Biochemical Pharmacology, Vol. 39, No. 4, pp. 775–780, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

A LECTIN-LIKE RECEPTOR ON MURINE MACROPHAGE IS INVOLVED IN THE RECOGNITION AND PHAGOCYTOSIS OF HUMAN RED CELLS OXIDIZED BY PHENYLHYDRAZINE

SARA HORN, JACOB GOPAS and NAVA BASHAN*

Departments of Biochemistry, Microbiology and Immunology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

(*Received* 25 *July* 1989; *accepted* 16 *October* 1989)

Abstract—Phenylhydrazine (Phz) is a powerful hemolytic agent which has several effects on both normal and G6PD deficient red blood cells (RBCs). We have studied the mechanism of removal of Phz-damaged human RBCs by murine macrophages. Phagocytosis of Phz-treated RBCs was found to be 50 RBCs/100 mac as compared to 2 RBCs/100 mac of the controls. EGTA and sodium azide inhibited the phagocytosis, indicating a requirement for both calcium ions and energy. Incubation of macrophages with sugars such as D-galactose or D-mannose reduced phagocytosis of Phz-treated RBCs by up to 60%, indicating the involvement of a macrophage lectin-like receptor in the recognition of Phz-treated RBCs. The presence of serum in the phagocytosis assay did not affect either phagocytosis of Phz-treated RBCs or inhibition by sugars. β-Galactosidase, but not neuraminidase, treatment of RBCs caused a significant inhibition in phagocytosis of Phz-treated RBCs. These results suggest that galactosyl residues are exposed on RBC membrane during oxidation, probably not as a result of desialization. We conclude that Phz-treated RBCs are detected as damaged cells mainly due to sugar changes on their membrane, which are directly recognized by lectin-like receptors on the macrophages.

Oxidative agents have been useful *in vitro* for understanding the pathophysiological events which cause red blood cell (RBC†) destruction in oxidant-induced hemolytic disease, such as glucose-6-phosphate dehydrogenase (G6PD) deficiency [1, 2].

Phenylhydrazine (Phz) is a powerful hemolytic agent which has several effects on RBCs. Reactions between Phz and hemoglobin are known to generate both superoxide and hydrogen peroxide [3]. These types of activated oxygen may cause various kinds of cellular damage, such as methemoglobin formation, disulfide bond formation between intracellular glutathione (GSH), and/or various proteins, or between hemoglobin and membranal proteins [1, 4]. As a result of the cellular changes induced by Phz, the RBC membrane is affected: membranal aggregates between proteins are formed, polymerization of spectrin and covalent binding of hemoglobin to spectrin are observed [5], leading to configurational and/or proteolytic changes in the integral anion channel band 3 and enhanced binding of autologous IgG to the RBC membrane [6, 7]. In order to correlate these changes with ultimate macrophage-related RBC destruction *in vivo*, it has to be assumed that the membranal modifications on Phz-treated RBCs can be recognized by macrophages, leading to increased phagocytosis.

The erythrophagocytosis assay has been useful in studying the interaction between human RBCs and autologous or xenogeneic macrophages *in vitro* in cases of normal aging RBCs [8–12] and in hemolytic anemias such as thalassemia or sickle cell anemia [13, 14].

Many different signals for recognition and clearance of damaged RBCs has been proposed. The suggestions include generation of desialysated glycoproteins [13, 15, 16], exposure of galactose residues [9–11, 17, 18], oligomerization of band 3 [6, 18, 19], exposure of hydrophobic domains [14, 20], or other topological changes in the RBC membrane [22]. These changes are recognized by specific macrophage receptors in a direct [9–11, 20, 21], or immunoglobulin mediated [12, 15, 17–19, 22], way. The signals generated on red cells by various oxidative stresses are less clear [6, 7, 21].

The present studies were undertaken using murine thioglycollate-elicited macrophages to analyse the recognition mechanism of human Phz-treated RBCs. The results show that the pronounced phagocytosis of Phz-oxidized RBCs is mediated by a lectin-like macrophage receptor which recognizes galactosyl and not sialic acid residues on the surface membrane of the damaged RBCs.

