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Loss of complement receptor type 1 (CR1) on ageing of erythrocytes

Studies of proteolytic release of the receptor

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Complement receptor type 1 (CR1) is a glycoprotein of M_r about 250000 present on erythrocytes and other cell types. CR1 acts as a cofactor in the factor I-mediated breakdown of complement fragment C3b to form iC3b. Using an assay of cofactor activity, a wide variation in mean CR1 levels between erythrocytes from individual donors is observed. CR1 levels also decrease on ageing of erythrocytes *in vivo*, and again the rate of loss is widely variable between individuals. However, variable loss of CR1 during ageing of erythrocytes is likely to make only a minor contribution to the observed variation in mean CR1 levels. CR1 is very sensitive to proteolysis, and random proteolytic removal of CR1 from erythrocytes is likely to be an important factor in loss of CR1 on ageing of red cells *in vivo*. *In vitro*, mild trypsin treatment, plasmin or thrombin digestion of erythrocytes results in the loss of the factor I cofactor activity from the cell surface, and appearance of this activity in the supernatant. We conclude that an active fragment of CR1 is released from the cell surface on proteolysis. Subsequent prolonged trypsin treatment destroys most of the activity of this fragment. Proteolytic removal of CR1 from red cells may account not only for loss on ageing of cells, but also for the acquired CR1 deficiencies observed by others in systemic lupus erythematosus.

INTRODUCTION

The proteolytic degradation of fluid-phase or surface-bound C3b is a key step in the control of the activation of complement system (for review see Reid, 1983). C3b is degraded by the di-isopropylfluorophosphate-insensitive serine proteinase factor I (Harrison & Lachmann, 1980; Sim *et al.*, 1981*a*). The resulting product, iC3b, no longer forms the alternative pathway C3/C5 convertase. Proteolysis of C3b by factor I requires the presence of a cofactor protein which is thought to form a complex with C3b: only C3b which is in the form of a C3b–cofactor complex is efficiently cleaved by factor I. Two proteins have been described which act as cofactors for the factor I-mediated cleavage of C3b. These are the serum protein factor H (Whaley & Ruddy, 1976; Pangburn *et al.*, 1977; Harrison & Lachmann, 1980), and a glycoprotein termed complement receptor type 1 (CR1) present on the surface of human erythrocytes and a variety of nucleated cells. This protein has been identified as being the receptor for C3b or the immune adherence receptor (Fearon, 1979, 1980). The breakdown of C3b to iC3b in the presence of factor I and cofactor involves the sequential proteolysis of two peptide bonds in the α' chain of C3b (M_r 108000), leading firstly to the formation of M_r 68000 and 46000 fragments, followed by the loss of M_r 3000 fragment from the *N*-terminus of the M_r 46000 fragment (Harrison & Lachmann, 1980; Sim *et al.*, 1981*a*; Davis & Harrison, 1982). Whether factor I and CR1 are involved in the further degradation of iC3b to fragments C3dg and C3c in physiological conditions is still controversial (Yoon & Fearon, 1985), the use of appropriate proteinase inhibitors seeming to be a crucial point in this study (Malhotra & Sim, 1984).

Erythrocyte CR1 is a polymorphic glycoprotein with at least four variants of M_r ranging from 160000 to 260000 (Dykman *et al.*, 1985; Wong *et al.*, 1983). These variants do not appear to differ significantly in their factor I cofactor activity (Holers *et al.*, 1984). The mean quantity of CR1 (or of immune adherence receptor) per erythrocyte has been shown by several techniques [e.g. radioimmunoassay with polyclonal or monoclonal antibodies (Iida *et al.*, 1982; Wilson *et al.*, 1982; Walport *et al.*, 1985), binding of dimeric C3b (Arnaout *et al.*, 1981) or immune adherence assays (Klopstock *et al.*, 1965; Miyakawa *et al.*, 1981)] to vary very widely among normal individuals. Fearon and colleagues have suggested that there is a bimodal distribution of erythrocyte CR1 levels in individuals, and that CR1 levels are genetically determined. They postulated that CR1 levels per erythrocyte were controlled by codominant alleles at a single locus (Wilson *et al.*, 1982). Other studies also suggest inheritance of CR1 levels (Klopstock *et al.*, 1965), but under polygenic control (Walport *et al.*, 1985).

