COMPLEMENT RECEPTOR 1 (CR1) ON ERYTHROCYTES OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Summary Ninety-five (85%) of the 112 Japanese patients with systemic lupus erythematosus (SLE) were negative for the complement receptor 1 (CR1) activities on erythrocytes, while 770 (91%) of the 847 normal subjects were positive for CR1, as determined by immune-adherence hemagglutination. Pedigree analyses of the normal population suggested that the phenotype of negative CR1 was determined by a autosomal recessive gene. Among 112 SLE patients, 73 (65%) showed persistently negative CR1 during remission for over 26 months of follow-up, although the CR1 levels did vary with the disease activity in 22 SLE patients. These results show that the relative risk for developing SLE in persons with negative CR1 is 19. CR1 activity appears to be an important genetic factor related the development of SLE.

Key Words complement receptor 1, systemic lupus erythematosus

INTRODUCTION

The pathogenesis of systemic lupus erythematosus (SLE) is apparently of multifactorial origin, including hereditary and environmental factors. A genetic predisposition to SLE has been suggested by the epidemiological studies of twins (Block *et al.*, 1975) and by animal models of inbred mice (Hahn, 1987).

Receptors for the third and fourth components of the activated complement system (complement receptor 1, CR1) are present on erythrocytes, neutrophils,

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monocytes, B lymphocytes, some T lymphocytes and renal podocytes (Fearon, 1980; Bianco and Nussenzweig, 1977; Ross, 1979; Wilson *et al.*, 1982a; Gelfand *et al.*, 1975; Kazatchkine *et al.*, 1982). Although the number of CR1 molecules expressed on each red cell is less than 1% of those on leukocytes, more than 90% of the total CR1 on the surface of the peripheral blood cells are associated with red cells, because the mass of erythrocytes far exceeds that of leukocytes (Fearon, 1980). CR1 has a role in processing and clearance of microorganisms coated with C3b/C4b as well as immune complexes (IC), and most of the IC in the circulation is bound on the surface of erythrocytes. Therefore, if the CR1 on the erythrocytes is defective, free IC are not removed from the circulation and possibly cause tissue damage (Siegel *et al.*, 1981; Cornacoff *et al.*, 1983).

Miyakawa *et al.* (1981) found that levels of CR1 on erythrocytes were low in patients with SLE. Because the low CR1 levels were persistent in patients irrespective of the disease activity, and because they were observed frequently in family members, the defect would appear to be heritable. Wilson *et al.* (1982b) estimated the number of CR1 sites on erythrocytes, using a monospecific anti-CR1 antibody. They found that CR1 levels showed a trimodal distribution among the normal population. Thus, CR1 expression was thought to be controlled by two allelic genes, designated high (H) and low (L), and expressed codominantly on the erythrocyte membranes.

Iida *et al.* (1982) noted an increase in CR1 levels on erythrocytes in 2 of the 4 patients with SLE and entering remission. Inada *et al.* (1982) showed an inverse correlation between CR1 levels and the concentration of immune complexes in the circulation. Moreover, Holme *et al.* (1986) pointed out that numbers of CR1 were different in each of the two of identical twins with SLE. These reports suggested that the defective CR1 was, at least in part, a reversibly changed and acquired condition rather than a genetically determined state.

We report here CR1 levels on erythrocytes from SLE patients and those of normal subjects. The objective of the study was to search for possible genetic factors associated with the development of SLE.

PATIENTS AND METHODS

Patients. We studied 112 Japanese patients with SLE, who had been admitted or who were seen in the clinic of the First Department of Medicine, Kyushu University. The diagnosis of SLE was made according to the revised criteria by American Rheumatism Association (ARA) (Tan *et al.*, 1982). One hundred and sixty-two workers of a company in Fukuoka city and 685 residents in Yonaguni and Hateruma Islands, Okinawa Prefecture, served as the healthy controls. Family studies were performed for 59 members from the 19 families of probands having SLE and 146 members from the 25 families of normal control subjects.