MATERIALS AND METHODS

Materials

RPMI 1640, fetal calf serum (FCS), glutamine, and penicillin-streptomycin were obtained from Biological Industries (Beth Haemek, Israel). Thioglycollate medium was obtained from Bacto, Difco

* Correspondence to: Prof. N. Bashan, Pediatric Research Laboratory, Soroka Medical Center, Beer-Sheva 84101, Israel.

† Abbreviations: Phz, phenylhydrazine; G6PD, glucose-6-phosphate dehydrogenase; RBC, red blood cell; EGTA, Ethylene glycol bis-(-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Mac, macrophage(s); GSH, reduced glutathione; FCS, fetal calf serum; PBS, phosphate buffered saline; Hct, hematocrit.

Labs. (Detroit, MI). Phenylhydrazine, sugar derivates, neuraminidase (from *Clostridium perfrigiens*), β-galactosidase (from *Escherichia coli*), and other chemicals were obtained from the Sigma Chemical Co. (St Louis, MO).

Balb/c mice (6–9 weeks old) were purchased from the Annilab Co. (Rehovot, Israel).

Methods

Macrophages. Peritoneal exudate cells were recovered from peritoneal cavities 4–5 days after the i.p. injection of 3 mL of 3% sterile thioglycollate by washing the cavities twice with RPMI containing 10 units heparin/mL. The cells were then washed twice with RPMI containing no heparin, counted and suspended in RPMI in the presence or absence of 2% FCS to a final concentration of 2×10^6 mac/mL.

The cell suspension (1 mL) was placed on glass slides in 35-mm Falcon plastic culture dishes and incubated for 2 hr at 37° in a 5% CO_2 moist incubator. Non-adherent cells were removed after incubation by gentle washing in serum free RPMI, and 0.9 mL of fresh RPMI was placed in each well.

Red blood cells. Freshly heparinized human blood (Rh-positive) was depleted of leukocytes by passage through cellulose columns [23], washed six times with cold phosphate buffered saline (PBS) (pH 7.4), and resuspended to 10% hematocrit (% Hct) in PBS containing 6 mM D-glucose. The RBC suspensions were then incubated with 3 mM Phz (unless otherwise specified) for 1 hr at 37°. After incubation the cells were washed three times with PBS and suspended in cold RPMI (10% Hct).

Incubation of RBCs with serum. One volume of Phz-treated RBCs were resuspended in one volume of fresh autologous serum for 30 min at 37°. The suspension was then diluted 1:5 (10% Hct) in RPMI before the addition to macrophage cultures.

Opsonization of RBCs. Washed RBCs, suspended in PBS at 10% Hct, were incubated with anti-D antibodies at a final dilution of 1:2 for 30 min at 37°. The cells were then washed three times in PBS and resuspended in cold RPMI (10% Hct).

Enzymatic treatment of RBCs. RBC suspensions (10% Hct) were incubated with different concentrations of neuraminidase or β-galactosidase for 30 and 60 min, respectively, at 37°. After incubation the supernatants were collected for sialic acid determination by the TBA method [24] and the cells were washed three times and resuspended in cold RPMI (10% Hct).

Phagocytosis assay. RBC suspension (0.1 mL) was added to the macrophage cultures and incubated for 1 hr at 37°. The medium was subsequently removed, the cell layer was twice washed with PBS and the non-internalized RBCs were removed by 20-sec lysis with double distilled water.

The macrophages were then stained with Giemsa and examined by light microscopy. The number of RBCs ingested in 200 randomly-observed macrophages was counted. All assays were performed in triplicate and all experiments were performed at least three times. The results are expressed as mean ± SD.

Phagocytosis inhibition assays. Sodium azide and EGTA were incubated with macrophage cultures for 30 min and removed with RPMI prior to RBC addition. Sugars at 25 mM final concentration (unless otherwise specified) were incubated with macrophage cultures for 30 min prior to RBC addition and during phagocytosis assay. The percentage of phagocytosis inhibition was determined by the relative decrease in phagocytosis compared to phagocytosis values of Phz-treated RBCs without any inhibition treatment.

