N. R. Cooper, and M. B. A. Oldstone. 1975. Immunologic injury of cultured cells infected with measles virus. I. Role of IgG antibody and the alternative complement pathway. *J. Exp. Med.* **141**: 761–774.
46. Johnson, F. R., V. Agnello, and R. C. Williams, Jr. 1972. Opsonic activity in human serum deficient in C2. *J. Immunol.* **109**: 141–145.
47. Pepys, M. B. 1974. Role of complement in induction of antibody production in vivo. Effect of cobra factor and other C3-reactive agents on thymus-dependent and thymus-independent antibody responses. *J. Exp. Med.* **140**: 126–145.
48. Ellman, L., I. Green, F. Judge, and M. M. Frank. 1971. In vivo studies in C4-deficient guinea pigs. *J. Exp. Med.* **134**: 162–175.
49. Leonhardt, E. T. G. 1967. Family studies in systemic lupus erythematosus. *Clin. Exp. Immunol.* **2**: 743–759.
50. Block, S. R., J. B. Winfield, M. D. Lockshin, W. A. D'Angelo, and C. L. Christian. 1975. Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. *Am. J. Med.* **59**: 533–551.
51. DeHoratius, R. J., and R. P. Messner. 1975. Lymphocytotoxic antibodies in family members of patients with systemic lupus erythematosus. *J. Clin. Invest.* **55**: 1254–1259.
52. DeHoratius, R. J., R. Pillarisetty, R. P. Messner, and N. Talal. 1975. Antinucleic acid antibodies in systemic lupus erythematosus patients and their families. Incidence and correlation with lymphocytotoxic antibodies. *J. Clin. Invest.* **56**: 1149–1154.