CLINICAL RESEARCH

Family study of the major histocompatibility complex in patients with systemic lupus erythematosus: importance of null alleles of C4A and C4B in determining disease susceptibility

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Abstract

The families of 29 patients with systemic lupus erythematosus and 42 normal subjects were studied to determine the inheritance of the HLA-A, B, C, and DR antigens and also the complement polymorphisms for C2, C4A, C4B, and Bf, which are encoded in the same region of the sixth chromosome. Null (silent) alleles for C4A, C4B, or C2 were found in 24 of the 29 (83%) patients compared with 18 of the 42 (43%) normal controls. HLA-DR3 was present in 20 (69%) of the patients and seven out of 39 (18%) of the normal controls. There was strong linkage disequilibrium between DR3 and the null alleles for C4A and C4B.

The data did not permit the relative contributions of DR3 and null factors of C4A and C4B as genetic risk factors to be distinguished. The known association of systemic lupus erythematosus with uncommon inherited and acquired deficiencies of complement components suggests, however, that the presence of null alleles for C4A and C4B, as well as C2, found in most of the patients, is relevant to their genetic susceptibility to this disease.

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Introduction

Although the aetiology and pathogenesis of human systemic lupus erythematosus remain obscure, both environmental and genetic factors appear to play a part.[1] Among the relevant genetic factors are the inherited complement deficiency states.[2 3] Of these, C2 deficiency is the most prevalent, and systemic lupus erythematosus has been observed in roughly one third of individuals with homozygous C2 deficiency.[2] Partial deficiency of C2, which is found in individuals with one C2 null allele (heterozygous C2 deficiency), may also be a risk factor.[4] Deficiencies of C1 esterase inhibitor, each classical pathway component, and the membrane attack complex components have also been reported in conjunction with systemic lupus erythematosus.[2]

Significant associations have been observed between HLA antigens and systemic lupus erythematosus.[1] In white populations the most consistent associations are with A1, B5, B7, B8, DR2, and DR3. The importance of these associations remains uncertain, but one possibility is that they reflect linkage disequilibrium with other loci that determine risk factors. In this context the HLA region encoded complement polymorphisms C2, C4A, C4B, and Bf may be relevant candidates. The polymorphism is particularly complex and extensive for the C4A and B loci.[5-7] In addition to the expressed polymorphic variations null (silent) alleles for C2 and both C4 loci have been described,[6 8] and these are associated with no detectable product. Furthermore, variation in haemolytic activity between the C4 gene products has been observed, and one, C4A6, is non-haemolytic when inherited in certain haplotypes.[9]

Proved complement deficiency states account for only a small minority of cases of systemic lupus erythematosus, but no

systematic studies have been done in the past to determine the prevalences of null and non-haemolytic alleles of the HLA region encoded complement polymorphisms. Possibly previously unsuspected heterozygous deficiency of one of the complement components or the presence of a functionally inefficient variant occurs in most patients. It is consistent with this idea that B8, one of the HLA antigens associated with systemic lupus erythematosus, has been reported to be in linkage disequilibrium with the C4A null allele.[6 10]

Direct study of complement polymorphisms in patients with systemic lupus erythematosus is hampered by their low serum concentrations during periods of disease activity. We therefore undertook a family study in which patients and their first degree relatives were haplotyped for HLA-A, B, C, and DR antigens and for polymorphisms of C2, C4A, C4B, and Bf. For comparison a group of normal families were similarly haplotyped. This paper describes our initial results.

Subjects and methods

PATIENTS

We studied 29 female patients with systemic lupus erythematosus from 26 white families. They were selected on the basis that they fulfilled at least four of the preliminary classification criteria for the disease of the American Rheumatism Association and had a family in which three or more first degree relatives were willing to provide blood samples and a clinical history. The mean age of the patients was 32 (range 13-69) and the mean age at onset of disease was 24 (range 9-67). All but two patients had had raised antibody titres to double stranded DNA (Farr assay 30% DNA binding)[11] on at least two occasions during their illness. Of the two who had not, one had had repeatedly raised antibody titres to La and the other to Ro and La antigens. Nineteen patients lived in the south east of the United Kingdom, three in the Midlands, three in the west, and two in East Anglia.

RELATIVES

A full clinical history was taken from each member of the patients' families. Particular attention was paid to symptoms associated with connective tissue diseases. The group of relatives comprised 45 parents, 70 siblings, and 14 children.

