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A possible role for antibodies against spectrin in the interaction between erythroblasts and macrophages *in vitro*

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Summary. The nature of serum factors which participate in the interaction *in vitro* between dimethylsulphoxide-induced Friend leukaemia erythroblasts (IFLE) and syngeneic mouse peritoneal macrophages was investigated. When heat-inactivated newborn calf serum (HI-NBCS) was depleted of IgG its activity to promote the association of neuraminidase-treated ^{59}Fe-labelled IFLE (^{59}Fe-IFLE) with macrophages was markedly reduced but could be restored by the addition of bovine IgG. Trypsin treatment of macrophages caused incomplete inhibition of their subsequent association with both untreated and neuraminidase-treated ^{59}Fe-IFLE in the presence of HI-NBCS. When spectrin, the major red cell cytoskeleton protein, was added to HI-NBCS there was a dose-related inhibition of the association with macrophages of both untreated and neuraminidase-treated ^{59}Fe-IFLE. Moreover a mouse monoclonal antibody against spectrin promoted the interaction of neuraminidase-treated ^{59}Fe-IFLE with macrophages. Mouse sera which supported the association of neuraminidase-treated ^{59}Fe-IFLE with macrophages were found to contain anti-spectrin antibodies. These results suggest that IgG antibodies mediate the interaction between erythroblasts and macrophages via trypsin-sensitive and trypsin-resistant receptors on the macrophage surface and that at least some of the antibodies show specificity for spectrin.

Keywords: spectrin, erythroblasts, macrophages, antibodies

The interaction between DMSO-induced Friend leukaemia cells (IFLE) and syngeneic mouse peritoneal macrophages *in vitro* provides a useful experimental model for investigating mechanisms which underlie ineffective erythropoiesis (Wiener & Wickramasinge 1982). Previous studies of this system showed that erythroblasts rendered defective by treatment with either inhibitors of protein synthesis (Wiener & Wickramasinghe 1983*a*) or neuraminidase (Wiener & Wickramasinghe 1983*b*), exhibit heightened susceptibility to phagocytosis by macrophages. Moreover the interaction of the desialated erythroblasts with the phagocytes was found to be dependent on a heat-stable serum factor which was probably in the γ-globulin fraction (Wiener & Wickramasinghe 1983*b*). The present paper provides evidence for the immunoglobulin G nature of this serum component. In addition it explores whether the recognition of defective erythroblasts by macrophages is mediated by antibodies which show specificity for defined proteins and glycoproteins of the red cell membrane.

Materials and methods

Chemicals and sera. Neuraminidase type V (EC 3.2 1.18) (purified from *Cl. perfringens*),

trypsin type III (EC 3.4 21.4) (purified from bovine pancreas), lectin from *Arachis hypogea* (peanut) (PNA), bovine serum albumin (BSA) and immunoglobulin G (IgG) were obtained from Sigma Chemical Co., St Louis, Mo. U.S.A. Heat-inactivated newborn calf serum (HI-NBCS), Hanks' balanced salt solution (HBSS) and nutrient mixture F-12 (Ham's) were purchased from Gibco Europe Ltd, Paisley, Scotland, mouse serum from Sera Lab Ltd., Crawley Down, West Sussex, and tissue culture medium 199 with antibiotics (TC20) from Wellcome Reagents Ltd, Beckenham, Kent.

Spectrin was purified from human red cells according to the method of Shotton *et al.* (1979). Most of the spectrin used in the experiments as well as a mouse ascites fluid containing monoclonal antihuman α-spectrin antibody (61a) were generous gifts from Dr D.M. Shotton, Department of Zoology, University of Oxford. Mouse ascites fluid containing monoclonal antihuman epidermal growth factor receptor antibody (EGR/G49) was kindly provided by Dr A.R. Rees, Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford.

Preparation of ^{59}Fe-labelled erythroblasts (^{59}Fe-IFLE). Friend leukaemia cells were induced to differentiate into erythroblasts and labelled with ^{59}Fe as described previously (Wiener & Wickramasinghe 1983*a*). In one experiment the cells were cultured in the absence of serum during the 12 h before their harvest.