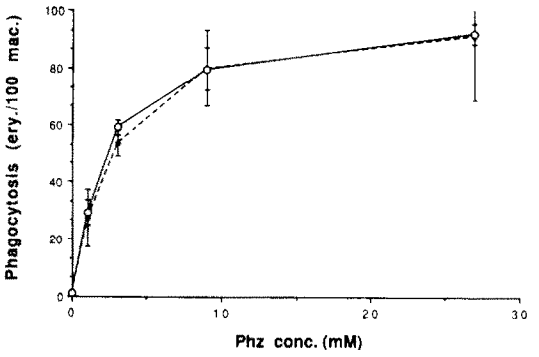

Fig. 1. Phagocytosis of Phz-treated RBCs. Normal RBCs were incubated with various concentrations of Phz without D-glucose (solid line) or with 6 mM D-glucose (broken line) in the incubation medium. Mean ± SD of three experiments are presented.

RESULTS

The effect of Phz on RBC phagocytosis

Phagocytosis of untreated RBCs by mouse peritoneal macrophages was found to be less than 2 RBCs/100 macrophages (% phagocytosis). Pretreatment of the RBCs with Phz for 1 hr in a serum-free medium caused a significant increase in phagocytosis in a concentration dependent manner up to values of 90% phagocytosis (Fig. 1). Addition of D-glucose (6 mM) or fresh autologous serum to the RBC suspensions did not significantly affect phagocytosis of Phz-treated RBCs. In all subsequent experiments, washed RBCs were oxidized with 3 mM Phz after removal of leukocytes in a serum-free medium. At this Phz concentration, significant phagocytosis occurs and no apparent hemolysis is observed (the viability of the cells was 90–95%).

Incubation of macrophages with a chelating agent such as EGTA, or with the metabolic inhibitor sodium azide, almost totally inhibited phagocytosis, indicating the need for calcium ions and normal metabolic activity of the macrophages in order to obtain phagocytosis (Table 1).

During incubation of RBCs with Phz, superoxide and hydrogen peroxide are known to be formed [3, 4]. In order to test whether these oxygen derived products non-specifically activate macrophages and enhance phagocytosis [25], macrophage cultures were exposed to 3 mM Phz, or to 2.5–5% hemolysate of Phz-treated RBCs, before the addition of untreated RBCs. These treatments did not affect phagocytosis (Table 1). Furthermore, phagocytosis values of Phz-treated RBCs were not reduced by the

Table 1. The effect of oxidation and metabolic inhibitors on erythrophagocytosis

Treatment of RBCs	Treatment of macrophages	Phagocytosis (RBCs/100 mac)
+Phz	Untreated	54.6 ± 11.8
+Phz	+EGTA	17.0 ± 10
+Phz	+sodium azide	10.6 ± 10.4
+Phz+catalase* 800 units/mL PC	Untreated	53.3 ± 1.3
Untreated	Untreated	0.6 ± 0.17
Untreated	+2.5% Hem.	1.2 ± 0.3
Untreated	+5.0% Hem.	4.2 ± 3.8
Untreated	+Phz	1.5 ± 0.9

Hem., hemolysate of Phz-treated RBCs; PC, packed RBCs.

Cultured adherent macrophages were incubated with 0.1 mM sodium azide, 2.5–5% hemolysate, or 3 mM Phz dissolved in RPMI for 30 min. The incubation media were then washed and replaced with new RPMI before the addition of 0.1 mL RBC suspensions (10% Hct). EGTA (4 mM) was handled in exactly the same way except it remained in the medium without being washed out before the addition of RBC suspensions.

* Samples of Phz-treated RBCs suspended in RPMI (10% Hct) were incubated with catalase for 5 min before being added to macrophages. Catalase was therefore present during phagocytosis.

addition of catalase to these cells, suggesting that hydrogen peroxide released from Phz-treated hemolysates is not directly responsible for the increase in the phagocytosis observed.