Immune-Adherence Hemagglutination (IAHA). The CR1 levels were measured according to the method of IAHA, as described by Miyakawa *et al.* (1981). Human

IgG was prepared from pooled sera by salt precipitation and chromatography on DEAE-cellulose and was stored at -70°C . A solution of IgG in phosphate-buffered saline (10 mg/ml) was heated at 65°C for 30 min. After the precipitates had been removed by centrifugation at $15,000\times g$ for 15 min, the supernatant was used as the aggregated human γ -globulin (AHG). Serial two-fold dilutions of AHG starting from 100 $\mu\text{g}/\text{ml}$ were prepared in a gelatin-veronal-buffer (GVB^{2+}) in a polystyrene U-bottom microtiter plate. Complement was prepared by dilution of guinea pig serum in GVB^{2+} containing 3 CH50 units/ml, 25 μl of which was added to each well. The plate was incubated at 37°C for 45 min. Thereafter, 25 μl of dithiothreitol solution (3 mg/ml) was added to protect the generated C3b sites from decay (Mayumi *et al.*, 1971). Subsequently, 25 μl of erythrocytes suspension (2×10^8 cells/ml in EDTA-GVB) was transferred to each well and the plate was incubated at 24°C for further 60 min. The pattern of hemagglutination was observed and the IAHA levels of erythrocytes were expressed by the highest dilution (2^N) of AHG that induced hemagglutination.

RESULTS

CR1 levels of SLE patients and normal controls

Figure 1 illustrates the distribution of IAHA titers of erythrocytes from the 112 SLE patients and the 847 normal controls. Seventy-three (65%) SLE patients showed no detectable IAHA levels, 38 patients had titers ranging from 2^4 to 2^8 , and 1 patient had a titer of 2^{11} . In contrast to the SLE patients, IAHA titers showed a trimodal distribution in normal controls and only 77 (9%) showed a negative IAHA. No overlaps between positive and negative groups were observed in either SLE patients or the normal controls.

Family study of CR1 levels in the normal population

Among 77 individuals in the normal population with a negative CR1, 25 families were available for pedigree analyses and CR1 levels on erythrocytes from 146 members were investigated by IAHA (Fig. 2). Forty (27%) of the 146 members were negative for CR1 and more than two members had a negative CR1 in 8 families.

CR1 levels of members of SLE families

CR1 levels on erythrocytes from the 59 relatives of 19 SLE patients were assessed by IAHA. As shown in Fig. 3, clustering of negative CR1 was observed in 9 families. Six (33%) of 18 siblings tested had a negative CR1, thereby indicating that the frequency of negative CR1 is about 3 times higher in siblings of SLE patients than that in normal controls. In one family (Fig. 3B) all members of the proband tested so far have a negative CR1. Here a younger sister of the proband was found to have SLE. The mother showed no clinical symptoms of SLE but the laboratory data were positive for the LE-test, hypergammaglobulinemia as well as leukopenia. She therefore may have had a strong genetic potential to develop SLE.

Fig. 1. Distribution of immune-adherence hemagglutination (IAHA) titers of erythrocytes from 112 patients with SLE (A) and 847 healthy controls (B).

Fig. 2. CR1 levels in a normal population. Positive CR1, $\square/\circ$; Negative CR1, $\blacksquare/\bullet$.

Fig. 3. CR1 levels in patients with SLE and in their relatives. Positive CR1, $\square/\circ$; Negative CR1, $\blacksquare/\bullet$.

Fig. 4. Clinical course of a 42 y.o. female patient with SLE.

Relationship between the CR1 levels and disease activity

CR1 levels on erythrocytes from 112 SLE patients were followed up for over 26 months. In 22 of the 95 SLE patients who were negative at the initial testing, the CR1 level varied with time during the course of the disease. Figure 4 shows the clinical course of a 42-year-old woman with SLE. CR1 levels in this patient were negative at the beginning of the study, and became positive as she went into the remission while on prednisolone.