CONTROLS

Families of members of hospital staff and a local general practice were approached, and those in which there were at least three healthy first degree relatives to provide blood samples and a clinical history were selected. Each of the 21 control families contained at least one woman aged 20 or over, none of whom gave a history suggestive of connective tissue disease. All but three of the 21 normal families were resident in the south east.

HLA TYPING

HLA-A, B, C, and DR antigens were defined by conventional microcytotoxicity tests as previously described.[12] Tests that were technically unsatisfactory were repeated as required. HLA-CW7 was not tested owing to a lack of suitable antisera. Haplotypes were assigned on the basis of the typing data and consideration of the segregating complement polymorphisms.

COMPLEMENT ALLOTYPES

Bf allotyping was carried out by high voltage agarose electrophoresis of serum in 0·8% weight/volume agarose (Sigma type 11) followed by immunofixation according to the method of Alper *et al.*[13]

C4 allotyping was performed on edetic acid plasma or serum with edetic acid added to 0·02 mol; samples were treated overnight at 4°C with neuraminidase from *Clostridium perfringens* (Sigma type V111) at a final concentration of 5 U/ml serum. Electrophoresis was performed on the desialised samples in 0·8% agarose (Seakem ME, Marine Colloids Division) in trometamol-glycine-barbital buffer pH 8·2 according to the method of O'Neill *et al*[14] with the addition of edetic acid (0·002 mol) to the gel buffer. The gels were run at about 30 V/cm for three hours until the haemoglobin marker had moved 7-8 cm from the origin. Immunofixation of the C4 bands was achieved using 2-3 μl/cm^2 rabbit antihuman C4 complement (DAKO immunoglobulins). Functional detection was performed using an overlay of 12 ml 0·6% agarose (Behringwerke Ag) in complement fixation test diluent (Oxoid Ltd, UK) containing 0·1% gelatin, 3×10^9 sheep cells sensitised with rabbit antisheep serum (Pasteur Institute), and 3% C4 deficient guinea pig serum. The method was based on that described by Lachmann and Hobart.[15] Lysis was visible after 30 minutes at 37°C.

Heterozygotes were detected by two dimensional rocket electrophoresis according to the method of Awdeh *et al*,[16] but the electrophoresis in the first dimension was extended to five or six hours to obtain good separation of the peaks.

C2 typing was performed by isoelectric focusing of sera in polyacrylamide gels using an LKB Multiphor 2117 and power pack 2103, essentially as reported previously.[17 18] Before application of the samples the gel was partially focused by stepwise increase of the voltage setting to 1300 V over one hour. Samples were applied to the surface of the gel on LKB application filters at the anodic end and the voltage increased stepwise to 2000 V over a further 90 minutes. This voltage was maintained for two hours. The runs, performed at 4°C, were of about five hours' duration.

The gels were washed as previously described[19] and the bands visualised with a functional overlay similar to that used for C4 but with 2·5% human serum deficient in C2 substituted for the C4 deficient guinea pig serum.

In the assignment of C2 alleles to haplotypes subjects who were phenotypically C2^1 (C2^c) were presumed to be homozygous unless they also possessed the haplotype HLA-DR2, C4A4, C4B2, BfS, HLA-B18, or HLA-A25, which is in strong linkage disequilibrium with the C2 null. In families in which this haplotype segregated serum C2 concentrations were measured direct by radial immunodiffusion and haemolytic plate assay (courtesy of Professor P J Lachmann).

Results

Table I gives the haplotypes of the patients and table II those of the parents of the normal families. Table I shows that 24 of the 29 patients (83%) carried a null complement allele, most at C4A, some at C4B, and one at C2. Twenty of the patients were positive for DR3 (69%). In contrast, 18 of the 42 parents of normal families (43%) possessed a C4 null allele, and seven out of 39 were DR3 positive (18%). DR typing was technically unsatisfactory in three controls. Comparing the prevalences of null alleles in patients and controls showed the differences to be highly significant ($\chi^2y = 9·7$; $0·01 > p > 0·001$). Furthermore, four of the patients but none of the controls had two null alleles at C4A. Similarly, the prevalences of DR3 in the patient and control groups differed highly significantly ($\chi^2y = 16·0$, $p < 0·001$).

There did not appear to be any clear clinical difference between the patients with and without detected null alleles. As only five patients were without null alleles, however, it is too early to draw firm conclusions. It may be relevant that one of the five (family 7) had a haplotype with a non-haemolytic form of C4A6.

As well as comparing the patients with normal subjects we also compared them with their unaffected sisters. The prevalences of DR3 and null complement alleles in these two groups (table III) were not significantly different, but the trend was towards lower prevalences in the unaffected subjects.

