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Erythrocyte complement receptor type 1 (CR1) expression and circulating immune complex (CIC) levels in hydralazine-induced SLE

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SUMMARY

Family studies were carried out to look at CR1 expression in 24 hydralazine-induced SLE patients (Hz Reactors), who had been off the drug for at least 1 year and were clinically well at the time of the study. Mean expression of CR1 was reduced by 27% in the group of hypertensives who had developed Hz-induced SLE compared with a group of 35 normal individuals. CR1 expression was also slightly reduced in the relatives of the Hz Reactors compared to the normal group. Using a solid-phase C1q binding assay, CIC levels were found to be elevated in the plasma of the Hz reactors and an inverse relationship was found between CR1 levels and CIC levels in this patient group. Both CR1 levels and CIC levels in Hz Reactors and normal individuals were constant over the 36 weeks studied. This study suggests that there is an association between an inability to deal efficiently with CIC and susceptibility to developing Hz-induced SLE.

Keywords circulating immune complexes CR1 drug-induced SLE hydralazine

INTRODUCTION

The first case of drug-induced SLE was reported by Huffman in 1945 as an adverse reaction to sulfadiazine. Since then over 30 drugs have been implicated in causing an SLE-like syndrome (Hughes, 1979; Amos, 1982). Hydralazine is one of the drugs most closely associated with SLE as an immunotoxic effect (Lee & Chase, 1975). The probability of developing hydralazine-SLE varies with sex, acetylator phenotype and is dose-dependent. Prospective studies reported the incidence to be 2 to 21% (Lee & Chase, 1975). A more recent study reported that 19·4% of women taking 200 mg hydralazine per day developed SLE-like symptoms; when the dose was reduced to 100 mg per day, the incidence among women was still 8% (Cameron & Ramsay, 1984). Up to 54% of hydralazine-treated patients develop antinuclear antibodies (ANA) (Lee & Chase, 1975) but again the incidence is affected by acetylator phenotype and drug dosage (Perry *et al.*, 1970; Mansilla-Tinoco *et al.*, 1982). When hydralazine is withdrawn, clinical symptoms improve within days, and disappear completely after a few weeks (Perry, 1973). Despite the high incidence of hydralazine-induced SLE the drug is still widely used in treatment of hypertension, normally in conjuction with a diuretic and beta-blocker (British National Formulary, 1981).

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Clinical and laboratory features in hydralazine-induced SLE resemble idiopathic SLE, and although the two syndromes almost certainly have different pathogenesis, understanding the mechanisms underlying drug-induced SLE may help elucidate the primary cause of idiopathic SLE. Genetic factors appear to be important in both forms of the syndrome since HLA Class II and III antigens have been shown to be associated with the idiopathic disorder (Fielder *et al.*, 1983; Reveille *et al.*, 1985), and Hz-induced SLE patients have an increased incidence of the class II antigen, DR4, and are almost exclusively slow acetylators (Batchelor *et al.*, 1980; Mansilla-Tinoco *et al.*, 1982). Although both the idiopathic and drug-induced syndromes are multi-factorial in nature, defects in the classical pathway are likely to contribute to the observed symptoms in both cases, since individuals with homozygous deficiencies of the early components of the classical pathway of complement are at increased risk of developing idiopathic SLE (reviewed by Ross & Densen, 1984; Rynes, 1982) and hydralazine has been shown to inhibit the covalent binding reactions of the complement component, C4 (Sim, Gill & Sim, 1984).

Idiopathic SLE patients have been shown to have low levels of the erythrocyte immune adherence receptor, CR1 (Miyakawa *et al.*, 1981; Inada *et al.*, 1982; Iida, Mornaghi & Nussenzweig, 1982; Wilson *et al.*, 1982; Takemura *et al.*, 1984; Walport *et al.*, 1985a; Holme *et al.*, 1985; Ross *et al.*, 1985). There has been much controversy as to whether the low levels of CR1 observed in these patients are acquired or inherited (Miyakawa *et al.*, 1981; Wilson *et al.*, 1981; Iida *et al.*, 1982; Walport *et al.*, 1985a; Holme *et al.*, 1985; Ross *et al.*, 1985). However erythrocyte CR1 has been shown to be important in primate immune complex handling (Cornacoff *et al.*, 1983) and reduced binding of anti-ds DNA/^{3}H-ds DNA by erythrocytes from idiopathic SLE patients was attributed to decreased levels of CR1 (Taylor *et al.*, 1983).

