Clin. exp. Immunol. (1992) **87**, 422–428

Peripheral catabolism of CR1 (the C3b receptor, CD35) on erythrocytes from healthy individuals and patients with systemic lupus erythematosus (SLE)

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(Accepted for publication 25 October 1991)

SUMMARY

The present study investigated the rate of catabolism of CR1 (the C3b receptor, CD35) on erythrocytes (E) *in vivo*, in relationship with the expressed number of CR1/E, the CR1.1. *Hin*dIII quantitative CR1 polymorphism, and cell age. The relationship between the number of CR1/E and cell age was analysed by measuring G6PDH activity in E that had been sorted according to high or low expression of CR1 (CD35), by assessing the expression of CR1 (CD35) on E separated according to cell density, and by comparing the number of CR1 (CD35) antigenic sites on reticulocytes and on E. A physiological catabolism of CR1 (CD35) manifested by a reduction in the number of CR1 (CD35) antigenic sites/E with cell ageing was consistently observed in healthy individuals. The number of CR1/E decreased with ageing of E according to a complex pattern that associated an exponential decay and an offset. Calculated half-lives of CR1 (CD35) ranged between 11 and 32 days in healthy individuals. A more rapid loss of CR1 (CD35) with cell ageing occurred on cells from individuals expressing high numbers of CR1/E. In patients with systemic lupus erythematosus (SLE), half-lives of CR1 (CD35) on E were in the same range as those of healthy individuals with a similar quantitative CR1 genotype; the number of CR1 (CD35) on reticulocytes was reduced and linearly related to the number of CR1/E, independently of the patients' quantitative CR1 genotype. Transfusion experiments with E bearing high or low amounts of CR1/E indicated the lack of preferential removal of E bearing high numbers of CR1 (CD35) in patients with SLE. These results indicate that the rate of loss of CR1 (CD35) from E with cell ageing is directly related to the quantitative CR1 phenotype and suggest that enhanced peripheral catabolism is not the sole mechanism of the acquired loss of CR1 (CD35) on E in patients with SLE.

Keywords CR1 CD35 systemic lupus erythematosus erythrocytes receptors complement physiology human diseases

INTRODUCTION

CR1 (CD35) is a widely distributed membrane glycoprotein which serves as cellular receptor for C3b and C4b [1]. On erythrocytes (E), CR1 (CD35) functions to transport C3b-bearing soluble immune complexes from the circulation to the liver [2–4]. Immune adherence of immune complexes to E depends on the amount of C3b and C4b that is bound to complexes and on the number of molecules of CR1 (CD35) expressed on E [5]. The organization in clusters of CR1 (CD35) on the E membrane favors high avidity multimeric ligand–receptor interactions [6,7]. The quantitative expression of CR1 (CD35) on E is determined by an autosomal biallelic codominant polymorphism that correlates with a *Hin*dIII restriction fragment length polymorphism (RFLP) within the CR1 gene [8]. E express an average of 550 CR1 (CD35) antigenic sites/cell (CR1/E). The mean number of CR1/ranges from 150 to 1200 and represents a stable phenotypic characteristic in healthy individuals.

CR1 (CD35) numbers are reduced on E of patients with SLE [9–15] and on E from primates subjected to immune complex-induced experimental glomerulonephritis [16,17]. Decreased expression of CR1 (CD35) in SLE is not inherited with the Hind III polymorphism [18,19]. The acquired loss of CR1 (CD35) was suggested to depend on an accelerated peripheral catabolism of the molecule on the E surface [20–22].

The present study analysed the rate of catabolism of CR1 (CD35) on E *in vivo* in healthy individuals and in patients with SLE.