It has been demonstrated that individuals suffering from systemic lupus erythematosus (SLE) have lowered erythrocyte CR1 levels. This was initially considered likely to be a genetically determined primary defect in SLE (Miyakawa *et al.*, 1981; Wilson *et al.*, 1982). Further studies, however, suggest that, in at least some SLE cases,

Abbreviations used: DGVB^{++}, 140 mM-glucose/71 mM-NaCl/2.5 mM-sodium 5,5-diethylbarbiturate/0.5 mM-$MgCl_2$/0.15 mM-$CaCl_2$, pH 7.5, containing lg of gelatin per litre; SLE, systemic lupus erythematosus. The NIH unit of thrombin activity is defined as follows: 1 unit will clot a 2.5 mg/ml fibrinogen solution in 15 s at 37 °C. The nomenclature of complement components and fragments is as recommended by the World Health Organisation (1968, 1981).

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the partial CR1 deficiency is acquired (i.e. a secondary defect) (Holme *et al.*, 1986; Ross *et al.*, 1985; Walport *et al.*, 1985).

It had earlier been reported that CR1 levels per erythrocyte decrease on ageing of erythrocytes *in vivo* (Wilson *et al.*, 1982; Sim *et al.*, 1983). It appeared likely that this phenomenon of loss of CR1 on ageing might have similarities to a mechanism of acquired loss of CR1 in SLE. We report here more detailed studies of the loss of CR1 on ageing of erythrocytes, together with an investigation of one mechanism, namely proteolysis, by which this might occur. Assays for CR1 were performed throughout by determining its factor I-cofactor activity (Sim & Sim, 1983), since this sensitive method is capable of detecting highly degraded, as well as intact, CR1 (Sim, 1985).

MATERIALS AND METHODS

Materials

Trypsin (type XI), soya bean trypsin inhibitor (type I-S), human thrombin (product no. T-6759), 1,10-phenanthroline, pepstatin A, di-isopropylfluorophosphate and kits for the assays of haemoglobin by the method of Drabkin & Austin (1935) and for the assay of glutamate:oxaloacetate transaminase by the method of Reitman & Frankel (1957) were purchased from Sigma Chemical Co., Poole, Dorset, U.K. Iodoacetamide was from Koch-Light, Colnbrook, Bucks., U.K., and Iodogen (1,3,4,6-tetrachloro-3,6-diphenylglycouril) was from Pierce & Warriner, Chester, U.K. $Na^{125}I$ (carrier-free) was from Amersham International, Amersham, Bucks., U.K. Human plasmin was purchased from AB KABI, Stockholm, Sweden.

C3 was purified from human plasma as described previously (Hammer *et al.*, 1981; Sim *et al.*, 1981*b*) and C3b was prepared by limited trypsin digestion of C3 (Sim & Sim, 1981). C3b was radioiodinated with ^{125}I by using Iodogen (Fraker & Speck, 1978) as described before (Sim & Sim, 1981). Factor I was prepared as described by Hsiung *et al.* (1982).

Erythrocyte preparation

Blood samples were obtained from healthy volunteer donors by venepuncture, or were kindly provided, in the form of plasma-reduced blood, by the Oxford Regional Blood Transfusion Service, John Radcliffe Hospital, Oxford. Additional plasma-reduced blood samples from heterozygous and homozygous C3-deficient individuals were supplied by Dr. J. J. Roord and Professor J. H. Edwards (Roord *et al.*, 1983).

Blood cells were washed five times by centrifugation (10 min, 2000 *g*) in 20 vol. of 0.15 M-NaCl, and the buffy coat was carefully removed by aspiration after each wash. The erythrocytes were then washed and resuspended in Alsever's solution (per litre: 8.0 g of trisodium citrate dihydrate, 19.0 g of D-glucose, 4.2 g of NaCl, 0.5 g of citric acid monohydrate) and passed through nylon wool (Cellselect Leukocyte Filters; Lorne Laboratories, Maidenhead, Berks., U.K.) to remove final traces of white cell material. Removal of white cell material was monitored by: (i) light microscopy, which indicated removal of at least 95% of leukocytes, and (ii) assay of leukocyte elastase (Wenzel *et al.*, 1980) in the detergent lysate (Sim, 1985) of the cells. This activity was not detectable in the final preparation. Erythrocytes were used for assay within 3 days after being collected. Erythrocytes were counted with a haemocytometer.

Separation of erythrocytes into pools of different ages

Erythrocytes from each of 14 individual volunteer donors were separated by centrifugation into five pools of different ages, using a procedure based on those of Kadlubowski & Agutter (1977) and Murphy (1973).

