Clin. exp. Immunol. (1986) **63,** 41–48.

Decreased C3b receptors (CR1) on erythrocytes from patients with systemic lupus erythematosus

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(Accepted for publication 18 June 1985)

SUMMARY

Using ^{125}I-F(ab')$_2$ anti-CR1 we have measured C3b receptors (CR1) on the erythrocytes of 56 normal individuals 26 patients with systemic lupus erythematosus (SLE) and 24 with rheumatoid arthritis (RA). The mean number of CR1 sites in SLE (1150/cell) but not RA (1460/cell) was significantly lower ($P < 0{\cdot}01$) than normal (2200/cell). Although the cumulative frequency curve for normals showed minor inflections at frequencies of 18% and 64%, these were not sufficiently marked to permit us to conclude that they distinguished subpopulations of different CR1 phenotypes. Measurement of CR1 numbers of two normal families and four families of SLE patients indicated that low CR1 numbers aggregated in families as did high CR1 numbers, a finding which suggests that CR1 numbers are under genetic control. However, certain observations in SLE patients indicated that low CR1 numbers could be an acquired abnormality. These included, (a) absent CR1 phenotype in a patient whose family had moderate and high CR1 numbers, (b) increasing CR1 numbers as SLE patients went into remission, (c) CR1 numbers were lower in patients with active compared with inactive disease and (d) CR1 numbers were different in each of two sets of identical twins (Fig. 4A).

Our conclusions are that, (a) genetic factors probably influence CR1 numbers in normal individuals, (b) that our findings were not inconsistent with the two codominant allele models (Wilson *et al.*, 1982), and (c) the low CR1 phenotype of SLE patients may be secondary to the disease process.

Keywords C3b receptors systemic lupus erythematosus

INTRODUCTION

Receptors (CR1) for the activated third component of complement (C3b) are present on the plasma membranes of human erythrocytes, polymorphonuclear leukocytes, mononuclear phagocytes, B lymphocytes, some T lymphocytes and renal podocytes (Fearon, 1984). CR1 has been isolated and shown to be a glycoprotein with mol. wt of 205,000 (Fearon, 1979). The presence of the receptor on phagocytic cells is important in promoting the phagocytosis of opsonized particles (Ehlenberger & Nussenzweig, 1977). CR1 on erythrocytes was originally shown by its ability to bind trypanosomes coated with antibody and complement (Duke & Wallace, 1930). Nelson later termed this phenomenon immune-adherence and suggested that its purpose was to remove complement-coated particles from the blood and to transport them to the reticulo-endothelial system (Nelson, 1963). Although the average number of CR1 molecules on each erythrocyte (approximately $1{\cdot}4 \times 10^3$ mol./

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cell) is less than 1% of those on leukocytes (approximately 2×10^5 mol./cell) (Iida, Mornaghi & Nussenzweig, 1982), the total number of circulating erythrocytes ($5{\cdot}5 \times 10^9$/ml exceeds the number of leukocytes ($7{\cdot}5 \times 10^6$/ml) to such an extent that over 90% of CR1 in the peripheral blood is associated with red cells. Thus immune complexes (IC) in the circulation would bind preferentially to erythrocytes, a conclusion that has been supported by the *in vitro* (Medof & Oger, 1982) and *in vivo* studies (Cornacoff *et al.*, 1983). The experiments of Cornacoff *et al.* (1983) showed that when IC were infused intravenously into baboons and rhesus monkeys they became bound rapidly to erythrocytes from which they were subsequently removed during passage through the liver and spleen.

Reduced erythrocyte CR1 numbers have been demonstrated in patients with systemic lupus erythematosus (SLE) (Miyakawa *et al.*, 1981; Wilson *et al.*, 1982; Inada *et al.*, 1982; Iida *et al.*, 1982; Walport *et al.*, 1985). The major area of dispute is whether this CR1 abnormality is inherited or acquired. The data of Miyakawa *et al.* (1981) and of Wilson *et al.* (1982) suggested that the abnormality is inherited, whereas the results of the studies of Inada *et al.* (1982) and Iida *et al.*, (1982) indicate an acquired disorder. In this study we report the results of studies of CR1 numbers in patients with SLE or theumatoid arthritis (RA). Our results suggest that low CR1 numbers in SLE is an acquired abnormality which is related to disease activity.