The involvement of sugars in phagocytosis of Phz-treated RBCs

Macrophages have the ability to recognize sugar derivatives such as D-galactose or D-mannose on various cells [9–11, 26–28]. In order to determine whether carbohydrate recognition is involved in the interaction between macrophages and Phz-treated RBCs, phagocytosis inhibition studies using various sugars were performed.

As can be seen in Fig. 2, addition of various sugars such as D-galactose, D-mannose, *N*-acetyl-D-glucose amine and *N*-acetyl-D-galactose amine to macrophage cultures inhibited the phagocytosis of Phz-treated RBCs. Sugars such as α-methyl-mannoside and D-glucose, however, did not significantly affect phagocytosis of these cells. Simultaneous addition of both D-galactose and D-mannose did not change inhibition values obtained with each individual sugar, indicating that the effect of those sugars is not additive, and therefore suggesting that they are recognized by the same receptor. Inhibition of phagocytosis by D-mannose (Fig. 3) was already observed at low sugar concentrations (0.1 mM) and it increased in a concentration-dependent manner up to a maximal inhibition of 63%.

In Table 2, it can be seen that the inhibition of phagocytosis by D-galactose or D-mannose was not affected by the addition of serum to RBCs. Moreover, the phagocytosis of anti-D opsonized RBCs, which is mediated by Fc receptors, was not

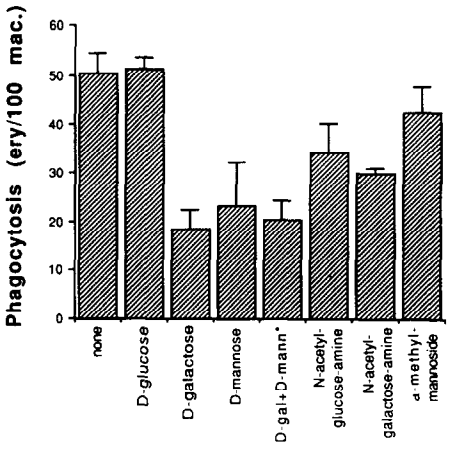

Fig. 2. The effect of sugars on phagocytosis of Phz-treated RBCs. Various sugars at 25 mM concentration were added to macrophage cultures. After 30 min incubation, 0.1 mL of Phz-treated RBCs (10% Hct) were added to the medium. (*) D-Galactose and D-mannose, each at 25 mM concentration, were added simultaneously to macrophage cultures. Mean ± SD of three experiments are presented.

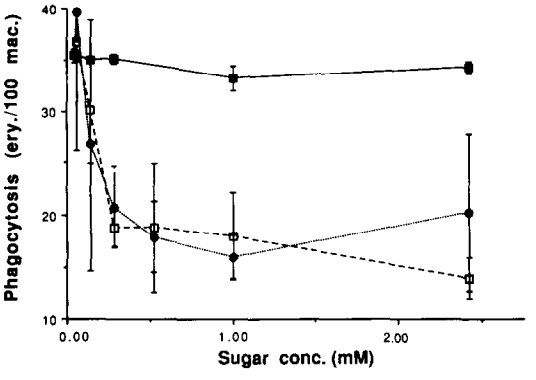

Fig. 3. The effect of sugars on phagocytosis of Phz-treated RBCs. Different sugar concentrations were added to macrophage cultures. After 30 min incubation, 0.1 mL RBCs were added to the medium, as indicated in Fig. 2, and phagocytosis was determined. Mean ± SD of eight experiments are presented. (—■—) D-glucose, (--□--) D-galactose, (----●----) D-mannose.

affected by the addition of D-galactose or D-mannose, which excluded the possibility that non-specific inhibition of macrophage phagocytic activity occurs in the presence of these sugars.

We thus conclude that the mechanism by which Phz-oxidized RBCs are recognized involves a lectin-like macrophage receptor.