Erythrocytes prepared as above were suspended at 9×10^9/ml in 0.15 M-NaCl. Two portions, of 4.5 ml, of this suspension were centrifuged at 30000 *g* for 1 h at 30 °C in a Beckman SW50 rotor. The resulting cell column was separated into five fractions by peristaltic pumping from the bottom of the tube. Fractions were designated I (bottom, 'oldest') to V (top, 'youngest'). The cell concentration in each fraction was established by haemocytometer counting, and the cells were resuspended in Alsever's solution at 1×10^9 cells/ml. To establish that age-dependent fractionation had been achieved, cells in each fraction were assayed for haemoglobin and glutamate:oxaloacetate transaminase, as described by Kadlubowski & Agutter (1977). The mean cell haemoglobin concentration was found to increase regularly from the 'youngest' to 'oldest' pool with a mean difference of 9.5% between fractions I and V. Glutamate:oxaloacetate transaminase activity decreased regularly with age, and the activity in fraction I was on average 36% lower than in fraction V. These results are in accordance with those of Kadlubowski & Agutter (1977).

Treatment of erythrocytes with proteinases

Erythrocytes, 2.5×10^8/ml in $DGVB^{++}$, were incubated with trypsin, thrombin or plasmin at 37 °C. The reaction was stopped at appropriate times by adding an equal volume of either (i) $DGVB^{++}$ containing a 5-fold molar excess over proteinase of soya bean trypsin inhibitor for stopping trypsin or plasmin digestion, or (ii) $DGVB^{++}$ containing 10 mM-di-isopropylfluorophosphate for stopping thrombin digestion. The cells were centrifuged (2000 *g* for 10 min at 4 °C), the supernatant was retained for assay, and the pellets were washed four times by centrifugation as above with 20 vol. of $DGVB^{++}$. Where indicated, the whole mixture of erythrocytes plus supernatant was retained for assay.

Assays of CR1

CR1 was assayed by measuring its capacity to act as an obligatory cofactor in the factor I-mediated proteolytic degradation of soluble C3b to form iC3b. CR1 activity was detected semi-quantitatively on cells or in supernatants by incubating erythrocytes or supernatants from proteolysis together with factor I and ^{125}I-labelled C3b for a fixed time at 37 °C. Samples were then analysed by SDS/polyacrylamide-gel electrophoresis and autoradiography, as described below, and the extent of C3b degradation was calculated by measuring the ^{125}I radioactivity associated with the C3b α' chain. For full quantification of CR1 activity on cells or in supernatants, a kinetic assay was done, in which the initial rate of degradation of C3b α' chain was determined in conditions where this rate is proportional to the concentration of cofactor present (Sim *et al.*, 1981*a*; Sim & Sim, 1983). To establish each time-point in the assay, erythrocytes (20–50 μl containing 10^7–10^8 cells) in $DGVB^{++}$, or supernatants from proteolysis (20–50 μl in

$DGVB^{++}$) were incubated with ^{125}I-labelled C3b (0.5 μg, 100000 c.p.m.) and 0.4 μg of factor I for appropriate times at 37 °C.

To avoid non-specific proteolysis which might result from the release of erythrocyte proteinases, all assays were done in the presence of proteinase inhibitors as follows: di-isopropylfluorophosphate (0.25 mM final concentration); iodoacetamide (5 mM), soya bean trypsin inhibitor (100 μg/ml); 1, 10-phenanthroline (0.2 mM) and pepstatin A (1 μg/ml). The reaction was stopped by adding an equal volume of 2% (w/v) SDS/8 M-urea/0.2 M-Tris/HCl, pH 8.0, containing 40 mM-dithiothreitol (Sigma). After reduction, samples were analysed by SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970). The gels were stained, destained, dried and autoradiographed as described before (Sim & Sim, 1983). The cofactor activity of the different samples tested was quantified as before (Sim *et al.*, 1981*a*; Sim & Sim, 1983). The initial rate of degradation of the α' chain of ^{125}I-labelled C3b was determined graphically by plotting the percentage decrease of the α' chain radioactivity as a function of time.

Effect of clotting of blood

For the comparison of the factor I cofactor of CR1 on human erythrocytes before and after blood clotting, 10 ml of blood was taken from a single donor and divided into three portions. One portion was immediately diluted in 1 vol. of Alsever's solution, and the other two portions were allowed to clot at room temperature for 1 h. One of the clotted samples was then incubated for 12 h at 37 °C to allow clot retraction to occur. To extract erythrocytes, clots were washed in $DGVB^{++}$, and gently dissociated. Erythrocytes from clotted and non-clotted samples were washed, passed through nylon wool as described above, and adjusted to 2.5×10^8 cells/ml.