MATERIALS AND METHODS

Patients. Twenty-six patients with SLE (Tan *et al.*, 1982), 24 patients with RA (Ropes *et al.*, 1959) and 56 normal individuals were included in the study. Immediately prior to venous blood sampling the SLE patients were examined for evidence of disease activity which was defined as continuing clinical activity of involved organ systems. These features included polyarthritis, rash, vasculitis, pleurisy, pericarditis, continuing neurological features supported by abnormal EEG, or continuing active urinary sediment with cells and casts alone, or in addition to proteinuria when present. Blood was taken for erythrocyte sedimentation rate (mm in first hr Westergren) haemoglobin concentration, white cell and platelet counts. 10 ml of blood was collected in a heparinized container for estimation of CR1 numbers. In addition an equal volume of blood was allowed to clot in a plain tube and the serum stored at -70 °C for estimation of antinuclear antibody and DNA binding capacity (Webb & Whaley, 1974), total haemolytic complement (CH50), C4, C3 and factor B levels (Whaley, 1985).

Erythrocyte CR1 numbers were measured at different intervals over the course of several months in seven SLE patients, three RA patients and three normal individuals.

Preparation of CR1 and its antiserum. CR1 was prepared from erythrocytes by the method of Fearon (1979) and an antiserum was prepared by the immunization of rabbits. The antiserum gave a single precipitin line when tested against solubilized human erythrocyte membranes. Autoradiographic analysis of anti-CR1 immunoprecipitates of the lysates of ^{125}I-labelled erythrocytes (Dykman *et al.*, 1983) showed a single band of 210,000 daltons. This band was not seen when normal rabbit serum was substituted for anti-CR1.

$F(ab')_2$ fragments of the anti-CR1 antiserum and non-immune rabbit IgG were prepared by pepsin digestion (Nisonoff 1964). $F(ab')_2$ anti-CR1 was radiolabelled with ^{125}I by the chloramine T procedure (McConahey & Dixon, 1966).

Measurement of CR1 numbers. Erythrocytes were separated from heparinized venous blood by centrifugation to remove the plasma and buffy coat. The red-cell pellet was then washed three times in RPMI.1640 (Flow Laboratories, Irvine, Ayrshire, UK) containing 1% w/v bovine serum albumin (BSA: RPMI.BSA) and resuspended to $2{\cdot}5 \times 10^8$ ml in RPMI.BSA. Aliquots (200 μl) of the suspension were transferred to a series of 12 microcap tubes. Unlabelled non-immune rabbit $F(ab')_2$ fragments (12·5 μg/tube) were added to tubes 1–6, whereas unlabelled anti-CR1 $(F(ab')_2$ fragments (12·5 μg/tube) were added to tubes 7–12. After 1 h at 37°C, incremental quantities of ^{125}I-anti CR1 $F(ab')_2$ (0·25 μg, 0·5 μg, 1 μg, 2 μg, 3 μg, 4 μg) were added to each set of six tubes. After a further hour at 37°C the samples from each tube were layered onto 300 μl of dibutylphthalate/dinoylphthalate (6 vol: 4 vol) in microfuge tubes. After centrifugation 10,000 ***g*** for 2 min the supernatant was removed,

the tubes were cut, and the pellets counted for cell-bound radioactivity. Unbound radioactivity was determined by subtracting cell-bound counts from the total radioactivity added to each tube. The amount of radioactivity bound to erythrocytes in the presence of non-immune $F(ab')_2$ fragments (tubes 1–6) and the amount bound in the presence of anti-CR1 $F(ab')_2$ (tubes 7–12) were plotted against input of anti-CR1 $F(ab')_2$ with each point being performed in triplicate. Tubes 1–6 represented specific and residual non-specific binding whereas those from tubes 7–12 measured non-specific binding. The difference between these two curves was due to the specific binding of ^{125}I-anti-CR1 $F(ab')_2$ to CR1. The number of antigenic CR1 sites per cell was obtained from the specific binding curve using Scatchard plot analysis (Scatchard, 1949). The number of antigenic CR1 sites per erythrocyte will be greater than the number of receptors as we have used a polyclonal anti-CR1 antibody which will detect more than one antigenic determinant per receptor. For this reason the numbers of antigenic CR1 sites in our control population are greater than those which were reported by Walport *et al.* (1985) who used a monoclonal antibody which recognized one epitope per receptor.