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MATERIALS AND METHODS

Study population

Blood samples were obtained from nine healthy individuals and 16 patients with active SLE whose symptoms met the disease criteria of the American Rheumatism Association [23]. The main clinical presentation of the patients was: arthralgias ($n=2$); skin and mucous membranes abnormalities ($n=4$); thrombopenia ($n=4$); lupus anticoagulant ($n=3$); central nervous system abnormalities ($n=2$); pericarditis ($n=1$). Four of the patients had mild proteinuria. None of the patients had renal failure. Anti-DNA antibodies were present in the serum of all patients as assessed by a Farr precipitation assay. Twelve patients had CH50 values below 70% of normal. Five patients had a positive Coombs test. Two patients had a significant degree of autoimmune haemolysis, as defined by a reticulocyte count above 3% of the total erythrocyte count. Haemoglobulin was above 9 g per 100 ml in all patients; none of the patients required transfusion. Twelve of the patients received 10 mg/day of prednisone or more; none of the patients received cytostatic agents at the time of the study. The study was approved by the Ethical Committee of the University of Reims. Informed consent was obtained from the patients and from healthy volunteers.

Quantification of CR1 (CD35) on erythrocytes

Quantification of CR1 (CD35) antigenic sites on E was performed using flow cytometry and a biotin–Avidin enhancing system for immunofluorescent staining, as previously described [24]. In brief, 10^6 E were sequentially incubated with biotinylated mouse anti-CR1 monoclonal antibody J3D3 [25], phycoerythrin-conjugated streptavidin, biotinylated anti-streptavidin antibody and phycoerythrin-conjugated streptavidin. Cells were washed between each of the staining steps with PBS containing 1% bovine serum albumin (BSA) and 0·1% NaN_3 (PBS-BSA-NaN_3). Cells were fixed in the presence of 0·1% formaldehyde and analysed using a Becton Dickinson Facstar I apparatus (Becton Dickinson, Mountain View, CA). Fluorescence was activated at 488 nm with an argon laser set at 500 mW power. The number of CR1/E refers to the number of MoAb molecules bound/E at saturation. The number was back-calculated from the results obtained in a cytofluorographic assay using E bearing known amounts of ^{125}I-labelled anti-CR1-binding sites/cell as a calibration curve.

Cell sorting

For cell sorting of E bearing high and low amounts of CR1 (CD35), E were stained as described above, except that the fixation step was omitted to allow enzymatic measurements. Windows were selected to sort approximately half of the cells that expressed the highest staining intensity and the 25% of the cells that expressed the lowest staining intensity. Sorted cells were counted to calculate the exact ratio of sorting in each experiment. The quality or sorting was verified by determining the distribution of CR1/E in the sorted fractions by a second flow cytometric analysis.

Glucose-6-phosphate dehydrogenase activity

Glucose-6-phosphate dehydrogenase (G6PDH) activity was determined in the sorted fractions using a photometric assay measuring the G6PD-dependent transformation of NADH to NAD (Boerhinger, Manheim, Germany).

Separation of erythrocytes according to cell density

Density separation of E was performed on continuous Percoll (Pharmacia-LKB, Uppsala, Sweden) gradients according to a published method [26] with the following changes: blood samples were collected into heparin, diluted 4 to 1 in PBS containing 5 mM diisopropylfluorophosphate and passed through columns containing equal amounts of alpha cellulose and microcrystalline cellulose in order to remove leucocytes [27]. Filtered cells were collected in PBS containing 10 mM EDTA, pelleted at 4°C and one volume of packed cells was mixed with 9 volumes of a Percoll solution that had a density of 1·10 g/l, an osmolality of 320 mosmol/kg in PBS containing 1 g/l D-glucose and 0·5 mM EDTA at pH 7·4. The suspension was centrifuged for 20 min at 40 000 ***g***. Separation E exhibited a discrete and characteristic banding pattern. The lower bands of the gradient contained older E of higher densities whereas the highest band contained almost pure reticulocytes. Six distinct fractions were recovered from each gradient. Separated fractions were washed three times with PBS containing 1 g/l D-glucose and 0·5 mM EDTA. Samples of fractionated and washed cells were run a second time on new gradients in small tubes at a haematocrit of 2% in 6 ml (15 min at 13 000 ***g***) to assess whether cells maintained their density or redistributed. Reruns further served to determine the relative densities of fractionated samples. A photographic picture was taken to determine the R_F value of banded E [26]. A relationship between the age of E and the density of the cells in separated fractions was established by measuring the creatine content of cells in each fraction [28]. Density-separated E from each fraction were analysed for CR1 (CD35) expression by flow cytometry. The kinetic pattern of the loss of CR1/E with cell ageing was analysed using the Grafit® Software (Leatherbarrow R.J. 1989. Graft Erythracus Software, Staines, UK).