RESULTS

Distribution of CR1 levels on erythrocytes from individual donors, as assessed by cofactor activity assay

The variation in factor I-cofactor activity of erythrocytes from 67 individual donors is shown in Fig. 1. A wide spread of values for cofactor activity/cell is observed, ranging from 10% up to 170% of the arithmetic mean. This range and pattern of variation is similar to those observed with measurement of CR1 by labelled antibody techniques (Iida *et al.*, 1982; Walport *et al.*, 1985), although there is not a pronounced bimodal distribution such as was observed by Wilson *et al.* (1982). Three homozygous C3-deficient individuals (Roord *et al.*, 1983) have CR1 levels in the middle of the range (Fig. 1).

Loss of CR1 activity on ageing of erythrocytes *in vivo*

Erythrocytes from 14 individuals were fractionated by centrifugation into age-related pools. The mean CR1 levels of the individuals chosen were spread across the entire range of variability shown in Fig. 1. For each individual it was found that the level of CR1 per erythrocyte, as measured by assays of cofactor activity, decreased regularly from the 'youngest' (fraction V) to

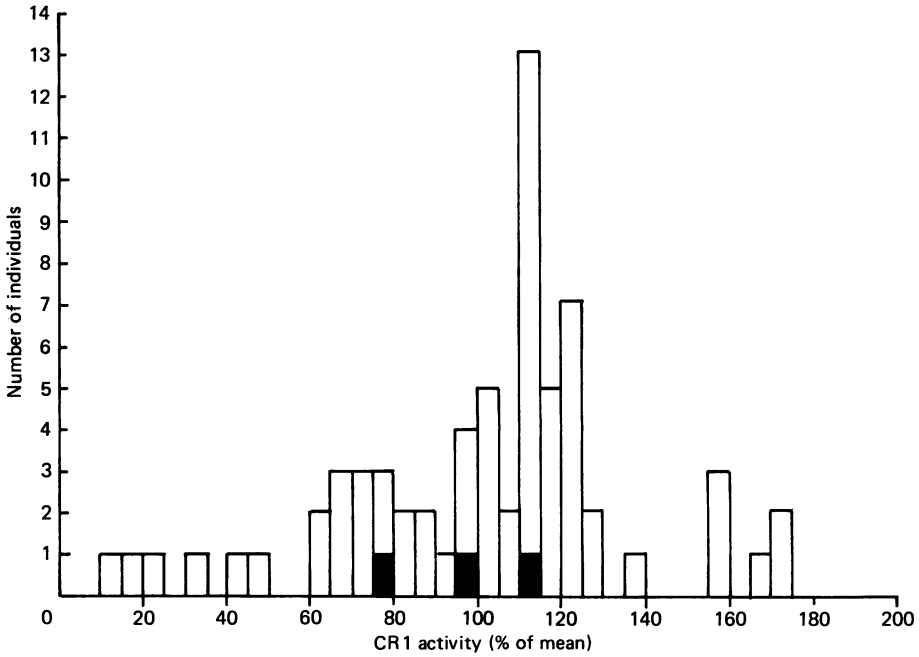

Fig. 1. Variability in CR1 activity on erythrocytes from individual donors

Erythrocytes were prepared from 67 individual blood samples as described in the Materials and methods section. The cofactor activity of CR1 was measured in each sample by determining the initial rate of breakdown of C3b α' chain by factor I in the presence of erythrocytes, as described in the Materials and methods section. Multiple samples (10^8 cells in 50 μl of $DGVB^{++}$) of erythrocytes from each individual were incubated for 0, 1, 2, 5, 10, 20 and 60 min at 37 °C with factor I plus ^{125}I-labelled C3b, and the samples analysed by SDS/polyacrylamide-gel electrophoresis and autoradiography, as described in the Materials and methods section. The initial rate of C3b α' chain cleavage was expressed as % cleavage/min per 10^8 cells. The arithmetic mean rate of cleavage was calculated, and the rate for each individual is expressed as a percentage of the mean. Solid areas represent the CR1 activity of three homozygous C3 deficient donors, as discussed in the text.