When the erythrocytes from patients or normals were stored at 0°C in RPMI.BSA and tested at different intervals up to 24 h the variation between results was never greater than 10%. As CR1 numbers in normals and in patient groups were distributed in a non-parametric fashion, data were analysed using the Mann-Whitney procedure.

RESULTS

CR1 levels in patients and controls

The distribution of erythrocyte CR1 antigenic sites in the controls was logarithmic (Fig. 1). The mean level was 2200 sites/cell with a range of 335–8009.

The number of CR1 sites in the SLE group (1150/cell; range 0–4553) was significantly lower than the number in the normal group ($P < 0{\cdot}01$). The mean number of receptor sites in the RA group (1460/cell; range 334–4259) did not differ significantly from the normal mean.

CR1 numbers of patients and controls were re-plotted as cumulative frequency curves (Fig. 2). The curve for the normals was sigmoidal with a number of minor irregularities. However, the largest inflection on the curve occurred at a cumulative frequency of 64%, which is similar to the position of the break in the frequency histogram of Wilson *et al.* (1982) and was used to distinguish the HH and

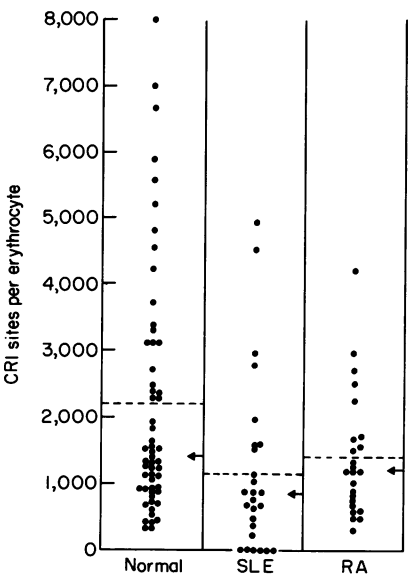

Fig. 1. Distribution of erythrocyte CR1 numbers in normal individuals, and patients with SLE and RA. The dashed lines represent the mean values and the arrows the median values.

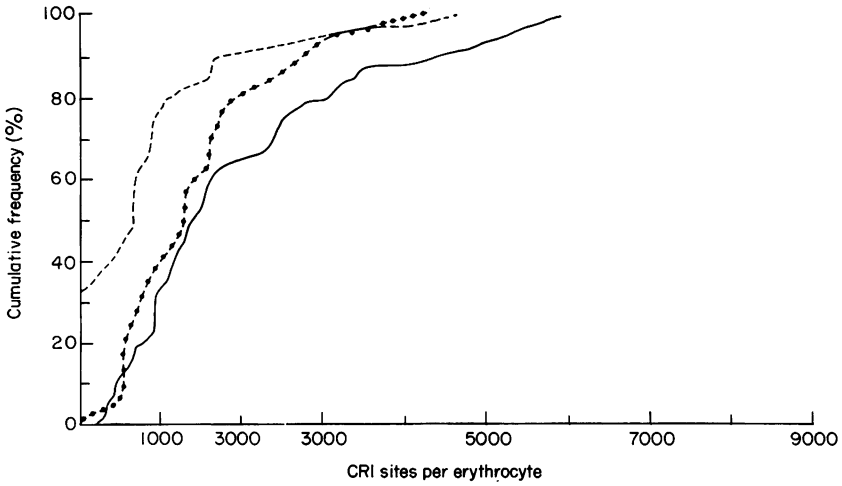

Fig. 2. Cumulative frequency curves of CR1 numbers in normal individuals (——), patients with SLE (– – –) and RA (-●- -●-). Although the inflections at 18% and 64% appear small, they are similar to the positions predicted by Wilson *et al.* (1982).