This study was set up to compare further the relationship between idiopathic SLE and Hz-induced SLE with respect to erythrocyte CR1 levels in order to determine the importance of low CR1 levels as a predisposing factor in drug-induced SLE. To investigate whether any genetic effect influences CR1 levels in Hz-SLE, former Hz-induced SLE patients who no longer showed any clinical signs of the syndrome and who had been off the drug for at least 1 year were investigated.

MATERIALS AND METHODS

Blood

Venous blood (5 ml) was collected into EDTA-coated tubes. Plasma was separated and stored at −70°C. Erythrocytes were washed three times in PBS (50 ml). The buffy coat was removed by aspiration at each stage. Erythrocytes were stored in phosphate-buffered saline (pH 7·4) (PBS) (20 ml) at 4°C for up to 3 days. For long-term storage erythrocytes were suspended in an equal volume of 40% glycerol in 5% trisodium citrate and frozen in liquid nitrogen. Immediately before use the erythrocytes were thawed and dialysed against 50 sample volumes of PBS. The cells were then washed three times in 50 ml of PBS.

Subjects

Categories of subjects for investigation were as follows:

Group 1. Twenty-four hydralazine (Hz) reactors (21 women, three men) fulfilling the following criteria: (A) Development of at least one of the symptoms in the ARA revised criteria for SLE (Tan *et al.*, 1982). (B) Presence of significant titres of ANA. (C) Reversal of clinical features after withdrawal of Hz.

Group 2. Twenty-five consanguinous relatives of the 25 Hz reactors.

Group 3. Five idiopathic SLE patients fulfilling the 1982 revised criteria for SLE (Tan *et al.*, 1982).

Group 4. Thirty hypertensives who have taken Hz for longer than 1 year without developing any adverse reactions (Hz control group). Seventeen of the Hz controls were still taking hydralazine at the time of the study, while the remaining 13 were no longer taking the drug.

Group 5. Seventeen consanguinous relatives of the Hz control group.

Group 6. Seventeen hypertensives who were being treated with drugs other than Hz.

Group 7. Thirty-five normal individuals.

Preparation and iodination of antibodies

The mouse monoclonal anti-human CR1 antibody, E11 (Hogg *et al.*, 1984), was kindly provided by Dr N. Hogg (ICRF, London, UK). E11 was isolated from ascites fluid by the method of Bruck *et al.* (1982). $F(ab')_2$ goat anti-human-IgG, IgA and IgM (GAH IgGAM) and $F(ab')_2$ goat anti-human IgG (GAH IgG) were obtained from TAGO, Tissue Culture Services, Slough, UK. All antibodies were radiolabelled in PBS with ^{125}I using the chloramine T method (McConahey & Dixon, 1966). E11 (30 μg) was incubated with chloramine T (15 μg) and 300 μCi of carrier-free ^{125}I (100 mCi/ml) in a total volume of 70 μl. A specific activity of $1{\cdot}4 \times 10^7$ to $3{\cdot}8 \times 10^7$ ct/min/μg was obtained. Both GAH IgGAM (100 μg) and GAHIgG (100 μg) were incubated with chloramine T (8 μg) and 300 μCi of carrier-free ^{125}I in a total volume of 240 μl. A specific activity of $5{\cdot}0 \times 10^6$ to $9{\cdot}3 \times 10^6$ ct/min/μg was obtained. All reactions were carried out at 0°C for 10 min and a 6-fold molar excess (over chloramine T) of sodium metabisulphite (20 mg/ml in PBS) was added to stop the reaction. In order to separate radiolabelled protein from unreacted ^{125}I, the reaction mixture was applied to a Sephadex G-25 column (Pharmacia, Milton Keynes, Beds, UK) which had been presaturated with 150 mg of bovine serum albumin (BSA) (Sigma Chemical Co., Poole, Dorset, UK) and equilibrated with PBS. The column was eluted using PBS/1% BSA.

Purification and iodination of human C1q

C1q-containing fractions were identified by SDS PAGE and by double radial immunodiffusion of samples (Ouchterlony & Nilsson, 1978) against sheep anti-human C1q (Serotec, Bicester, UK). The euglobulin fraction was isolated from human serum: C1q-containing fractions were pooled and dialysed into starting buffer (57 mM sodium phosphate, 5 mM EDTA, pH 7·4), for application to a DEAE cellulose column as described for the purification of C1r and C1s (Sim, 1981). C1q was eluted in the starting buffer. The major C1q-containing fractions were pooled and applied in the same buffer to a Superose 6H FPLC column (Pharmacia, Milton Keynes, Beds. UK). The pooled C1q obtained from this step was more than 90% pure, although it was shown to have two minor contaminating bands by SDS-PAGE under reducing conditions. The C1q was stored in 1 ml aliquots (60 μg/ml in starting buffer) at −70°C until required.