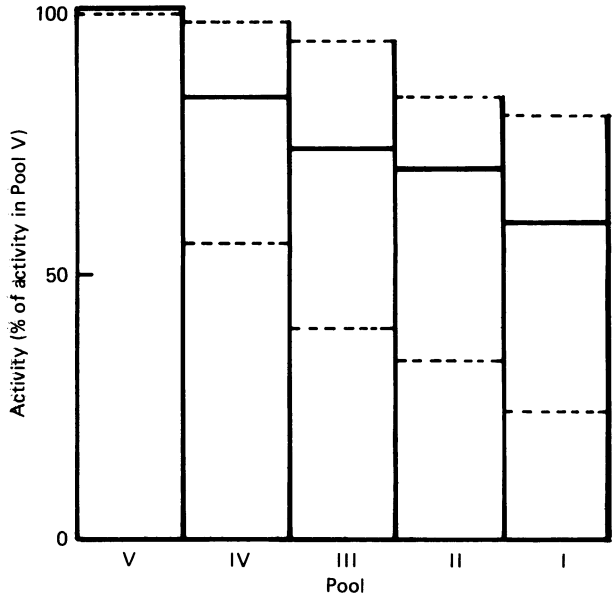

Fig. 2. Loss of CR1 activity on ageing of erythrocytes

Erythrocytes from 14 donors were separated into five age-related fractions as described in the Materials and methods section. The CR1 activity per 10^8 cells was measured as described in the Materials and methods section and in the legend to Fig. 1. For each individual, the activities in pools I–IV were expressed as a percentage of the activity in pool V. Pool I represents the oldest cells, pool V the youngest. The solid line represents the average result for the 14 donors, and broken lines represent the two individuals showing the highest and lowest percentage loss of CR1 activity, as discussed in the text.

the 'oldest' (fraction I) pool of cells (Fig. 2). The precentage difference between 'oldest' and 'youngest' cells also varied widely between individuals, and the two extremes of variations are shown in Fig. 2 to illustrate this.

Since CR1 is unusually susceptible to proteolysis (Sim, 1985), it was considered possible that the loss of CR1 from erythrocytes on ageing might occur by the action of proteolytic enzymes. A more detailed investigation of the fate of CR1 on proteolysis of erythrocytes was undertaken.

Removal of CR1 activity from erythrocytes on treatment with trypsin

Treatment of human erythrocytes with increasing concentrations of trypsin for a fixed time (30 min) at 37 °C results in progressive decrease of the CR1 cofactor activity detectable on the erythrocytes (Fig. 3). At concentrations of trypsin greater than 100 μg/ml, essentially all of the CR1 cofactor activity is removed from the cell. The cofactor activity of CR1 becomes readily detectable in the supernatant, even at very low trypsin concentrations (2 μg/ml; Fig. 3). At high trypsin concentrations, however, the cofactor activity of CR1 appears to be destroyed, both in the supernatant and on the erythrocytes. To eliminate the possiblity that the CR1 activity detectable in the supernatant represented CR1 associated with small membrane vesicles, the supernatants were assayed before and after centrifugation for 1 h at

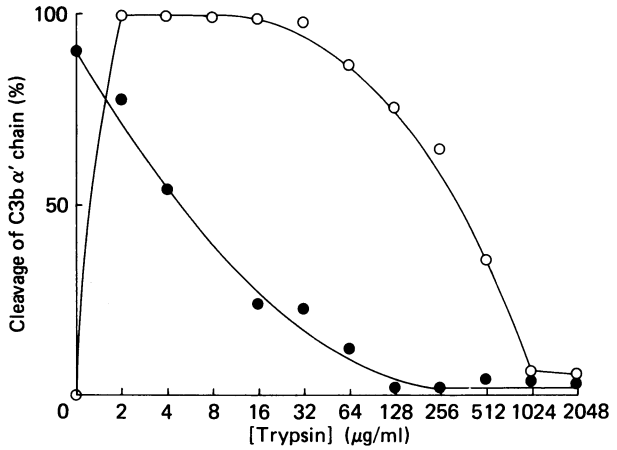

Fig. 3. Effect of increasing concentrations of trypsin on factor I cofactor activity of human erythrocytes

Erythrocytes (2.5×10^8) were incubated with increasing concentrations of trypsin (from 2 μg/ml to 2 mg/ml final concentration) in a total volume of 50 μl of DGVB^{++} for 30 min at 37 °C. Reaction was stopped by addition of 50 μl of DGVB^{++} containing a 5-fold molar excess of soya bean trypsin inhibitor over trypsin. Cells were pelleted by centrifugation at 2000 *g* for 10 min, the supernatant was removed and the cells were washed four times in DGVB^{++}. Cells were resuspended in 100 μl of DGVB^{++} and 20 μl (5×10^7 cells) was taken for assay of cofactor activity; 20 μl of the supernatant of trypsin-treated cells was used for assay. The semi-quantitative assay for detection of cofactor activity was performed as described in the Materials and methods section. All incubations were done for 90 min at 37 °C. The extent of cleavage of the α' chain of C3b was determined by measuring the percentage of the total radioactivity associated with the α' chain of ^{125}I-labelled C3b as described in the Materials and methods section. Control assays with no factor I were done to verify the specificity of the assay in detecting factor I cofactor activity. ●, Cofactor activity in the cell pellet; ○, cofactor activity in the supernatant. Trypsin concentration is shown on a logarithmic scale.