Table I shows that all the 20 patients positive for DR3 also had a C4 null allele (17 were C4AQO, three were C4BQO), and in 19 of these the null allele was on the same haplotype as the DR3. Evidently a strong linkage disequilibrium exists between C4AQO and DR3 in this group of patients. By direct counting the respective gene prevalences for DR3 and C4AQO were 0·414 and 0·379. The expected prevalence of haplotypes containing both DR3 and C4AQO was therefore 0·157, and the observed prevalence was 0·345 (for linkage disequilibrium = 0·188). Similarly, in the normal controls (table II) the gene prevalences of DR3 and C4AQO were equal at 0·090; the expected prevalence for a haplotype with both was therefore 0·0081, and the observed was 0·051 (for linkage disequilibrium = 0·043). The most striking example of linkage disequilibrium in this study was the

TABLE I—*HLA region haplotypes of 29 patients with systemic lupus erythematosus (null alleles indicated by QO)*

Family No	HLA-DR	C4A	C4B	C2	Bf	HLA-B	HLA-C	HLA-A
1	3	QO	1	1	S	8	—	1
	7	3	1	1	F	44	—	29
2	3	QO	1	1	S	8	—	1
	7	3	1	1	S	40	—	9 (24)
3	3	QO	1	1	S	40	3	2
	6	3	1	1	F	37	6	28
4	2	3	1	1	S	51	—	11
	7	3	1	1	F	44	4	9
5	3	QO	1	1	S	8	—	1
	2	3	1	1	S	7	—	3
6	2	3	QO	1	S	7	—	2
	1	3	4	1	S	15	3	2
7	7	6	1	1	S	17	—	1
	5	3	1	1	S	7	—	2
8	3	QO	1	1	S	8	—	1
	4	3	1	1	S	21	6	32
9	3	QO	1	1	S	8	—	1
	3	QO	1	1	S	8	—	1
10	2	3	1	1	S	7	—	3
	3	3	QO	1	F1	18	—	2
11a*	2	3	1	1	S	7	—	2
	7	3	1	1	F	44	—	29
11b*	3	QO	1	1	S	8	—	1
	7	3	1	1	F	44	—	29
12a*	3	QO	1	1	S	8	—	1
	3	3	1	1	S	44	—	3
12b*	3	QO	1	1	S	8	—	1
	4	3	2	2	S	16	—	26
13	3	QO	1	1	S	8	—	1
	6	QO	2	1	S	40	3	2
14	2	3	1	1	S	40	3	2
	TF	3	2	1	S	14	—	29
15	2	4	2	QO	S	18	—	25
	2	3	1	1	S	7	—	2
16	3	QO	1	1	S	8	—	1
	2	3	1	1	S	35	4	2
17	5	3	QO	1	S	51	—	28
	3	3	1	1	S	35	4	33
18	3	QO	1	1	S	8	—	1
	3	QO	1	1	S	51	2	11
19	1	3	QO	1	S	35	4	11
	4	3	1	1	F	44	—	29
20	2	3	1	1	S	51	3	30/31
	4	4	2	2	S	15	3	2
21	3	QO	1	1	S	8	—	1
	2	3	1	1	S	7	2	29
22	3	QO	1	1	S	8	—	2
	4	3	1	1	S	40	—	2
23	7	6	1	1	S	8	—	1
	3	3	QO	1	F1	18	—	30
24	8	QO	1	1	S	8	—	2
	4	3,2†	1	1	F	35	4	11
25a*	3	QO	1	1	S	8	—	1
	4	3	1	1	S	40	3	31
25b*	3	QO	1	1	S	8	—	32
	1	4	2	2	S	8	—	31
26	3	QO	1	1	S	8	—	1
	3	QO	1	1	S	16	—	3

*These families contained two affected patients.
†Homoduplicated locus.[26]
TF = Technical failure, when there was no opportunity for repeat testing.

extended haplotype HLA-DR3, C4AQO, C4B1, C2[1], BfS, HLA-B8, HLA-CW-, and HLA-A1, which had a prevalence of 0·259 in the patients and 0·038 in the controls.

Discussion

The principal finding in this study was a highly significant increase in the prevalence of null complement alleles in patients with systemic lupus erythematosus. In 23 of the 24 patients reported on here the null alleles were located at the C4A or the C4B locus (18 at C4A and five at C4B). Nineteen out of 24 of these null alleles were located on major histocompatibility complex haplotypes encoding DR3, which we have also found to be strongly associated with systemic lupus erythematosus. These data did not allow us to distinguish between the relative contribution of C4 null alleles and HLA-DR3 as risk factors for the development of the disease or to determine whether both are important.