The effect of β-galactosidase and neuraminidase treatments of RBCs on phagocytosis

To further establish the involvement of D-galactose recognition in phagocytosis, control and Phz-treated RBCs were incubated with β-galactosidase. Figure 4a shows that indeed β-galactosidase treatment of oxidized RBCs reduced the phagocytosis to

Table 2. The effect of serum and D-galactose or mannose on phagocytosis of Phz-treated RBCs

Treatment of macrophages	Phagocytosis (erythrocytes/100 mac)		
	Phz-RBCs		Opsonized RBCs
	−Serum	+Serum*	(anti-D)†
Untreated	50.5 ± 4.1	53.2 ± 6.3	60.3 ± 12.5
+D-Galactose‡	18.6 ± 3.9	19.4 ± 4.5	67.4 ± 11.9
+D-Mannose‡	23.3 ± 9.0	24.2 ± 8.0	65.0 ± 10.4

* One volume of Phz-treated RBCs were resuspended in one volume of fresh autologous serum for 30 min at 37°. The suspension was then diluted 1:5 (10% Hct) in RPMI before the addition to macrophage cultures.

† RBCs were incubated with anti-D antibodies (1:2 dilution) for 30 min at 37°. The cells were then washed and resuspended in RPMI (10% Hct) before being added to the macrophages.

‡ Cultured adherent macrophages were incubated with 25 mM of D-galactose or D-mannose, as described in the legend to Fig. 2.

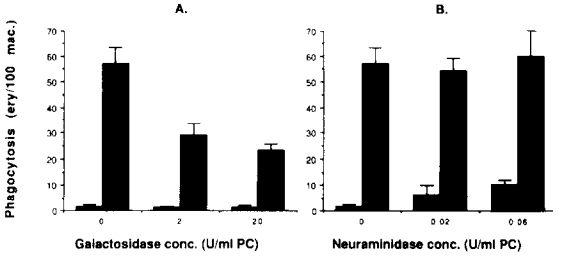

Fig. 4. The effect of β-galactosidase and neuraminidase on phagocytosis of Phz-treated RBCs. Control (solid columns) and Phz-treated (striped columns) RBCs (10% Hct) were incubated with β-galactosidase (A) or neuraminidase (B), for 60 or 30 min at 37°, respectively. After incubation the cells were washed, suspended in RPMI, and added to macrophage cultures for phagocytosis. Mean ± SD of three experiments are presented.

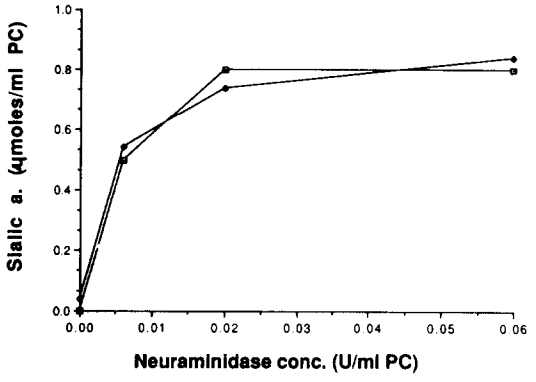

Fig. 5. The effect of neuraminidase treatment of RBCs on sialic acid removal. Normal or Phz-treated RBCs were incubated with various concentrations of neuraminidase for 30 min at 37°. After incubation the cells were centrifuged and the supernatants were collected. The sialic acid content removed from the RBCs membranes into the supernatant was determined by the TBA method. (—◆—) Sialic acid content in supernatant of control RBCs, (—⊡—) sialic acid content in supernatant of Phz-treated RBCs.

a degree similar to that obtained with D-galactose or D-mannose (almost 50% inhibition) and did not affect the phagocytosis of untreated RBCs. Phagocytosis of Phz+β-galactosidase-treated RBCs was not further inhibited in the presence of D-galactose (60% inhibition), indicating that the galactosyl residues removed by β-galactosidase are significant in the recognition of the oxidized RBCs by lectin-like macrophage receptors.