100000 *g*. Centrifugation did not alter the results. In each assay for cofactor activity on erythrocytes or in supernatants, a control with no factor I was included. No proteolytic cleavage of C3b was observed in the absence of factor I, confirming that the assays are specific for cofactor-dependent factor I cleavage.

The results shown in Fig. 3 were obtained by assessment of C3b α'chain cleavage at a single fixed time of incubation with cofactor and factor I. This is not a full quantitative measurement. In order to quantify the yield of cofactor activity released into the supernatant, erythrocytes were incubated for varying times with 50 μg of trypsin/ml, and the cofactor activity on the cells and in the supernatant was measured by determining the rate of cleavage of C3b α' chain (Fig. 4). CR1 cofactor activity is rapidly released from the cells, and appears in the supernatant. After 4 min approx. 50% of the original cofactor activity is still associated with the cells, and 50% is in the supernatant. Thereafter activity both in the supernatant and on the erythrocytes declines. The pattern of overall decrease in cofactor activity is consistent with studies on the proteolysis of isolated CR1 (Sim, 1985) where it was shown that initial cleavages of CR1

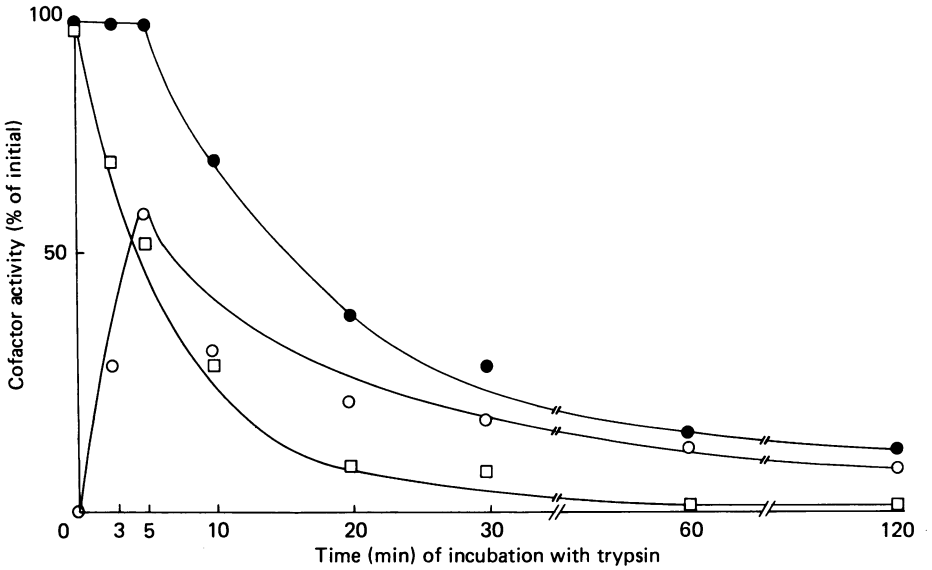

Fig. 4. Loss of factor I cofactor activity from erythrocytes and appearance in the supernatant on treatment with trypsin

Multiple samples of 2.5×10^8 erythrocytes were treated with trypsin (50 μg/ml final concentration) in a final volume of 50 μl of DGVB^{++} at 37 °C. At various times of incubation the reaction was stopped by adding 50 μl of DGVB^{++} containing a 5-fold molar excess of soya bean trypsin inhibitor over trypsin. The cells were pelleted, the supernatant was removed, and the cells were washed four times in DGVB^{++} and resuspended in 100 μl of DGVB^{++}. For some samples the whole digestion mixture of cells plus supernatant was conserved. A quantitative kinetic assay for cofactor activity was then performed as described in the Materials and methods section. For each sample (erythrocytes, supernatants or unseparated digestion mixture), five portions of 20 μl were incubated with factor I plus ^{125}I-labelled C3b for 0, 3, 5, 10, or 20 min at 37 °C, and the initial rate of C3b α' chain cleavage was assessed after analysis on SDS/polyacrylamide gels as described in the Materials and methods section. Cofactor activity of CR1 is expressed as a percentage of the activity present in the erythrocytes before trypsin treatment. □, Activity associated with cells; ○, activity in supernatant; ●, activity in unseparated cells and supernatant.