HL CR1 phenotypes. The curve for the SLE patients was similar in shape to the normal curve but was displaced to the left. In contrast the curve for the RA patients overlapped the lower end of the normal curve, but at the upper end it deviated towards the SLE curve. This observation indicates that RA patients do not have very low or very high CR1 numbers.

Family Studies

Erythrocyte CR1 numbers were measured in the families of two normal individuals and four patients with SLE. For ease of presentation of data we have divided individuals into three groups,

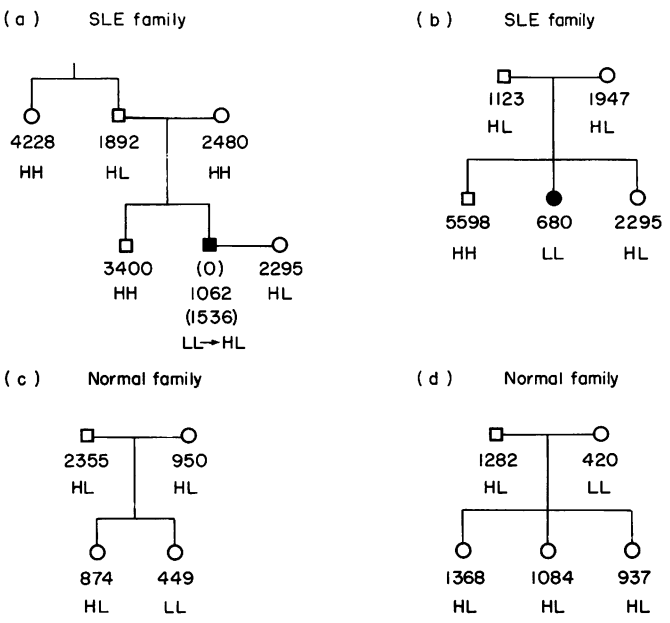

Fig. 3. Family studies of CR1 numbers in normal (c and d) and SLE (a and b) families. The phenotypes shown below the CR1 numbers have been designated tentatively on the possibility that the inflections in the cumulative frequency curve for normal individuals (18% and 64%) distinguish the phenotypes LL, HL and HH (Wilson *et al.*, 1982).

on the basis of CR1 numbers (low < 700: medium 700–2400; high > 2400). These divisions were made at the points where the greatest inflections occurred in the cumulative frequency curve (at 18% and at 64%).

The normal family shown in Fig. 3 was chosen because the individual had low CR1 numbers (449). Her sister (874), mother (959) and father (2355) had medium numbers of receptors. The two sisters and the father of the control individual in the other normal family (Fig. 3d) all had medium CR1 numbers, whereas the mother had low receptor numbers. None of these individuals had antinuclear or DNA antibodies in their sera, and serum CH50, C4, C3 and B levels were normal. The distribution of CR1 numbers in these families fits the two codominent-allele model proposed by Wilson *et al.* (1982).

At the time the family was studied, the first SLE patient (Fig. 3a) had medium CR1 numbers, as did his father. His brother, mother and paternal aunt had high numbers of receptors. This patient was studied 4 months earlier when his disease had been active. At that time he had undetectable receptors. The patient in family b had low numbers of CR1 (Fig. 3b). All his first degree relatives had medium CR1 numbers. The two remaining SLE families each contained a set of identical twins. Both twins in family a had SLE. However, only one member of family b had clinical SLE, whilst the other twin (Fig. 4 $^{+}$●) had only serological evidence of SLE. Monozygosity was established on the basis of physical appearance, HLA tissue typing and analysis of the blood groups ABO, Rh, Kell, Lewis, Duff and Kidd. In the first set (Fig. 4a) one twin had medium (1977) and the other had high (4954) numbers of receptors. The mother and brother of the twins had high CR1 numbers. In the second set of twins (Fig. 4b) one twin had medium (1047) and the other had low (377) CR1 numbers.