C1q (12 μg) was radiolabelled, as described above for the antibodies, by incubation with 100 ng of chloramine T (10 μg/ml) and 400 μCi of carrier-free ^{125}I (100 mC/ml). The specific activity obtained was $2{\cdot}6 \times 10^5$ to $5{\cdot}5 \times 10^5$ ct/min/μg. The Sephadex G-25 column was equilibrated with the elution buffer (50 mM Tris-HCl/120 mM NaCl, pH 7·2) before separation of the iodination mixture.

Quantification of erythrocyte CR1

Binding of ^{125}I-E11 to erythrocytes was analysed by a modification of the method of Iida *et al.* (1982). Scatchard analysis (Scatchard, 1949) showed that 75% to 85% of the radiolabelled protein had antibody activity. It was found that 250 ng of ^{125}I-E11 was sufficient to saturate greater than 95% of receptors on $2{\cdot}5 \times 10^8$ erythrocytes. This is in agreement with Hogg *et al.* (1984). This amount was used in the single point binding assays described below. Non-specific uptake of ^{125}I-E11 in the presence of 100-fold molar excess of cold E11 was less than 1%. This was in agreement with background ct/min levels when sheep erythrocytes were substituted for human erythrocytes. Sheep erythrocytes were used as a control in subsequent binding assays.

Single point binding assay

This was carried out according to the method of Hogg *et al.* (1984): erythrocytes ($2{\cdot}5 \times 10^8$) were incubated (21°C for 30 min) with 250 ng of ^{125}I-E11 with specific activity of $1{\cdot}0 \times 10^6$ to $3{\cdot}0 \times 10^6$

ct/min/μg in a total volume of 250 μl of PBS/1% BSA/0·1% sodium azide. Triplicate 70 μl aliquots were then layered onto 300 μl of dibutyl phthalate in microcentrifuge tubes, centrifuged (1 min at 9000 ***g***), and the tubes were frozen in methanol/dry-ice. The tips of the tubes, containing cell-bound ^{125}I-E11, were then counted on an LKB rackgamma counter.

Calculation of erythrocyte number

Erythroctes were diluted 1/100 (v/v) (a) in distilled water for absorbance measurements at 541 nm, and (b) in PBS for counting the number of cells using an Improved Naubauer haemocytometer. OD_{541} readings were plotted against cell number to construct a standard reference curve. From the standard reference curve of absorbance vs cell number, 1×10^9 cells/ml diluted 1/100 (v/v) in distilled water, gave an absorbance of 0·4 at 541 nm. An absorbance of 0·7 at 541 nm was assumed for 10^9/ml sheep erythrocyte diluted 1/5 (v/v) in distilled water.

Circulating immune complex (CIC) assays

Two different C1q binding assays were used to look at the levels of CIC.

Solid-phase C1q binding assay
This was a modification of the method of Hay, Nineham & Roitt (1976).

Preparation of the C1q-coated plate. C1q was diluted to 10 μg/ml in PBS and 100 μl was added to each well of a 96-well flat-bottomed PVC flexi-plate (Sterilin Ltd, Teddington, Middlesex, UK). After incubation for 18 h at 4°C the C1q was emptied from the plate by inversion, and the wells were washed three times with 250 μl of ice-cold PBS/0·25% Tween 20 (Sigma Chemical Company, Poole, Dorset, UK).

Preparation of the plasma samples. In order to dissociate endogenous C1, the test plasma samples (50 μl) were incubated with 200 mM EDTA, pH 7·5, (100 μl) at 37°C for 30 min and then diluted 1/9 (v/v) in ice-cold PBS/0·25% Tween 20. Diluted plasma (100 μl) was then added to duplicate wells of the C1q-coated plate, and incubated at 37°C for 1 h followed by 30 min at 4°C. The plate was then washed as above. ^{125}I-iodinated $(Fab')_2$ GAH-IgG (100 ng) or ^{125}I-iodinated GAH-IgGAM (100 ng) was added to each well in 100 μl of PBS/Tween 20. The plate was again incubated for 60 min at 37°C followed by 30 min at 4°C, washed three times, dried, and individual wells were counted in a gamma counter. The levels of CIC were expressed as ^{125}I ct/min bound per well.