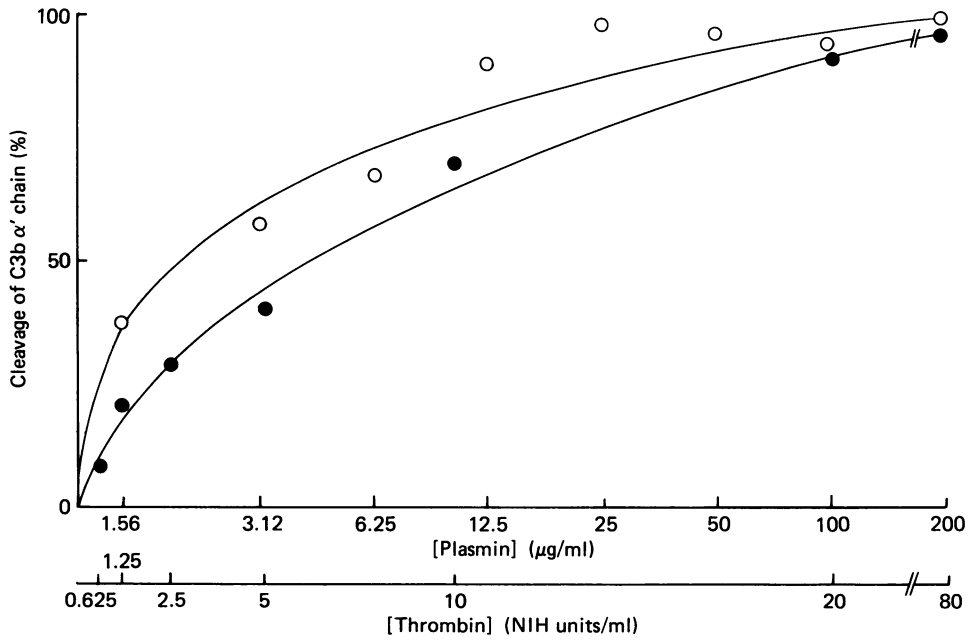

Fig. 5. Effect of plasmin and thrombin digestion: release of CR1 cofactor activity into the supernatant

Multiple samples of 2.5×10^8 erythrocytes were incubated in a final volume of 50 μl of DGVB^{++} containing either plasmin (0.75–200 μg/ml) or thrombin (0.62–80 NIH units/ml) for 2 h at 37 °C. The reaction was stopped as described in the Materials and methods section and the cells were pelleted. Cofactor activity was detected semi-quantitatively in the supernatants by incubating 20μl of the digest supernatants for 90 min at 37 °C with factor I and ^{125}I-labelled C3b as described in the Materials and methods section. The percentage cleavage of C3b by supernatants from plasmin (○) and thrombin (●) digests is indicated. Plasmin concentrations are on a logarithmic scale. Control assays were performed without factor I to verify the specificity of the assay.

by trypsin result in only a small decrease in cofactor activity, while prolonged digestion, which degrades CR1 to multiple fragments of M_r less than 25000, results in a loss of about 70% of the cofactor activity. The results shown in Fig. 4 are consistent with the efficient proteolytic release of an active CR1 fragment into solution, and this is subsequently degraded with loss of most of its cofactor activity.

Plasmin and thrombin treatment of erythrocytes

Incubation of erythrocytes with human thrombin or plasmin also resulted in release of cofactor activity into the supernatant (Fig. 5). No apparent destruction of cofactor activity, such as is seen in Figs. 3 and 4, was observed with plasmin and thrombin, although this may have occurred if higher concentrations of proteinase had been used.

Since plasmin and thrombin are generated during clotting and clot retraction in blood, we examined, as described in the Materials and methods section, whether CR1 was lost from erythrocytes during clotting of whole blood. However there was no significant difference between the CR1 cofactor activity levels on control erythrocytes and on erythrocytes derived from clotted samples. Thus although plasmin and thrombin can cleave CR1, insufficient free thrombin and plasmin is generated during clotting *in vitro* to cause significant release of CR1 from erythrocytes.

DISCUSSION

Measurement of CR1 by assay of factor I cofactor activity indicates a similar range of variation between individuals (Fig. 1) as is detected by other CR1 assay methods. A cumulative frequency curve (not shown), constructed as discussed by Holme *et al.* (1986), for the data presented in Fig. 1 indicates that the data are not compatible with the single locus inheritance model for CR1 levels suggested by Wilson *et al.* (1982).

Studies of the loss of CR1 activity from erythrocytes during ageing *in vivo* indicated a considerable variation in the lifetime of CR1 between individuals (Fig. 2). A 40% loss of activity was found on average when comparing the 'oldest' with the 'youngest' cells of an individual donor, but variations between 20% and 75% loss were observed.