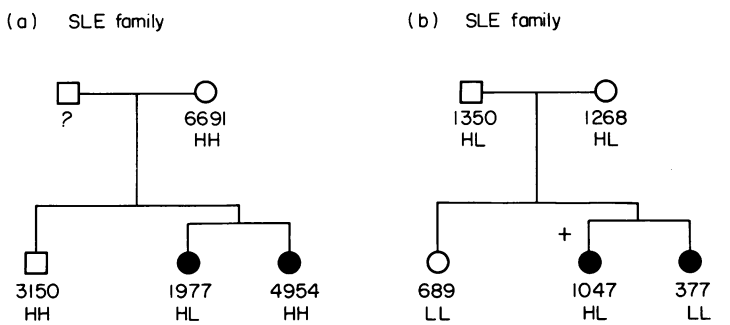

Fig. 4. Distribution of erythrocyte CR1 numbers in the family members of two sets of identical twins with SLE. (+) This twin had serological SLE only. See legend to Fig. 3 for the designation of proposed phenotypes. We were unable to get a blood sample from the father in family a.

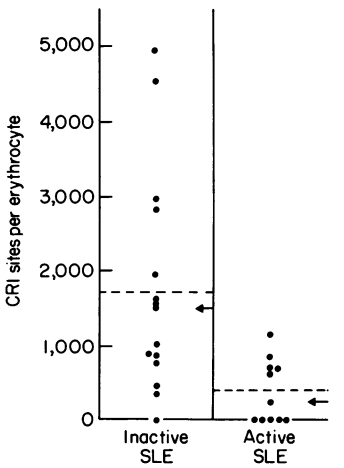

Fig. 5. Distribution of CR1 numbers in SLE patients with inactive and active disease. The dashed lines represents the mean, and the arrows the median values.

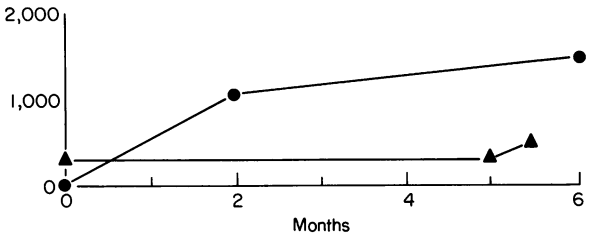

Fig. 6. Serial studies of CR1 numbers in a normal individual (▲) and a patient with SLE (●). At time 0 the patient had active disease. After 2 months the disease was beginning to enter a remission phase.

The sister of the twins also had low CR1 numbers, but both parents had medium numbers of receptors. Except for the twins none of the members of either family had clinical or serological evidence of SLE.

Relationship between CR1 numbers and disease activity

The mean number of erythrocyte CR1 in those patients with SLE who had clinical evidence of disease activity at the time the first blood sample was taken had lower CR1 numbers (mean 389) than those whose disease was clinically inactive (mean 1766) ($P < 0{\cdot}01$) (Fig. 5). No correlation was found between CR1 numbers and serum levels of circulating immune complexes, CH50, C4, C3 or B.

Over a 6-month period, serial measurements of CR1 numbers were made on three normal individuals. It was found that receptor numbers remained fairly constant (Fig. 6). In contrast serial studies of three SLE patients who had no receptors at the time of the initial examination showed that CR1 could be detected as the disease went into remission (Fig. 6).