In support of our hypothesis that null C4 alleles may themselves be important risk factors, however, there are the associations of homozygous complement component deficiencies of the classical pathway and heterozygous C2 deficiency with systemic lupus erythematosus.[2] Secondary deficiency of C1, C2, and C4 resulting from inherited deficiency of C1 esterase inhibitor (hereditary angio-oedema) may also be associated with an

TABLE II—*HLA region haplotypes of 42 normal individuals (null alleles indicated by QO)*

Family No		HLA-DR	C4A	C4B	C2	Bf	HLA-B	HLA-C	HLA-A
N1	A	7	3	1	1	F	40	3	1
	B	4	6	1	1	S	7	3	1
	C	3	3	1	1	S	8	—	2
	D	6	3	1	1	F	44	—	2
N2	A	5	3	QO	1	S	44	—	10 (26)
	B	8	3	6	2	S	44/-	—	2
	C	2	3	1	1	S	27	—	2
	D	4	3	QO	1	S	44	—	2
N3	A	2	3	1	1	S	7	—	3
	B	1	3	1	1	S	51	—	3
	C	1	2	1	1	S1	21	6	30
	D	4	3	1	1	S	40	—	2
N4	A	8	6	1	1	S	17	6	1
	B	1	2	1	1	S	14	—	28
	C	4	3	1	1	S	35	4	10
	D	4	3	1	1	S	41	—	2
N5	A	6	3	1	1	S	7	—	3
	B	6	3	1	1	F	44	—	3
	C	6	3	1	1	S	8	—	1
	D	2	3	1	1	S	7	—	9 (24)
N6	A	1/-	3	1	1	S	44	—	2
	B	1	2	QO	1	F	35	4	3
	C	4	3	QO	1	S	44	—	2
	D	2	4	2	1	S	44	—	32
N7	A	6	3	1	1	S	15	—	2
	B	6	3	1	1	F	44	—	3
	C	4	3	1	1	S	45	—	2
	D	2	3	1	1	S	7	—	3
N8	A	7	QO	2	1	F	7/-	—	3
	B	2	3	1	1	S	7	—	3
	C	5	QO	2	1	S	40	—	2
	D	5	3	1	1	S	18	—	24
N9	A	3	QO	1	1	S	8	—	1
	B	2	3	1	1	S	22	3	2
	C	4	4	2	1	F	35	4	2
	D	5	4	2	1	S	40	3	2
N10	A	4	3	1	1	S	7	—	1
	B	2	3	1	1	S	7	—	9
	C	1	3	1	1	S	44	5	2
	D	4	3	QO	1	S	44	5	2
N11	A	4	3	1	1	S	22	1	2
	B	4/-	3	QO	1	S	44	1/-	3
	C	1	4	2	1	S	35	4	11
	D	4	4	2	1	S	15	3	2
N12	A	2	3	1	1	S	7	—	2
	B	1	3	QO	1	S	35	4	11
	C	3	3	1	1	S	8	—	1
	D	2	3	1	1	S	7	—	2
N13	A	4	3	QO	1	S	44	—	2
	B	4	3	1	1	S	15	—	3
	C	7	6	1	1	S	37	6	1
	D	6	3	1	1	S	40	2	32
N14	A	TF	3	1	1	S	7	—	3
	B	TF	3	1	1	S	16 (38)	—	2
	C	TF	3	1	1	S	15	—	29
	D	TF	3	1	1	S	35	—	3
N15	A	6	2	1	1	S	16 (39)	—	1
	B	5	3	1	1	S	21 (49)	2	9
	C	3	3	1	1	F	16	—	9 (24)
	D	3	2	1	1	S	16	—	3
N16	A	3	QO	1	1	S	8	—	1
	B	7	6	1	1	S	17	6	1
	C	4	3	1	1	S	15	1	9 (24)
	D	5	3	1	1	S	35	4	2
N17	A	6	3	QO	1	S	35	4	9 (24)
	B	1	3	1	1	S	22	—	11
	C	7	1	QO	1	F	17	—	3
	D	4	6	1	1	S	17	6	1
N18	A	1	3,2*	QO	1	F	35	4	11
	B	7	6	1	1	S	17	6	1
	C	TF	3	1	1	S	44	5	2
	D	TF	4	2	2	S	37	—	1
N19	A	4	4	2	1	S	18	5	9 (24)
	B	4	4	2	1	S	44	—	2
	C	2	3	1	1	S	7	—	3
	D	2/-	3	1	1	S	7/-	—	2
N20	A	7	3	1	1	S	17	—	1
	B	5	3	1	1	S	18	—	24
	C	8	QO	1	1	S	27	2	11
	D	8	3	1	1	S	18	—	1
N21	A	4	4	2	1	S	22 (56)	3	24
	B	3	QO	1	1	S	8	—	1
	C	7	6	QO	1	S	17	6	2
	D	3	QO	1	1	S	8	—	2

*Homoduplicated locus.[26]
TF = Technical failure, when there was no opportunity for repeat testing.