DISCUSSION

As shown in Fig. 1, when CR1 levels among the normal controls were depicted as a frequency histogram, 77 (9%) showed negative IAHA, 558 (66%) had titers ranging 2^3 to 2^{10} and 212 (25%) had titers of 2^{11} . This distribution seemed to be trimodal and suggested the existence of two codominantly expressed alleles that determined high (H) and low (L) levels of CR1 originally proposed by Wilson *et al.* (1982b). Persons with high (2^{11}) IAHA titers would be homozygous for the high receptor allele and would have the HH phenotype, those with negative IAHA would be homozygous for the low allele and would have the LL phenotype, and those with intermediate (2^3 to 2^{10}) IAHA titers would be heterozygotes and would have the HL phenotype. However, the expected frequencies of these phenotypes in our

Table 1. Summary of observed phenotypes of a positive and a negative CR1 in 30 families.

Phenotypes of parents	Phenotypes of children	Number of families
P × P	(N)	4
	(P,P,N)	2
	(P,P,P,N)	1
	(P,P,N,N)	1
	(P,P,P,P,N)	1
	(P,P,P,P,P,N)	1
P × N	(P)	7
	(N)	1
	(P,P)	6
	(P,N)	2
	(P,P,P)	1
	(P,N,N)	1
	(N,N,N)	1
N × N	(N)	1

P, positive CR1; N, negative CR1.

Table 2. Tests for goodness of fit of the inheritance of CR1 on the hypotheses of (A) an autosomal recessive inheritance for a positive CR1, or (B) and (C) an autosomal recessive inheritance for a negative CR1.

(A) Backcross (P×N)				
Sib size	Number of P	Observed	Expected	χ^2
1	1	7	7	0
2	1	2	5.333	2.083
2	2	6	2.667	4.167
3	1	1	0.857	0.024
3	2	0	0.857	0.857
3	3	1	0.286	1.786
Total		17	17.000	8.917

(B) Backcross (P×N)				
Sib size	Number of N	Observed	Expected	χ^2
1	1	1	1	0
2	1	2	1.333	0.333
2	2	0	0.667	0.667
3	1	0	0.857	0.857
3	2	1	0.857	0.024
3	3	1	0.286	1.786
Total		5	5.000	3.667

(C) Intercross (P×P)				
Sib size	Number of N	Observed	Expected	χ^2
1	1	4	4	0
2	1	0	0	0
2	2	0	0	0
3	1	2	1.459	0.200
3	2	0	0.486	0.486
3	3	0	0.054	0.054
4	1	1	1.234	0.044
4	2	1	0.617	0.238
4	3	0	0.137	0.137
4	4	0	0.011	0.011
5	1	1	0.519	0.447
5	2	0	0.346	0.346
5	3	0	0.115	0.115
5	4	0	0.019	0.019
5	5	0	0.001	0.001
6	1	1	0.433	0.742
6	2	0	0.361	0.361
6	3	0	0.160	0.160
6	4	0	0.040	0.040
6	5	0	0.005	0.005
6	6	0	0.000	0.000
Total		10	9.997	3.406

(A): (D.F.=3) $p < 0.05$. (B): (D.F.=3) $p > 0.25$. (C): (D.F.=5) $p > 0.50$.

normal subjects, as calculated with Hardy-Weinberg equilibrium and assuming this two-codominant-allele model, were 34, 49 and 17% for HH, HL and LL phenotypes, respectively, and differed significantly from the observed frequencies. The IAHA method was semi-quantitative and there appeared to be overlaps between high (2^{11}) and intermediate (2^3 to 2^{10}) IAHA titers. Even if IAHA titers higher than 2^8 were assumed to be HH phenotype, there was a significant difference between expected and observed frequencies. Therefore, we considered that HH and HL phenotypes were indistinguishable by the IAHA method and the frequency histogram of CR1 levels among normal controls showed a bimodal distribution. Segregation of a positive and a negative CR1 among 30 families is summarized in Table 1.