Since desialization is known to be one of the mechanisms for sugar exposure on RBCs membranes, we determined: (i) the effect of desialization on phagocytosis; (ii) the release of sialic acid during oxidation; and (iii) the amount of sialic acid on the oxidized cell membrane. The results show that neuraminidase treatment of RBCs caused only a slight increase in the phagocytosis of non-oxidized RBCs (from 2% to 10%) and did not significantly affect the phagocytosis of Phz-treated RBCs (Fig. 4b). In parallel, no sialic acid was detected in the medium during Phz oxidation (data not shown), and the amount of sialic acid released from both untreated and Phz-treated RBCs was found to be similar (Fig. 5). These data suggest that the galactosyl residues recognized by the macrophages are not exposed by the removal of sialic acid.

DISCUSSION

In this paper the mechanism of recognition and phagocytosis of oxidized RBCs was studied in order to further our understanding of the basis of macrophage removal of oxidized RBCs. For this purpose, a heterologous system was used involving mouse thioglycollate-elicited peritoneal macrophages that interacted with human RBCs. It has been previously shown that mouse peritoneal macrophages efficiently phagocytose senescent, thalassemic, and sickle cell anemic RBCs [9, 11, 13, 14]. This system was chosen since the phagocytic activity of these macrophages is high and consistent from experiment to experiment.

RBCs treated with the oxidative agent Phz are phagocytosed by macrophages (Fig. 1) [7, 21]. However, the mechanism(s) by which these cells are recognized is still unclear.

Since enhanced phagocytosis of Phz-treated RBCs is not due to non-specific macrophage activation by oxidative agents, such as hydrogen peroxide or Phz (Table 1), specific mechanisms that could mediate the interaction between the oxidized cells and the macrophages were searched for.

One of the known mechanisms of interaction between cells involves recognition of membrane sugars by lectin-like receptors. This mechanism has also been reported in the recognition of senescent RBCs, bacteria or yeast by macrophages [9, 11, 26, 27]. It was therefore reasonable to test whether macrophage lectin-like receptors are also involved in the recognition of Phz-treated RBCs. Phagocytosis of Phz-treated RBCs, but not opsonized RBCs, was found to be inhibited by several sugars, most efficiently by D-galactose and D-mannose (Figs 2 and 3). These results imply the involvement of galactosyl/mannosyl lectin-like receptor(s) in the recognition of these cells, which differ from the classical Fc receptor mechanism. The binding ability of these receptors is calcium-dependent, since the chelator agent, EGTA, almost totally inhibited the phagocytosis of Phz-treated RBCs (Table 1), as previously shown with senescent RBCs [9].

Inhibition of phagocytosis was also obtained by removal of galactosyl residues from the cell surface with β-galactosidase (Fig. 4a). These results complement the previous sugar inhibition experiments and further support the role played by sugars in recognition and phagocytosis. Taken together, these data imply that during oxidation, sugar residues are exposed on RBC membrane and are recognized by the lectin-like receptors. Since phagocytosis was inhibited by D-galactose/D-mannose in the presence of serum as well (Table 2), it seems that sugar recognition of oxidized RBCs by macrophages may play a role *in vivo*.

Various explanations have been reported for exposure of sugars. Among these are desialization of the RBC membrane (as in thalassemic and senescent RBCs) [13, 15, 16], by the presence of contaminating glycosidases [29], and by enhanced proteolysis occurring during Phz oxidation [30, 31].

Our results do not support desialization as the main mechanism for D-galactose/D-mannose exposure since sialic acid residues were apparently not removed during oxidation (Fig. 5) and high levels of phagocytosis to the extent observed with Phz-treated RBCs could not be elicited by neuraminidase treatment of control RBCs (Fig. 4b).

Contaminating glycosidases of leukocyte origin [29] are probably not responsible for the exposure of sugars, since all RBC preparations were freed from leukocytes by cellulose columns [23].

Enhanced proteolysis has been reported in erythrocytes treated with hydrazines [30, 31]. It is conceivable that the proteolytic removal of oxidatively-damaged proteins could contribute to the exposure of cryptic sugars on the cell surface. This possibility remains to be tested.