DISCUSSION

Patients with inherited deficiency of C1 subcomponents, C4, C2 and C3 appear to be at risk to the development of SLE and other immune complex diseases (Schifferli & Peters, 1983). It has been suggested that the sera of these patients are unable to prevent the formation of large insoluble complexes which would become deposited in tissues causing injury (Schifferli & Peters, 1983). As erythrocyte CR1 is thought to play a role in the transport of IC to the reticulo-endothelial system (Cornacoff *et al.*, 1983), the findings that immune adherence was defective (Miyakawa *et al.*, 1981) and erythrocyte CR1 numbers were low in SLE patients (Wilson *et al.*, 1982) suggested another possible pathogenetic factor in this disease. The potential importance of low CR1 numbers in SLE was emphasized by the fact that both the Japanese (Miyakawa *et al.*, 1981) and Boston (Wilson *et al.*, 1982) workers suggested that the abnormality was inherited. Our observations are not wholly in agreement with those of these earlier workers. Like Wilson and his colleagues (Wilson *et al.*, 1982) we found that the range of the number of erythrocyte CR1 sites in normal individuals was wide (Fig. 1). However, although our cumulative frequency curves did indicate inflections at frequencies of 18% and 64% these inflections were small, and could represent sampling errors. Measurements of CR1 numbers in a larger number of normal individuals would resolve this issue. The Boston study (Wilson *et al.*, 1982) suggested that erythrocyte CR1 numbers were controlled by two autosomal codominant alleles which determine high and low CR1 numbers respectively. The HH phenotype was present in 37%, the HL phenotype in 48% and LL phenotype in 17% of the normal population. If their hypothesis is correct then the inflections of our normal cumulative frequency curve could indicate the different CR1 phenotypes in our normal population. The studies of the normal families of two individuals showed that the number of CR1 sites tends to be similar in first degree relatives (Fig. 3c, d). When the numbers of these families were classified with respect to their CR1 phenotype (i.e. HH, HL, LL) the patterns of inheritance were consistent with the two codominent-allele model. The studies of the four SLE families were also compatible with the Boston hypothesis (Figs 3, 4). However, certain data also suggested that the CR1 phenotype in SLE patients was affected by environmental factors. The patient in Family a had no CR1 detectable in during exacerbation, but

the receptors reappeared during remission, and CR1 numbers were different in identical twins with SLE.

In contrast to the situation in normal individuals, low CR1 numbers in SLE in many cases appears to be an acquired defect. A number of pieces of evidence support this conclusion:

1. Family studies have shown that, (a) the mother and father of an SLE patient with low CR1 numbers had high and medium CR1 numbers respectively (Fig. 3a). Similar results from more extensive family studies of SLE patients have been reported recently (Walport *et al.*, 1985). (b) Identical twins with SLE did not have the same CR1 numbers (Fig. 4).
2. CR1 numbers were lower in patients with active than those with inactive disease (Fig. 5).
3. Serial studies showed that in some patients CR1 numbers are low during exacerbation but increase as their disease enters the remission phase (Fig. 6).

The lack of CR1 on the glomerular podocytes of SLE patients with diffuse proliferative glomerulonephritis, but not with non-proliferative glomerulonephritis also suggests an acquired abnormality (Kazatchkine *et al.*, 1982).

Thus our data on SLE patients indicate that CR1 numbers may be influenced by environmental factors. The explanation for the fall in CR1 numbers during disease activity has not been ascertained. Possibilities include receptor blockade by antigen-antibody complexes or autoantibodies to CR1 and the degradation of receptors by plasma proteases. Other possibilities include the removal of CR1 along with antigen-antibody complexes as erythrocytes traverse the reticuloendothelial system. Finally as CR1 numbers are greater on younger erythrocytes, the possibility exists that an older than normal population is present in the blood of SLE patients. CR1 blockade by complexes is unlikely as $F(ab')_2$ anti-CR1 will displace erythrocyte-bound complement-coated BSA-anti-BSA complexes. Wilson *et al.* (1982) have shown that SLE patients do not have an increased proportion of aged erythrocytes in their circulations, and that CR1 autoantibodies are not present in SLE sera. Thus it would appear that erythrocyte CR1 numbers in active SLE are reduced because of proteolytic removal in the circulation or in the reticulo-endothelial system.

The observations that CR1 numbers in SLE are modified by environmental factors suggests that similar mechanisms might influence receptor numbers in normal individuals. Environmental factors might mask the genetic component controlling CR1 numbers, and explain why we do not see the distinct subgroups observed by Wilson *et al.* (1982).

This study was supported by a grant from the Scottish Home and Health Department. AF is supported by the Arthritis and Rheumatism Council.

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