CR1 (CD35) expression on reticulocytes

The reticulocyte-enriched fraction was the upper fraction from continuous self-generated Percoll gradients of 1·086 density in saline solution, recovered by ultracentrifugation at 19 000 ***g*** for 20 min. After washing cells with PBS containing 1% AB serum, 0·1% NaN_3, cells were incubated with biotinylated anti-CR1 (CD35) antibody J3D3 for 45 min, washed three times with PBS-BSA NaN_3, and incubated with phycoerythrin-conjugated streptavidin and FITC-conjugated anti-transferrin receptor (CD71) antibody (Immunotech, Marseille, France). Stained cells were washed with PBS-BSA-NaN_3 and fixed in buffer containing 0·1% formaldehyde. Cells were analysed using a Facstar flow cytometer with an argon laser emitting at 488 nm and conventional filter sets and fluorescence compensations for dual-labelling analysis. The window of analysis for reticulocytes was defined by using light-scattering parameters and by determining the cells which simultaneously stained with anti-transferrin receptor and anti-CR1 (CD35) antibodies.

Determination of half lives of erythrocytes in vivo

The half-lives of E expressing high and low numbers of CR1/cell were simultaneously assessed in four patients with SLE. Cells from normal donors expressing 800 CR1/E and cells from donors expressing approximately 200 CR1/E were labelled with

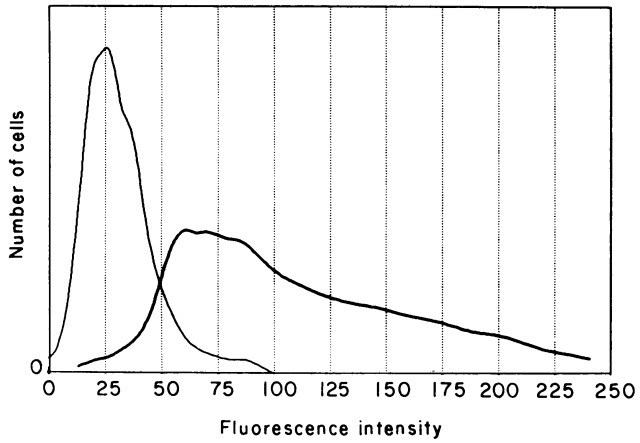

Fig. 1. Number of CR1 (CD35) antigenic sites per cell (CR1/E) on erythrocytes from a normal individual, following sorting of cells stained with anti-CR1 antibody, according to high and low expression of CR1. Sorted erythrocytes were re-analysed for CR1 (CD35) expression by cytofluorometry. Left curve: sorted cells expressing low CR1 (CD35) numbers. Right curve: sorted cells expressing high CR1 (CD35) numbers.

^{51}Cr and ^{111}In, respectively. The half lives of autotransfused ^{51}Cr and ^{111}In-labelled E were determined in healthy individuals. Donors were regular volunteer blood donors of blood group O Rh d. Two ml of labelled red cells (50 μCi) from each type were injected into patients. Blood samples were then obtained from the patients every day for the first week and every second day for 2 weeks. Gamma radiation counting windows and compensating systems were set to allow simultaneous assessment of ^{51}Cr and ^{111}In activities.