Fluid phase C1q binding assay
This was carried out exactly as previously described by Zubler *et al.* (1976).

Statistical analysis

For comparison of CR1 distributions between groups, analysis of variance was done using the GENSTAT library implemented on an ICL 2988 computer. This was followed by a 2-sample Student *t*-test, using the error term derived from the analysis of variances, to compare the means of the individual groups. Correlation between data groups was analysed using linear regression analysis, and the correlation coefficients were calculated.

RESULTS

Erythrocyte CR1 distribution in normals and Hz reactors. The CR1 distribution curve for normal individuals falls into three distinct groups (Fig. 1a), if sufficiently small intervals (50 sites) are chosen: 12% of normal individuals have 0–400 CR1 per erythrocyte, 51% have 400–800 CR1 per erythrocyte and the remaining 37% have 800–1250 CR1 per erythrocyte. This distribution is very similar to the trimodal distribution reported by Wilson *et al.* (1982) which formed the basis for

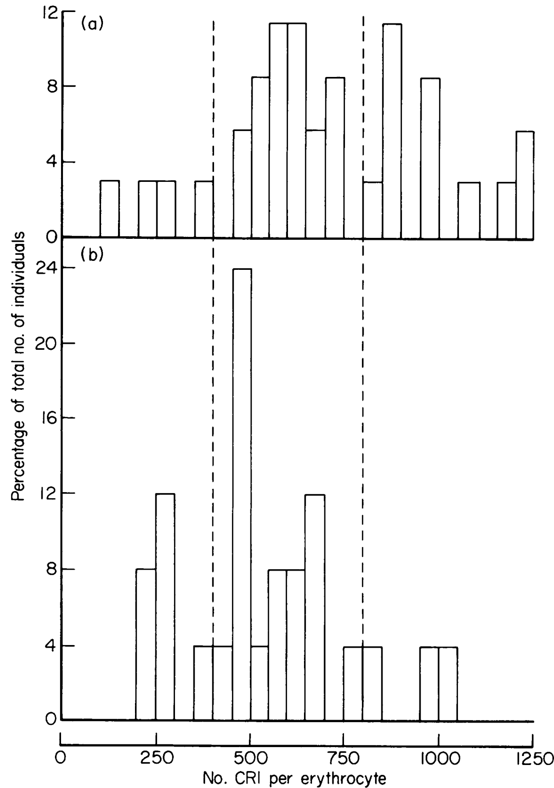

Fig. 1. The distribution of erythrocyte CR1 levels in (a) normal individuals and (b) in Hz reactors.

proposing the autosomal codominant two-allele model for control of CR1 expression. However subsequent reports have suggested a more complex genetic control of CR1 expression (Iida *et al.*, 1982; Takemura *et al.*, 1984; Ross *et al.*, 1985; Ripoche & Sim, 1986) and if larger group intervals (150 sites) are chosen then a very close approximation to a normal unimodal distribution is obtained (figure not shown). In the CR1 distribution histogram for Hz reactors (Fig. 1b), a larger percentage of individuals fall into the low and intermediate expressor groups (24% and 64% respectively), as designated by Wilson *et al.* and fewer individuals fall into the high expressor group (12%).

When the distributions are plotted as a cumulative frequency (Fig. 2b) the Hz reactor and normal curves are sigmoidal in shape and there is a small inflection in the normal curve around 57%, correlating with the break in the distribution histogram between the intermediate and high CR1 expressor groups. However, the expected inflection around 12% correlating with the break between the intermediate and low expressors is much less marked.

Erythrocyte CR1 levels in Hz reactors and control groups. The mean number of CR1 per erythrocyte (Fig. 2a) in the Hz reactor group was 541 ±48 (range 213–1001). This was significantly lower ($t(142)=2{\cdot}71$; $P<0{\cdot}01$) than the mean number of CR1 in the normal control group (mean = 712 ± 41, range 130–1250). The mean number of CR1 per erythrocyte (660 ± 45) in the Hz control group did not differ significantly ($P>0{\cdot}25$) from the normal control group. The values for Hz controls still taking Hz and those no longer on the drug were pooled because no difference was observed between the two groups when they were treated separately. The consanguinous relatives of the Hz reactor group had a lower mean number of CR1 (670 ± 48, range 380–1034) than normal individuals, whereas the consanguinous relatives of the Hz controls had a higher mean number of CR1 (766 ± 58). The difference in means from the normal control group was not statistically significant ($P>0{\cdot}25$) in either case. The mean number of CR1 per erythrocyte in hypertensives