Sugar residues on Phz-treated RBCs could be directly recognized by the putative lectin-like receptor on the macrophages, or indirectly through binding of antibodies. Recent reports have shown binding of immunoglobulins to Phz-treated RBCs, but it is not established whether this binding is essential for the observed phagocytosis [6, 7, 21]. Our results support the interpretation that immunoglobulins are not essential for the enhanced phagocytosis of Phz-treated RBCs, since: (i) phagocytosis of extensively-washed Phz-treated RBCs, takes place in the absence of serum and is not affected by its addition (Fig. 1, Table 2); and (ii) immunoglobulins are not detected on RBC membranes, as tested by immunoblots (data not shown). Studies using other oxidative agents such as diamide have shown the involvement of anti-band 3 antibodies and complement in the recognition and phagocytosis of RBCs [19, 22]. Therefore, it seems that a variety of oxidative agents may elicit a variety of changes in RBC membranes, leading to different patterns of RBC recognition by macrophages [7, 19–22].

We conclude that RBCs damaged by Phz oxidation are detected by macrophages mainly through lectin-like receptors which directly recognize sugar changes in RBC membrane in the absence of specific binding of immunoglobulins.

Acknowledgements—Thanks are due to Dr H. U. Lutz for enlightening discussion and generous advice.

REFERENCES

1. Bashan N, Pothashnik R, Feozer R and Moses SW, The effect of oxidative agents on normal and G6PD deficient red blood cell membranes. In: *Advances in Red Cell Biology* (Eds. Weatheral DJ, Fiorelly G and Gorini S), pp. 365–374. Raven Press, New York, 1982.
2. Flynn TP, Gerhard JJ and Allen DW, Mechanism of decreased erythrocyte deformability and survival in D-glucose-6-phosphate dehydrogenase mutants. *Erythrocyte Membranes* **2**: 231–245, 1981.
3. Jain SK and Hochstein P, Generation of superoxide radicals by hydrazine. Its role in phenylhydrazine induced hemolytic anemia. *Biochim Biophys Acta* **586**: 128–134, 1979.
4. Vilsen B and Nielsen H, Reaction of phenylhydrazine with erythrocytes. *Clin Pharm* **33**: 2739–2748, 1984.
5. Arduini A and Stern A, Spectrin degradation in intact red blood cells by phenylhydrazine. *Biochem Pharmacol* **34**: 4238–4289, 1985.
6. Low PS, Waugh SM, Zinke K and Drenckhahn D, The role of hemoglobin denaturation and band 3 clustering in red blood cell aging. *Science* **227**: 531–533, 1985.
7. Magnani M, Stocchi V, Cucchiarini L, Chiarantini L and Fornaini G, Red blood cell phagocytosis and lysis following oxidative damage by phenylhydrazine. *Cell Biochem Function* **4**: 263–269, 1986.
8. Klausner MA, Hirsch LJ, Leblond PF, Chamberlain JK, Klemperer MR and Segel GB, Contrasting splenic mechanism in the blood clearance of red blood cells and colloidal particles. *Blood* **46**: 965–976, 1975.
9. Schlepper-Schafer J, Kolb-Bachofen V and Kolb H, Identification of a receptor for senescent erythrocytes on liver macrophages. *Biochem Biophys Res Commun* **115**: 551–559, 1983.
10. Vaysse N, Gattegno L, Bladier D and Aminoff D, Adhesion and erythrophagocytosis of human senescent erythrocytes by autologous monocytes and their inhibition by galactosyl RBC derivates. *Proc Natl Acad Sci USA* **83**: 1339–1343, 1986.