RESULTS

Peripheral catabolism of CR1 (CD35) in healthy individuals is more intense on erythrocytes expressing high numbers of CR1/cell

Erythrocytes from six healthy donors were stained with anti-CR1 (CD35) MoAb and sorted according to the quantitative expression of CR1 (CD35) antigen on the cells (Fig. 1). The relative cell age of the half of the cells that expressed the highest staining intensity and of the 25% of the cells that expressed the lowest staining intensity was then determined by measuring G6PDH activity in sorted cells. Cells sorted for low expression of CR1 (CD35) contained low amounts of G6PDH activity as compared with cells sorted for high CR1 (CD35) expression, indicating that aged E express lower numbers of CR1 (CD35) per cell than younger cells (Table 1). The slight decrease in cell volume which occurs with cell ageing could not account for the differences in G6PDH activity that were observed between cells expressing high and low numbers of CR1 (CD35). The ratio between the E G6PDH activity in the high CR1/E and in the low CR1/E sorted fractions, was directly related to the mean number of CR1/E in unfractionated E from each donor ($r = 0{\cdot}84$).

To further assess the relationship between the number of CR1/E and cell ageing, we measured the expression of CR1 (CD35) on density-fractionated E from five normal individuals. A sample of density-separated E was recentrifuged on self-forming Percoll® gradients to verify that fractionated cells maintained their density and did not redistribute. Fractions of lowest density contained the cells with the highest creatine content (data not shown). Density-separated E exhibited decreasing numbers of CR1/E with increasing cell density (Fig. 2). The relationship between the expression of CR1/E and cell age fitted a model of an exponential decay associated with an offset. The half-life of CR1 on E was calculated from the data of Fig. 2 using the following equations: $Y = a \cdot e^{-bx} + k$ and $T_{1/2} = \ln 2/b$, where Y (number of CR1 antigenic sites) and X (relative migration through the gradient) were variables, a, b, and k were parameters, and e the symbol for 'exponential'.

The half-lives of CR1 on E ranged between 11 and 32 days in healthy individuals. The half-life of CR1 on E was inversely related to the mean number of CR1/E in unfractionated cells from each donor ($r = 0{\cdot}92$) (Fig. 3).

Table 1. Glucose-6-phosphate dehydrogenase (G6PDH) activity in erythrocytes sorted for high and low CR1 (CD35) expression (normal individuals)

Mean number of CR1/E	G6PDH activity*		CR1/E genotype†
	Low CR1 fraction	High CR1 fraction	
1100	45	115	HH
726	68	106	HH
691	71	119	HH
539	80	160	LH
314	166	264	LH
180	150	191	LL

* U/10^6 erythrocytes.

† As determined from the *Hin*d III CR1-1 RFLP.

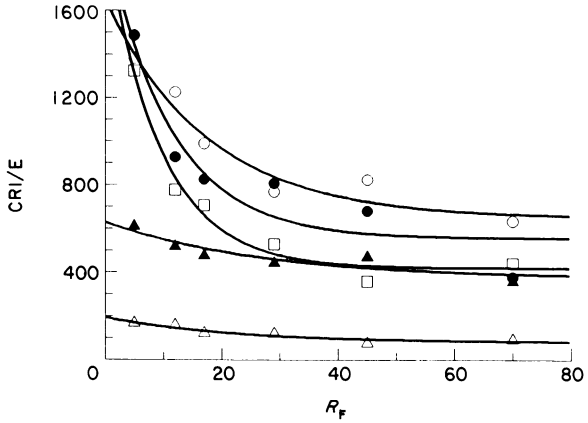

Fig. 2. Relationship between the number of CR1/E and the relative density of cells separated on continuous self-forming Percoll® gradients in five normal individuals. Each symbol denotes a single individual. The density of erythrocytes in separated fractions was estimated from their relative migration in the gradient and expressed as R_F values. R_F values were calculated by dividing the distance of migration by the length of the gradient and multiplying the ratio by 100. The first fraction on the left of the graph predominantly contained reticulocytes.