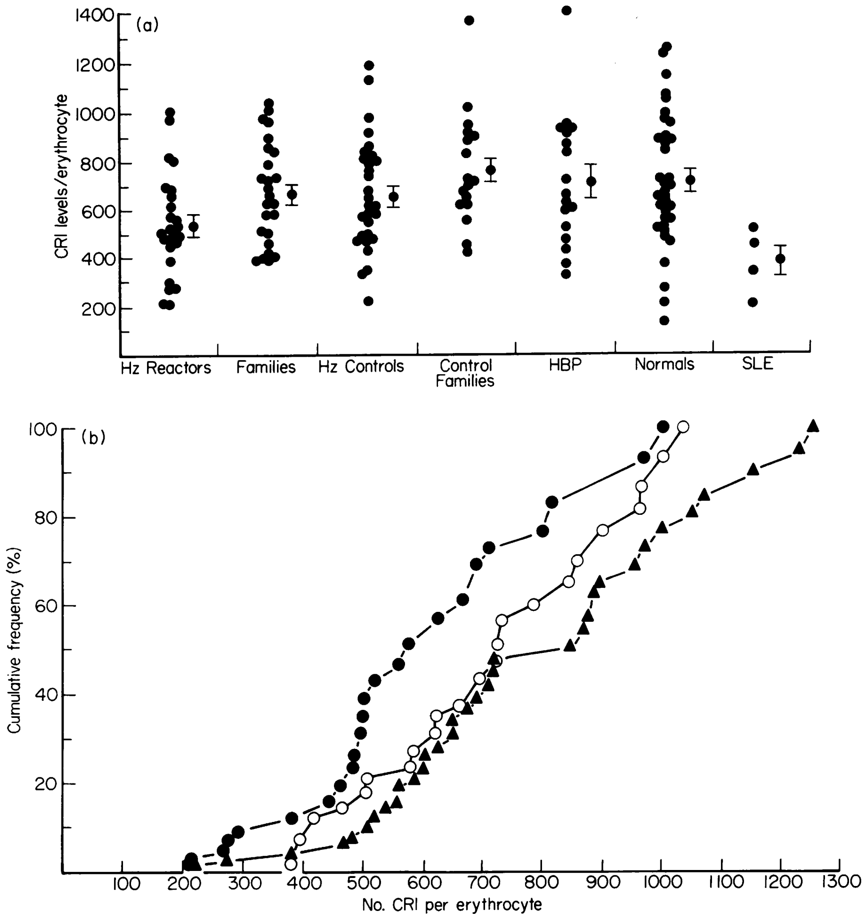

Fig. 2(a) Comparison between CR1 levels in Hz reactors, the relatives of the Hz reactors, Hz controls, the relatives of the Hz controls, hypertensives (HBP) not on hydralazine, normal individuals, and patients with idiopathic systemic lupus erythematosis (SLE). The groups are as described in the methods section. Each dot represents CR1 levels in an individual, and is the average of two determinations. Bars represent means ± s.e.m. (b) Cumulative frequency curve of the number of CR1 per erythrocyte in the group of normal individuals (▲) Hz reactors (●) and the consanguinous relatives of the Hz reactors (○).

(715 ± 66, range 315–1404) who were not on Hz was very close to the mean found in the group of normal individuals. CR1 levels were very low (mean = 388 ± 60, range 248–502) in the idiopathic SLE patients tested.

From the cumulative frequency curve (Fig. 2b), it can be seen that the Hz reactor curve is displaced to the left of the normal curve. The curve depicting the consanguinous relatives of the Hz reactors falls between the curve for normal individuals and that of the Hz reactors, and although the displacement is not significant, an inherited factor may contribute to the low CR1 levels found in the Hz reactors.

Family studies. In both the Hz reactors and Hz control families, first degree relatives tend to have similar CR1 levels. In one control family (Fig. 3b), a high expressor mother and low expressor father had two children who had intermediate CR1 levels. In the one full idiopathic SLE family studied (Fig. 3a) all the members had CR1 levels which were below the normal mean. Although sufficient family members were available in only a few cases, no patient with low CR1 levels had two parents who were high expressors.