It was initially considered possible that the variation between individuals in half-life of CR1 on erythrocytes (Fig. 2) might contribute significantly to the wide variation in mean CR1 levels (Fig. 1). If this were the case, it would be expected that, e.g. individuals with low mean CR1 levels would show a rapid loss of CR1on ageing of cells *in vivo*, while individuals with high mean CR1 levels would show a slow rate of loss on ageing. However there was no correlation of this type between mean CR1 levels and half-life of CR1 on erythrocytes. It is likely, therefore, that in healthy donors the variation in rate of loss of CR1 from erythrocytes on ageing makes only a minor contribution to the overall variability in mean CR1 levels.

The fact that loss of CR1 activity from erythrocytes can be observed on ageing *in vivo* indicates that there is a general, non-pathological mechanism for inhibition, removal or destruction of CR1. The same mechanism may also be responsible, in pathological conditions, for accelerated loss of CR1, and acquisition of CR1 deficiency in SLE. Several mechanisms for loss or blockage of CR1 in pathological conditions can be postulated, as discussed by Holme *et al.* (1986) and Ross *et al.* (1985). These include blockage of CR1 by large complement-coated immune complexes or other fixed ligands, development of autoantibodies against CR1, or proteolytic destruction of CR1. Although our own studies do not exclude the possiblity that CR1 activity on red cells is blocked by adherent immune complexes, other investigators have regarded this as unlikely (Ross *et al.*, 1985). Autoantibodies to CR1 are unlikely to be found widely, although there is a single report of an anti-CR1 autoantibody in an SLE patient (Wilson *et al.*, 1985). It has however been known for many years that immune adherence activity on erythrocytes is destroyed by treatment with trypsin, chymotrypsin or papain (Nelson, 1963), and in view of the high sensitivity of purified CR1 to proteolysis (Sim, 1985), random proteolysis is a plausible mechanism for destruction of CR1 during erythrocyte ageing.

Studies of proteolysis of erythrocytes by trypsin (Figs. 3 and 4) indicate that, even at very low trypsin concentrations, the factor I cofactor activity of CR1 is efficiently removed from the cells, and the activity becomes detectable in the supernatant. Further trypsin treatment leads to destruction of the activity which was released into the supernatant. Plasmin and thrombin also cause release of CR1 activity from the erythrocyte surface into the supernatant (Fig. 5). Preliminary attempts were made to characterize the active material released into the supernatant after trypsin treatment of erythrocytes. This was done by radioiodination of the proteins in the supernatant, followed by analysis by ion exchange and gel filtration chromatography. The material possessing CR1 activity was more basic than intact, detergent-solubilized CR1, as judged by chromatography on DEAE-Sephacel. Apparently intact CR1, which has been reported to circulate in a soluble form in plasma (Yoon & Fearon, 1985), was not released into the supernatant after brief trypsin treatment. Further attempts to determine an approximate M_r for the active fragment have failed because of adsorption of the material on gel filtration media. However, it is clear that trypsin, plasmin or thrombin treatment of erythrocytes leads to the release into the supernatant of a fragment of CR1, which still possesses factor I cofactor activity. On prolonged trypsin treatment, this activity is destroyed. The overall pattern of loss of cofactor activity on trypsinization of erythrocyte-bound CR1 is similar to that previously observed on trypsin treatment of soluble CR1 (Sim, 1985). Soluble CR1 is degraded by trypsin to form disulphide-linked fragments of M_r 65000 and 160000. The M_r 65000 fragment is subsequently broken down to M_r 38000 and 25000 peptides and finally to products of M_r less than 25000. This occurs with retention of much of the cofactor activity of the intact molecule. Only these last fragments are released from disulphide linkages to the M_r 160000 fragment, and would be suitable candidates for an active fragment released into the supernatant on trypsinization of erythrocyte-bound CR1. Prolonged trypsin digestion of soluble CR1 also results in sequential formation of non-disulphide-linked M_r 140000, 90000 and 70000 fragments derived from the M_r 160000 region of CR1, but generation of these fragments is slow, and occurs mostly after major loss of cofactor activity. These

fragments are therefore less likely to possess cofactor activity.

Proteolysis of CR1 is a likely mechanism for removal of CR1 from erythrocytes in physiological conditions ('ageing') and in pathological conditions (acquired deficiencies in SLE). Since erythrocytes have an important role in sequestering of immune complexes and transporting them to the reticulo-endothelial system in liver and spleen (Cornacoff *et al.*, 1983) it is possible that proteolysis of CR1 occurs randomly on contact with proteinase-rich tissue phagocytes. This contact would be increased in diseases, such as SLE, in which there is an elevated level of immune complex formation.

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