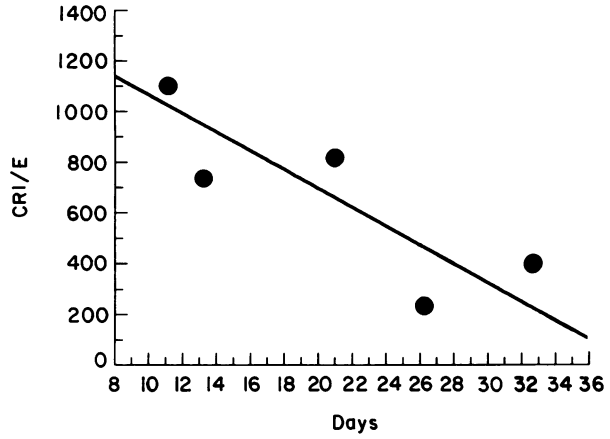

Fig. 3. Relationship between the half-life of CR1 (CD35) on E and the mean number of CR1/E in healthy individuals.

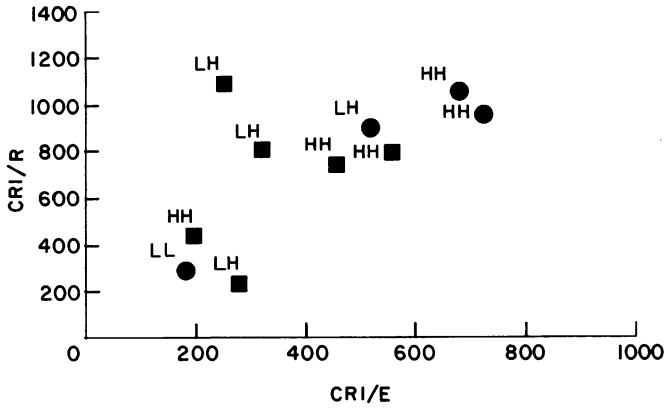

Fig. 4. Relationship between the mean number of CR1 (CD35) on reticulocytes (CR1/R) and on erythrocytes (CR1/E) in healthy individuals (●) and in patients with SLE (■). Analysed by cytometry on cells separated by their light scattering properties and expression of CD71, as described in Materials and Methods. The CR1/E genotype was determined by analysis of the Hind III CR1.1 polymorphism.

CR1 (CD35) expression on reticulocytes was quantified using dual labelling with anti-CD71 and anti-CR1 MoAbs and flow cytometry analysis. Mean numbers of CR1/R were compared with mean numbers of CR1/E. The mean number of CR1/R was directly related to that of CR1/E ($r = 0.95$) (Fig. 4).

Taken together, these results indicate that the number of CR1/E decrease with ageing of the cells in normal individuals and that the relative decrease in expression of CR1 (CD35) with cell ageing is more pronounced in individuals in whom circulating E express high numbers of CR1/E.

Peripheral catabolism of CR1 (CD35) on erythrocytes from patients with SLE

The expression of CR1 (CD35) on cells from most patients was lower than would have been predicted from their genotype (Table 2).

Table 2. Half-life of CR1 on erythrocytes from healthy individuals and patients with SLE*

Individuals	$T_{1/2}$ CR1 (RF units)	$T_{1/2}$ CR1 (days)	Mean number of CR1/E†	Quantitative CR1 Genotype
SLE	5·88	10·29	246	HH
Normal	6·37	11·14	1100	HH
SLE	6·89	12·06	158	HH
Normal	7·56	13·23	734	HH
SLE	8·12	14·22	239	LH
SLE	10·30	18·02	220	HH
SLE	11·03	19·31	280	HH
Normal	12·00	21·00	814	HH
Normal	15·09	26·40	180	LL
SLE	16·77	29·34	1289	HH
Normal	18·71	32·74	459	LH

* Half lives (RF units) were calculated from the data presented in Figs 2 (normal individuals) and 5 (SLE patients) using the equations described in Results. $T_{1/2}$ (days) were calculated from $T_{1/2}$ (RF units) assuming that one RF unit corresponds to 1·75 days of erythrocyte life time.

† Unfractionated erythrocytes.

E from patients with SLE were sorted according to high and low expression of CR1/E and G6PDH activity was then measured in the sorted fractions. The same linear relationship was observed between the number of CR1/E and the ratio of G6PDH activity between cells in the sorted fractions as that found in healthy individuals (data not shown).