The mode of inheritance for a negative CR1 was analyzed by the maximum likelihood scoring method (Morton, 1959) using informative, 29 normal families. The hypothesis that a positive CR1 is controlled by an autosomal recessive gene should be discarded because many children with negative CR1 were segregated from the matings of parents with positive CR1. Moreover, there was a significant difference between observation and expectation ($p < 0.05$) in 17 informative backcross families (Table 2A). Five informative backcross families were then analyzed assuming the hypothesis of an autosomal recessive gene for a negative CR1. There was no significant difference between observation and expectation in segregation of a negative CR1 in these families ($p > 0.25$), thereby indicating that the hypothesis of an autosomal recessive gene for a negative CR1 cannot be ignored (Table 2B). The analysis of 10 informative intercross families ($p > 0.50$) (Table 2C) also supports this hypothesis. All these data lend credence to the idea that a negative CR1 is of a simple recessive character.

If the results of our studies are compared to those reported by Wilson *et al.* (1982b), the negative IAHA could correspond to the LL phenotype and the positive IAHA to the HL or HH phenotype. Table 3 shows segregation of the phenotypes in 19 normal families. Data on persons with IAHA titers ranging from 2^8 to 2^{10} were ignored because of possible overlaps. Persons with high (2^{11}), intermediate (2^3 to 2^7) and negative IAHA titers were considered to have HH, HL and LL pheno-

Table 3. Segregation of phenotypes for CR1 in 19 families.

Phenotypes of parents	Number of families	No of children with respective phenotypes		
		HH (2^{11})	HL (2^3 - 2^7)	LL (2^9)
HH × HL	2	0	2	0
HH × LL	1	0	2	0
HL × HL	5	1	3	5
HL × LL	10	1	13	7
LL × LL	1	0	0	1

types. A two codominant allele model of inheritance was supported by this appropriate segregation of the phenotypes. Therefore, there seems to be no contradiction between our data and Wilson's two codominant allele hypothesis.

This study on CR1 levels on erythrocytes in SLE patients suggests that a negative CR1 is a simple recessive character as in the normal population. However, the positive CR1 phenotypes in three families were apparently derived from both parents with a negative CR1 (Fig. 3C), a finding incompatible with this hypothesis. In 22 of the 95 SLE patients who had a negative CR1 at the initial testing, the levels became positive during the course of the study. Therefore, the negative CR1 in SLE patients seems to be heterogeneous and acquired factors such as disease activity might contribute to the levels of expression of CR1 molecules on erythrocytes. In over a 2 year follow up, 73 (65%) of the 112 SLE patients, as compared to 77 (9%) of 847 normal controls, showed a persistently negative CR1. Thus, the major factor responsible for the negative CR1 in SLE patients seems to be a genetic one.

Kajiyama (1981) performed an epidemiological study for the purpose of assessing gene-environmental interaction in the development of SLE. He reported that disorders, presumably due to autoimmune mechanisms, hypergammaglobulinemia and unusual immunologic reactions were more frequently found among relatives of SLE patients than in normal individuals and their relatives who served as the controls. Lippman *et al.* (1982) also investigated genetic factors predisposing to autoimmune disease. Significant differences between relatives and spouses were found in case of occurrence of autoimmune disease, high-titer of antinuclear antibody and anti-single-strand DNA antibody. These observations suggest that genetic rather than environmental factors are more likely to be linked to clinical and serologic differences.

We estimated the relative risk for developing the disease in persons with negative CR1 to be 19, although it was the maximum estimation. CR1 on erythrocytes in some cases might be blocked even in a remission. This finding was remarkably high, compared with previously obtained data on HLA antigens and C2 deficiencies that gave the relative risk as between 3 and 6. Erythrocytes CR1 deficiency might cause various immunological disorders regarding liberation of free immune complexes into the circulation. Since a negative CR1 was found to be clustered among SLE patients as well as their relatives, this negative CR1 is assumed to be an important genetic factor linked to the development of SLE.

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