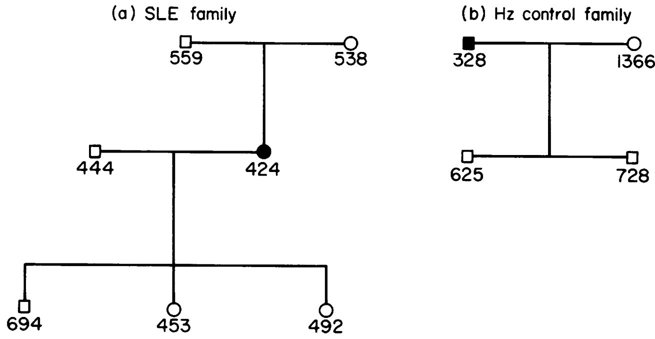

Fig. 3. Family studies of CR1 levels in (A) an idiopathic SLE family and (B) in a Hz control family. The numbers below each symbol represent erythrocyte CR1 levels. The solid symbols designate the patient.

Longitudinal studies. CR1 levels were measured on more than one occasion in seven normals and in six Hz reactors (Fig. 4). The interval between observations ranged from 8 to 42 weeks for the patient group (mean = 26 weeks), and from 1 to 18 weeks for normal individuals (mean = 10 weeks). For normal individuals, the correlation between paired observations was highly significant ($r = 0{\cdot}987$, $P < 0{\cdot}001$). The correlation between paired observations in the Hz reactor group was not significant ($r = 0{\cdot}137$, $P > 0{\cdot}25$). This was due to one point which fell far to the left of the line. If this individual (who was suffering from influenza at the time of the rebleed) is excluded from the statistical analysis, then the correlation between the paired observations from the five remaining Hz reactors is also significant ($r = 0{\cdot}981$, $P < 0{\cdot}01$).

No correlation was found between erythrocyte CR1 levels and either the dosage of hydralazine taken per day or the length of time that the patient had been off the drug.

Measuring IgG, IgM and IgA-containing immune complexes. Circulating immune complexes were measured (Fig. 5) using a solid-phase C1q binding assay. When $F(ab')_2$GAH IgGAM was used to detect immune complexes bound to the plate, the plasma of the Hz reactor group contained highly significant ($P < 0{\cdot}001$) levels of immune complexes (mean = $37{\cdot}0 \pm 0{\cdot}93$), compared to the Hz control group (mean = $31{\cdot}8 \pm 0{\cdot}78$). The Hz control group also had lower levels of circulating immune complexes than their own relatives (mean = $34{\cdot}0 \pm 0{\cdot}66$) and those of the Hz reactors

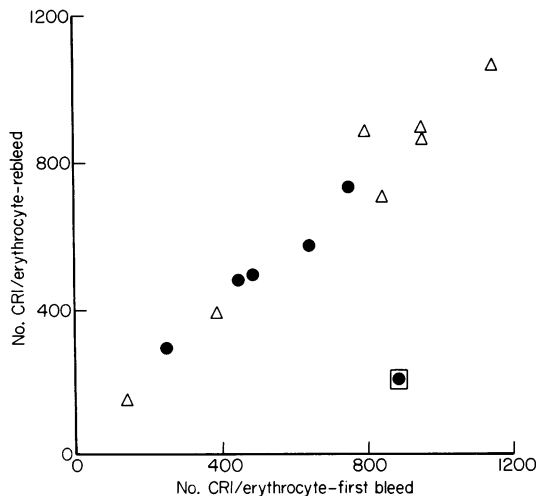

Fig. 4. Longitudinal studies of CR1 levels in normal individuals (△) and Hz reactors (●). The boxed symbol represents a Hz reactor who was suffering from influenza at the time of the rebleed.