E from eight SLE patients were analysed for CR1 (CD35) expression following density separation on self-generated continuous Percoll® gradients. In two patients expressing less than 100 CR1/E in unfractionated E, density-separated cells exhibited low numbers of CR1/cell in all fractions obtained from the gradients, including the fraction containing the youngest E. In six remaining patients, CR1 (CD35) expression decreased on cells of increasing cell density, as it had been observed in normal individuals (Fig. 5). The relative decrease in CR1/E with cell ageing was less pronounced on E from patients with low numbers of CR1/E in unfractionated E. Thus, the relationship between the relative cell age and expression of CR1 on E in individuals with SLE is independent of the CR1.1 Hind III polymorphism.

The half-lives of CR1 on E calculated in six patients, ranged between 11 and 29 days. In five of the six patients with low number of CR1/E, the half-lives of CR1 were in a range similar to that which had been found for CR1 (CD35) on E from healthy individuals homozygous or heterozygous for the high quantitative CR1 genotype (Table 2) (Fig. 6).

CR1 (CD35) expression was measured on reticulocytes (R) from six of the SLE patients. The mean number of CR1/R was directly related to that of CR1/E in five of the patients, as was found in healthy individuals (Fig. 4). In the sixth patient (patient M.V.), the mean number of CR1/R was higher than that which would have been expected from the analysis of healthy individuals, suggesting that enhanced peripheral catabolism of CR1 (CD35) was present (Fig. 4). The numbers of CR1/R and CR1/E were linearly related, except for patient M.V.

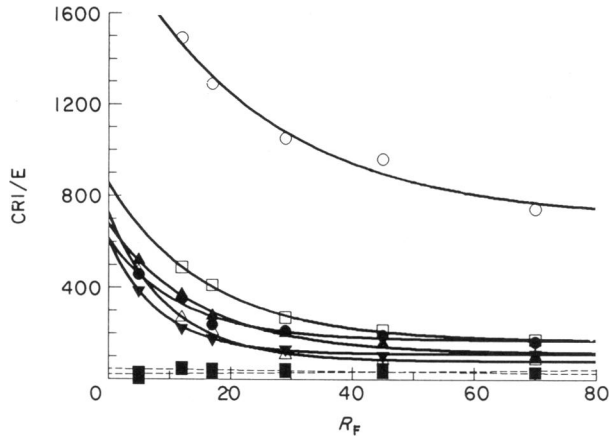

Fig. 5. Relationship between the number of CR1/E and the relative density of cells following separation on continuous self-forming Percoll® gradients. Patients with SLE. Results are expressed as in Fig. 2. The fractions containing cells of lowest density were not available for analysis in two of the patients due to the presence of cellular debris.

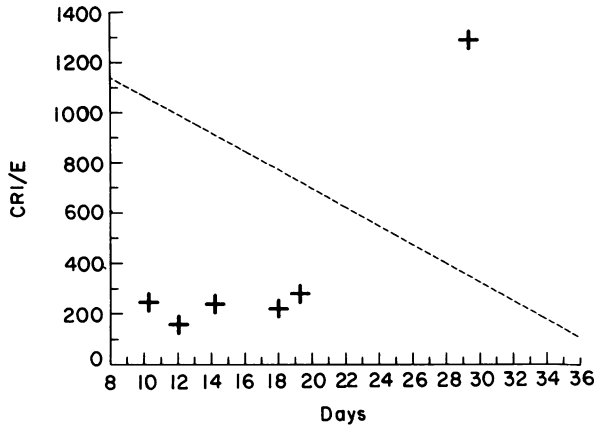

Fig. 6. Relationship between the half-life of CR1 (CD35) on E and the mean number of CR1/E in patients with SLE. The dotted line indicates the relationship that was observed in healthy individuals (Fig. 3).