11. Kyoizumi S and Masuda T, A lectin-like receptor on murine macrophage cell line cells Mm_1: involvement of sialic acid-binding sites in opsonin-independent phagocytosis for xenogeneic red cells. *J Leucocyte Biol* **37**: 289–304, 1985.
12. Kay MMB, Aging of cell membrane molecules leads to appearence of an aging antigen and removal of senescent cells. *Gerontology* **31**: 215–235, 1985.
13. Knyszynski A, Danon D, Kahane I and Rachmilewitz EA, Phagocytosis of nucleated and mature B thalassaemic red blood cells by mouse macrophages *in vitro*. *Br J Haem* **43**: 251–255, 1979.
14. Schroit AJ, Tanaka Y, Madsen J and Fidler IJ, The recognition of red blood cells by macrophages: role of phosphatidylserine and possible implications of membrane phospholipid asymmetry. *Biol Cell* **51**: 227–238, 1984.
15. Alderman EM, Fudenberg HH and Lovins RE, Isolation and characterization of an age-related antigen present on senescent human red blood cells. *Blood* **58**: 341–349, 1981.
16. Kelm S, Shukla AK, Paulson JC and Schauer R, Reconstitution of the masking effect of sialic acid groups on sialidase treated erythrocytes by the action of sialyltransferases. *Carb Res* **149**: 59–64, 1986.
17. Galili U, Korkesh A, Kahane I and Rahmilewitz EA, Demonstration of a natural antigalactosyl IgG antibody on thalassemic red blood cells. *Blood* **61**: 1258–1264, 1983.
18. Galili U, Flechner I, Knyszynsky A, Danon D and Rachmilewitz EA, The natural anti-galactosyl IgG on human normal senescent red blood cells. *Br J Haem* **62**: 317–324, 1986.
19. Lutz HU, Fasler S, Stammler P, Bussolino F and Arese P, Naturally occurring anti-band 3 antibodies and complement in phagocytosis of oxidatively-stressed and in clearence of senescent red cells. *Blood Cells* **14**: 175–195, 1988.
20. McEvoy L, Williamson P and Schlegel RA, Membrane phospholipid asymmetry as a determinant of erythrocyte recognition by macrophages. *Proc Natl Acad Sci USA* **83**: 3311–3315, 1986.
21. Beppu M, Ochiai H and Kikugawa K, Macrophage recognition of the erythrocytes modified by oxidizing agents. *BBA* **930**: 244–253, 1987.
22. Lutz HU, Red cell clearance (a review). *Biomed Biochim Acta* **46**: S65–S71, 1987.
23. Beutler E, West C and Blume KG, The removal of leukocytes and platelets from whole blood. *J Lab Clin Med* **88**: 328–333, 1976.
24. Aminoff DO, Methods for the quantitative estimation of *N*-acetylneuraminic acid and their application to hydrolysates of sialomucoids. *Biochem* **81**: 384–392, 1961.
25. Harada RN, Vatter AE, Repine JE, Macrophage effector function in pulmonary oxygen toxicity: hyperoxia damages and stimulates alveolar macrophages to make and release chemotaxins for polymorphonuclear leukocytes. *J Leucocyte Biol* **35**: 373–383, 1984.
26. Kean EL and Sharon N, Inhibition of yeast binding to mouse peritoneal macrophages by wheat germ agglutinin: a novel effect of the lectin on phagocytic cells. *BBRC* **148**: 1202–1207, 1987.
27. Perry A, Keisari Y and Ofek I, Liver and macrophage surface lectins as determinants in blood clearance and cellular attachment of bacteria. *FEMS Microbiol Lett* **27**: 345–350, 1985.
28. Sharon N, Surface carbohydrates and surface lectins are recognition determinants in phagocytosis. *Immunol Today* **5**: 143–147, 1984.
29. Brovelli A, Seppi C, Bardoni A, Balduini C and Lutz HU, Re-evaluation of the structural integrity of red-cell glycoproteins during aging *in vivo* and nutrient deprivation. *Biochem J* **242**: 115–121, 1987.
30. Cola D, Sacchetta P and Battista P, Proteolysis in human erythrocytes is triggered only by selected oxidative stressing agents. *Ital J Biochem* **37**: 129–138, 1988.
31. Runge-Morris MA, Iacob S and Novak RF, Characterization of hydrazine-stimulated proteolysis in human erythrocytes. *Toxicol Appl Pharmacol* **94**: 414–426, 1988.