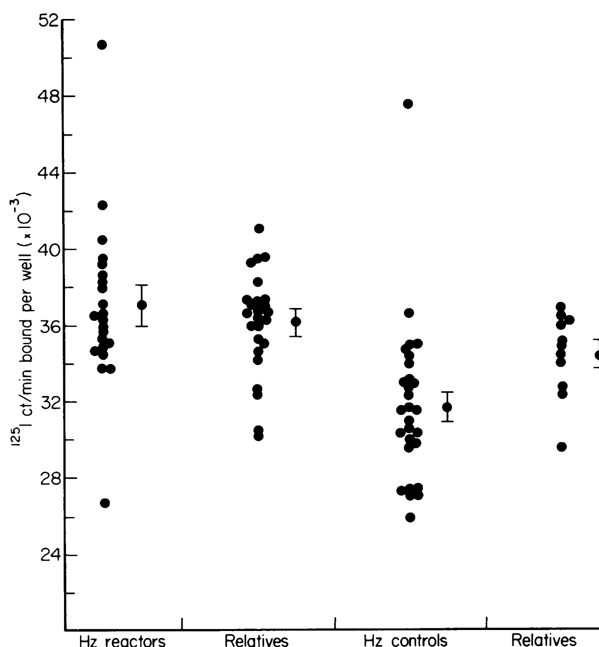

Fig. 5. Distribution of CIC in Hz reactors, the relatives of the Hz reactors, Hz controls and the relatives of the Hz controls. The assay was carried out as described in the methods section. The specific activity of the ^{125}I-GAH IgGAM was 5×10^6 ct/min per μg protein, and 5×10^5 ct/min was supplied to each well. The dots represent the average of two determinations of plasma CIC levels from one individual. The bars represent the mean $\pm$ s.e.m.

(mean $= 36{\cdot}2 \pm 0{\cdot}54$). CIC levels in sera vs plasma were consistently lower for single individuals, in agreement with Taylor *et al.* (1985).

When the relationship between circulating immune complex levels and CR1 levels was examined by linear regression analysis (Fig. 6), a negative correlation was found ($r = -0{\cdot}571$, $P < 0{\cdot}01$) for the Hz reactor group (Fig. 6b). There was no correlation ($r = 0{\cdot}140$, $P > 0{\cdot}25$) found between the two parameters when the Hz Control group values were examined by linear regression (Fig. 6a). CIC levels were found to be constant ($r = 0{\cdot}956$, $P < 0{\cdot}001$) in seven Hz reactors bled on two separate occasions.

When the solid phase C1q binding assay was carried out using ^{125}I F(ab$'$)$_2$ goat anti-human IgG to detect immune complexes bound to the C1q-coated plate, levels of circulating immune complexes in the Hz reactor group were again found to be raised but this time the difference between Hz reactors and Hz controls was not so marked. The fluid phase binding assay was unable to discriminate between Hz reactors and Hz controls.

DISCUSSION

Low levels of CR1 are associated with Hz-induced SLE, even though there are no clinical symptoms of the disease in the patients studied. Over 80% of the patients who presented were women, in contrast to previous studies where the percentage of women ranged from 40% to 60%. Possibly the reduction in recommended dose of hydralazine has increased the incidence of women developing hydralazine-induced lupus relative to men. The reduction in CR1 levels in the Hz reactors is less than has been observed in idiopathic SLE. In idiopathic SLE it has been found that CR1 levels rise during periods of remission (Iida *et al.*, 1982) such that patients with active disease have lower CR1 levels than patients with inactive disease (Holme *et al.*, 1985). In addition CR1 levels were found to