Half life of erythrocytes expressing high or low numbers of CR1/ cell transfused into normal individuals and patients with SLE

E from three normal individuals which expressed a mean number of 180, 540 and 820 CR1/E respectively, were labelled with ^{51}Cr or ^{111}In and simultaneously autotransfused in order to obtain reference values for half-lives of ^{51}Cr and ^{111}In-labelled E in healthy individuals infused with both isotopes. The mean half-life of ^{51}Cr-labelled E was 27 ± 2 days. The half life of ^{111}In-labelled E was 8 ± 1 days. To investigate whether the half-life of E depends on the relative expression of CR1/E in patients with SLE, four SLE patients were transfused with ^{51}Cr-labelled E expressing 800 CR1/E simultaneously with ^{111}In-labelled E expressing 200 CR1/E obtained from two Rh compatible healthy individuals. Single order decay was observed for ^{51}Cr in three of the patients during 3 weeks of observation following infusion. In one of the patients, the decay of ^{51}Cr was biphasic with a second phase of accelerated decay occurring from the third week after transfusion. ^{111}In radioactivity was detected in

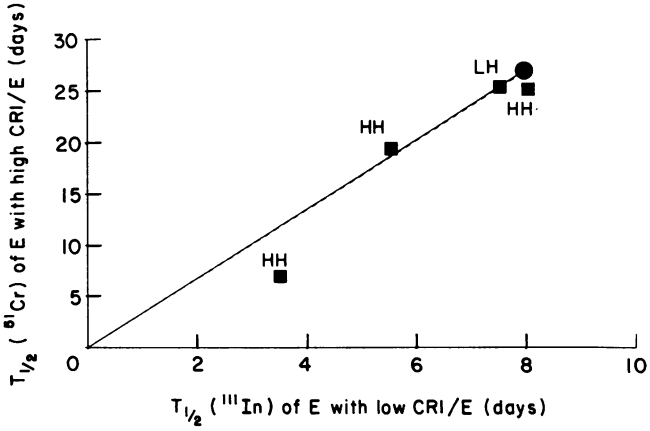

Fig. 7. Half-life of erythrocytes expressing high and low numbers of CR1/E simultaneously transfused into patients with SLE. Erythrocytes from a normal donor expressing 800 CR1 (CD35) sites/E were labelled with ^{51}Cr. Erythrocytes from a donor expressing 200 CR1 (CD35) sites/E were labelled with ^{111}In. The ordinate and the abcissa depict the half-lives of ^{51}Cr-labelled and of ^{111}In-labelled cells respectively. Closed squares depict patients with SLE. The circle depicts the half-life of ^{51}Cr-labelled and of ^{111}In-labelled autologous erythrocytes re-infused into three normal individuals. The CR1/E genotype was determined by analysis of the *Hin*dIII CR1.1 RFLP.

peripheral blood for 8 days. The half-lives of transfused E were similar to those observed in normal individuals in two of the patients (Fig. 7). The patients exhibited no overt haemolytic anemia. In two other patients who exhibited ongoing haemolysis, the half-lives of ^{51}Cr-labelled and of ^{111}In-labelled transfused E were decreased proportionally (Fig. 7). Both patients were of the HH CR1.1 genotype. Thus, E expressing high numbers of CR1 are not preferentially removed from the circulation in patients with SLE and autoimmune haemolytic anaemia.

DISCUSSION

The present study investigated the rate of catabolism of CR1 (CD35) on circulating E *in vivo* in relationship with the expressed number of CR1/E, the CR1.1. *Hin*dIII polymorphism and cell age in healthy individuals and in patients with SLE.

We investigated the relationship between the number of CR1/E and cell age by two reciprocal approaches: one analysed the age of E sorted according to CR1 (CD35) expression; the other, analysed CR1 (CD35) expression on young and aged cells separated according to cell density. In the first series of experiments, E were sorted into fractions expressing high and low numbers of CR1/E and analysed for their content in G6PDH activity. In healthy individuals, aged E containing low amounts of G6PDH activity were consistently found to express low numbers of CR1/E. The difference between the relative content in G6PDH activity of E expressing high and low numbers of CR1/E in a given individual was greater in individuals expressing higher numbers of CR1/E in unfractionated cells. A decrease in the number of CR1/E with cell ageing has previously been shown by Ripoche and Sim [29]. By using density separation on continuous self-forming Percoll® gradients, we were able to determine the kinetics of CR1 (CD35)