TABLE III—*Prevalences of HLA-DR3 and complement null alleles in patients with systemic lupus erythematosus and their unaffected sisters*

	No	No (%) with DR3	No (%) with null allele	No (%) with DR3 and null allele
Patients	29	20 (69)	24 (83)	20 (69)
Sisters	32	15 (47)	19 (57)	15 (47)

There was no significant difference between the two groups as judged by the χ^2 test.

illness similar to systemic lupus erythematosus.[20 21] C1 esterase inhibitor is not encoded within the major histocompatibility complex. This also suggests that susceptibility to the disease may be due to deficiency of a complement component rather than another gene product of the HLA region.

Earlier studies of HLA associations with systemic lupus erythematosus have shown the strongest associations to be with HLA-B8 and DR3.[1] Previous work has shown HLA-B8 to be in strong linkage disequilibrium with the C4A null allele.[6 10] Our data are the first to show by direct genotyping that the commonest extended haplotype in white patients with the disease is HLA-DR3, C4AQO, C4B1, C2[1], BfS, HLA-B8, HLA-CW-, HLA-A1. Testing of patients in other racial groups will be necessary to determine with certainty which element in this extended haplotype is of direct pathogenic relevance to the disease.

It would be premature to embark on an extensive discussion about possible mechanisms of how partial or complete deficiencies of complement proteins may predispose to systemic lupus erythematosus. It has been suggested, however, that complement deficiencies may allow an infectious agent or immune complexes to persist, resulting in a prolonged immunological stimulus.[2] The kinetics of antigen-antibody lattice formation and solubilisation are influenced by both the classical and alternative pathways of complement.[22 23] It might be expected that a deficiency of complement would be associated with an abnormality of localisation and clearance of immune complexes. It is already known that B lymphocyte memory responses require the localisation of antigen-antibody complexes within germinal centres by C3b receptor bearing cells. This localisation depends on activation of C3 via the classical pathway.[24]

Other diseases presumed to be mediated by immune mechanisms and associated with HLA-B8 or DR3 or both in white patients include chronic active hepatitis, idiopathic Addison's disease, Graves disease, type 1 diabetes mellitus, gold induced nephropathy, and coeliac disease.[25] Because of the strong linkage disequilibrium between these antigens and the C4A null allele the possible relevance of C4 null alleles in the pathogenesis of these diseases also needs to be considered.

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ONE HUNDRED YEARS AGO We are very glad to see that Dr. Danford Thomas made very sensible comments *à propos* of the death of a child in the Central District of London this week, found dead in bed, and whose death was attributed to atrophy from non-assimilation of food, and apparently also from injudicious feeding. It is one of the many advantages of the appointment of medical men as coroners that they are able to fulfil what is really the essential function of the coroner, that is, to determine the cause of death irrespective of the criminal applications of the inquiry, and of turning their knowledge in their judicial position to great public advantage by conveying information and advice which comes with a better grace and with more force from them than from any other quarter. It is unquestionably true that there still exists a vast amount of ignorance among mothers of all classes—the richer as well as the poorer—on the subject of the feeding of infants, and that the untiring energy of advertisers in puffing starchy foods for children, and the somewhat injudicious assistance which they occasionally receive from medical friends who have imperfectly acquainted themselves with the composition of such articles, lead extensively to the use of various so-called foods for infants, which are very ill suited for the purpose of nourishing children in early life. The difficulty with which farinaceous food is assimilated by infants is well known, as a rule, to medical men, but apparently they have not succeeded in instilling this information with sufficient force into the minds of mothers generally. A complete elimination of three-fourths of the infants' foods now extensively sold would, probably, lead to a remarkable diminution in the present infant mortality. There is no food for infants, in ninety-nine cases out of a hundred, where they have to be brought up artificially, which can be compared to ordinary condensed milk properly diluted. (*British Medical Journal* 1883;i:70.)