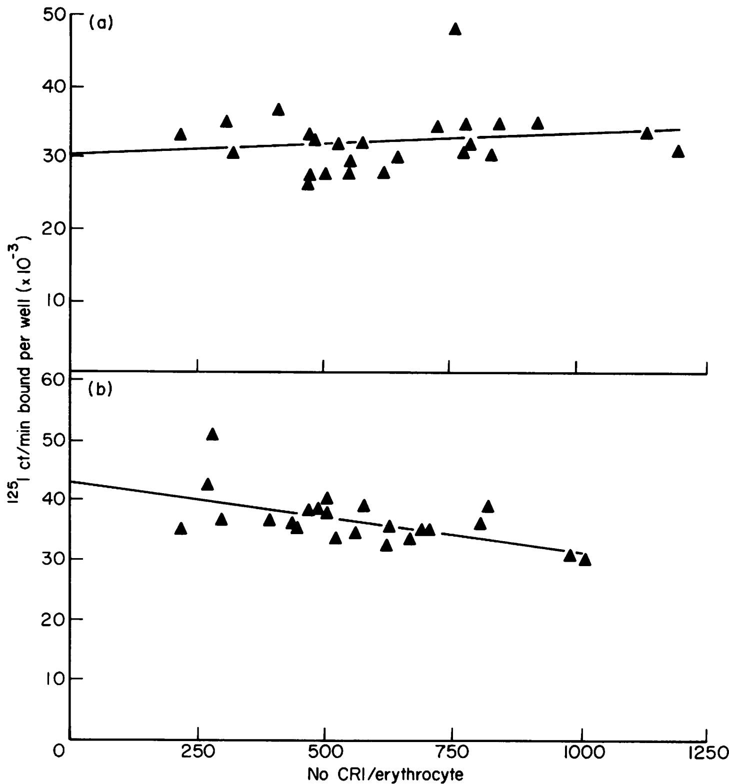

Fig. 6. Linear regression correlation analysis of the relationship between the number of CR1 per erythrocyte and CIC levels (a) in Hz controls and (b) in Hz reactors. Erythrocyte CR1 levels and CIC levels were determined as described in the methods section. Each symbol represents one individual. Correlation coefficient: (a) $r = 0{\cdot}140$; (b) $r = -0{\cdot}571$.

correlate inversely with CIC (Iida *et al.*, 1982; Inada *et al.*, 1982). Family studies have shown several idiopathic SLE patients with very low CR1 who would be expected to have high levels if CR1 expression were solely determined by inheritance (Walport *et al.*, 1985a; Holme *et al.*, 1985). Direct evidence of an acquired reduction was reported in a study in which erythrocytes transfused into patients with SLE showed a significant loss of CR1 within a week (Walport *et al.*, 1985b). The combined results support an acquired mode of reduction of CR1 but they do not exclude inherited low levels as a prediposing factor in SLE. Indeed earlier family studies found that the CR1 defect persisted in remission (Miyakawa *et al.*, 1981) and that reduced levels of CR1 were found in the relatives of SLE patients and from this it was concluded that low levels of CR1 in SLE are inherited (Wilson *et al.*, 1982).

In the present study, inheritance cannot be excluded as a contributing factor in low CR1 expression since CR1 levels are slightly reduced in relatives of the Hz reactors; conversely, CR1 levels are slightly elevated in the relatives of the Hz controls. In addition, no fluctuation was found in CR1 levels when the Hz reactors were rebled. This is in contrast to previous reports of longitudinal studies of SLE patients in which fluctuations in CR1 levels correlated with disease activity. However all the patients in the present study were well throughout the investigation. It is possible that CR1 levels are reduced further during the reaction to Hz comparable to levels found in idiopathic SLE. In order to test such a hypothesis it would be necessary to carry out a prospective study of patients receiving hydralazine.

The presence of elevated CIC correlates with low CR1 levels in idiopathic SLE, as mentioned above, and although clinical symptoms improve within days of a Hz Reactor being taken off the

drug, laboratory symptoms can take longer to resolve: for example it has been reported that ANA can persist for up to 10 years after withdrawal of hydralazine (Perry, 1973), although ANA titres usually return to normal within several months (Stratton, 1985). However no information was given on CIC in these studies. In order to investigate whether persisting immune complexes might contribute to the low CR1 levels observed in the Hz reactors, CIC were measured and compared with CR1 levels. Hz reactors, in contrast to controls, did show increased levels of plasma immune complexes. Moreover in the Hz reactor group, again in contrast to controls, increased levels of CIC were found to correlate with low CR1 levels. CIC levels did not vary with time in the Hz reactor group and no correlation was found between CIC and the length of time for which the patient had been off the drug. This was illustrated by one Hz reactor who had very low levels of CR1 and greatly elevated CIC levels; both parameters remained constant over 36 weeks and the patient had not taken hydralazine for over 5 years. The results from these longitudinal studies suggest that if recovery from an acquired pathology is being observed, the recovery phase is exceptionally prolonged. Such an explanation seems unlikely in view of the lack of correlation between CIC and cessation of hydralazine therapy (range, 1–5 years). Further investigation of the role of inheritance in CR1 expression is being carried out at the genomic level. In conclusion, in agreement with laboratory findings in idiopathic SLE, CIC levels correlate with low CR1 expression in Hz-SLE. In contrast to idiopathic SLE however, elevated CIC levels are found in a subgroup of disease-free Hz reactors, and not only during periods of disease activity. Therefore it is tempting to speculate that susceptibility to Hz-induced SLE is associated with an inability to deal efficiently with an immune complex load. If Hz then acts to compromise further immune complex clearance, for example, by causing a functional deficiency of the complement component C4, immune complex deposition in the tissues may occur, leading to SLE-like symptoms.

In order to study this group of patients further we are at present carrying out an extended HLA haplotype study to determine whether inheritance of a particular allele, for example a C4 null which could affect the handling of immune complexes, is associated with the low CR1 levels observed in this study.

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