catabolism and calculate the half-life of CR1 (CD35) on E. The decay of CR1 (CD35) on E followed a pattern associating an exponential decay and an offset which differed between individuals. Calculated half lives of CR1 (CD35) on E ranged between 11 and 32 days in healthy individuals. The shortest half-lives were observed in individuals expressing high numbers of CR1/E in unfractionated E. Comparative analysis of CR1 (CD35) expression on E and reticulocytes indicated that the mean number of CR1 (CD35) on R was linearly related to that of CR1 (CD35) on E in healthy individuals. These results provide quantitative data on CR1 (CD35) catabolism on E *in vivo* and indicate that the peripheral catabolism of CR1 (CD35) is not the mechanism that governs the genetically-determined quantitative expression of the molecule on E.

The catabolism of CR1 (CD35) was also investigated in 16 patients with SLE by one or several of the methodologies that had been used for the analysis of CR1 (CD35) in healthy individuals. Fifteen of the patients expressed lower numbers of CR1/E than would have been expected from their CR1 Hind III RFLP quantitative genotype. Several lines of evidence indicated that in most patients enhanced peripheral catabolism of CR1 (CD35) on E may not be the only mechanism explaining the decrease in the number of CR1/E: (1) in three patients whose E were sorted according to high or low expression of CR1 on E, the same relationship between CR1 (CD35) expression and the relative content in G6PDH of E was found as that which had been observed in normal individuals; (2) cells of two patients expressed very low numbers of CR1 (CD35) (i.e. below 100/cell) even in fractions of highest density containing the youngest cells; (3) the half-life of CR1 (CD35) on E of patients with lower numbers of CR1/E than would have been expected from their genotype was within the same range as that of healthy individuals who were homozygous or heterozygous for the high quantitative CR1 genotype. The half-life of CR1 (CD35) on E from these patients could not be calculated; (4) reticulocytes from five of six patients with SLE that were analysed, expressed low numbers of CR1/R that were directly related to the number of CR1 (CD35) expressed on E; (5) experiments in which Rh compatible E expressing high and low numbers of CR1/E were transfused into patients with SLE, showed that half-lives of transfused cells were similar to those observed in healthy individuals in two SLE patients without overt haemolytic anaemia, and a proportional shortening in the half-lives of transfused cells expressing high and low numbers of CR1/E in two other patients in whom haemolytic anaemia was present. Thus, the clearance of cells expressing high numbers of CR1/E is not specifically enhanced in patients with SLE and with haemolytic anaemia.

In one patient whose clinical presentation did not differ from the other patients and in whom no overt haemolytic anaemia was present, high numbers of CR1/R were present with low numbers of CR1/E, indicating the presence of a predominant peripheral catabolism of CR1 (CD35) as a possible mechanism for the decreased expression of CR1 (CD35) on E.

An accelerated catabolism of erythrocytic CR1 (CD35) in SLE has previously been suggested by the observation of reduced numbers of CR1/E on E transfused into two patients with SLE and warm haemolytic anemia [21].

Results from the present study suggest that the rate of CR1 (CD35) catabolism on E in patients with SLE corresponds to that which may be expected from the quantitative CR1 (CD35) geotype of the patients. Thus, an enhanced peripheral catabolism of CR1 (CD35) is unlikely to be the sole or major mechanism for the acquired loss of CR1 (CD35) on E in patients with SLE expressing low numbers of CR1/E. A decreased expression of CR1 (CD35) on neutrophils and B lymphocytes has been described in patients with SLE [30]. The data are compatible with the hypothesis that an altered biosynthesis and/or membrane expression of CR1 (CD35) in bone marrow cells may contribute to the acquired decrease in the expression of CR1 (CD35) on peripheral blood cells in SLE.

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM) and the Agence Nationale de Recherches sur le SIDA (ANRS), France. We wish to thank Ms F. Bougy, F. Philbert, B. Reveil, and M. Champagne for expert technical assistance.

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