Interaction between erythroblasts and macrophages. The interaction between DMSO-induced Friend leukaemia erythroblasts (IFLE) with syngeneic mouse peritoneal macrophages was studied in the *in vitro* system described by Wiener & Wickramasinghe (1983*b*) and estimated as the association of ^{59}Fe-labelled IFLE (^{59}Fe-IFLE) with the phagocytes. The procedure adopted previously was slightly modified in order to reduce the required number of macrophages and volume of reagents. Briefly, peritoneal macrophages were cultured in Linbro multiwell tissue culture plates, each with 24 flat-bottom wells (Flow Laboratories), individual wells being initially seeded with 7×10^5 peritoneal cells. After 48 h of culture in 2 ml of TC20 with 20% HI-NBCS and 100 μg nystatin/ml, the macrophage monolayers were washed and exposed for 1 h to 2×10^5 ^{59}Fe-IFLE in 200 μl of TC20 containing the various serum components, monoclonal antibodies, or spectrin. After removal of the medium which contained the unassociated ^{59}Fe-IFLE, the macrophage monolayers were washed six times with 1 ml of HBSS, digested in NaOH and their ^{59}Fe content counted as described previously. The extent of association of the ^{59}Fe-IFLE with the culture wells in the absence of metabolically active macrophages (non-specific uptake) was assessed using empty culture wells or wells with monolayers of heat-killed macrophages (Wiener & Wickramasinghe 1982). Heat-killed macrophages were used in experiments where the association between ^{59}Fe-IFLE and macrophages was studied in the presence of IgG-depleted HI-NBCS. Under these circumstances non-specific uptake was considerably lower with wells containing heat-killed macrophages than with empty ones. In the presence of unfractionated HI-NBCS, non-specific uptake was similar with both kinds of culture dishes. The association of the ^{59}Fe-IFLE with macrophages was defined as the difference between the radioactivity in the cultures with monolayers of living macrophages and the non-specific uptake. It was expressed as a percentage of the total radioactivity added (percentage ^{59}Fe-IFLE-macrophage association).

Treatment of ^{59}Fe-IFLE with neuraminidase. ^{59}Fe-IFLE were treated with neuraminidase as described previously (Wiener & Wickramasinghe 1983*a*) employing 0.20 units enzyme/ml.

Binding of PNA to ^{59}Fe-IFLE. Washed neuraminidase-treated ^{59}Fe-IFLE were resuspended in PBS to a concentration of

2.5×10^7 cells/ml. Aliquots of the cell suspensions were incubated with equal volumes of PNA in PBS (1 mg/ml) at room temperature for 15 min. The cells were finally washed twice in PBS.

Treatment of macrophages with trypsin. Forty-eight hour macrophage cultures were washed twice with 1 ml of HBSS, incubated for 15 min at 37°C with 200 μl TC20 containing trypsin in different concentrations and washed three times with 2 ml of HBSS.

Preparation of IgG-depleted HI-NBCS. Ammonium sulphate was added to HI-NBCS at a concentration of 45% (w/v) and the mixture allowed to stand at room temperature for 30 min. The sediment was removed by centrifugation (3000 *g*, 30 min) and the supernatant dialysed extensively against phosphate-buffered saline (PBS), pH 7.4. The procedure was repeated once and the final product concentrated *in vacuo*, dialysed against HBSS, and made up to its original volume with HBSS (Johnstone & Thorpe 1982). Immuno-electrophoresis of the IgG-depleted HI-NBCS revealed total absence of the IgG line.

Absorption of HI-NBCS with rabbit and human red cells. Freshly drawn rabbit and human red cells were washed three times in PBS. One volume of HI-NBCS was incubated with one volume of packed red cells at 37°C for 30 min. The red cells were sedimented by centrifugation and the sera collected and stored at 4°C until used.

Enumeration of macrophages in monolayers. In order to assess the effect of trypsin treatment of macrophages on their subsequent interaction with ^{59}Fe-IFLE, it was important to determine its influence on the number of macrophages in the monolayers. To this effect trypsin-treated and control macrophage cultures were incubated at 37°C with 200 μl TC20 containing 50% HI-NBCS, in the absence of any ^{59}Fe-IFLE. After 1 h the medium was removed and the monolayers washed six times with 1 ml of HBSS. The macrophages were incubated at room temperature for 15 min with 1 ml of Isoton (Coulter Electronics Ltd, Luton) containing three drops of Zapoglobin (Coulter Electronics Ltd)/10 ml in order to lyse the cells and release their nuclei (Newman *et al.* 1980). The latter were counted in a Coulter Counter Model FN (Coulter Electronics Ltd).

Determination of antispectrin antibodies in mouse serum. A standard ELISA assay was performed on double dilutions of mouse sera using human red cell spectrin-coated flat 96-well plates (Sterilin Ltd, Teddington, Middlesex). Alkaline phosphatase-conjugated rabbit anti-mouse IgG (affinity-isolated antigen-specific antibody) (Sigma Ltd) was employed as the second antibody with *P*-nitrophenylphosphate (Sigma Ltd) as the substrate. The product of the enzyme reaction was quantified by measuring its optical density at 405 nm using a Titertek Multiscan ELISA plate reader (Flow Labs, Irvine, Scotland). Negative controls consisted of serum dilutions in wells not coated with spectrin. Mouse ascitic fluid containing monoclonal anti-human α-spectrin antibody served as a positive control.

Results

^{59}Fe-IFLE-macrophage interaction in the presence of IgG-depleted HI-NBCS.

HI-NBCS was IgG-depleted and tested for its activity to promote the association of untreated and neuraminidase-treated ^{59}Fe-IFLE with macrophages. Following the removal of IgG from the serum there was only an insignificant decrease in the percentage association of untreated ^{59}Fe-IFLE with the phagocytes (Fig. 1). However, when the erythroblasts had been cultured in serum-free medium during the 12-h period before their harvest, ^{59}Fe-IFLE-macrophage interaction in the presence of IgG-depleted HI-NBCS was only 60% of that with unfrac-

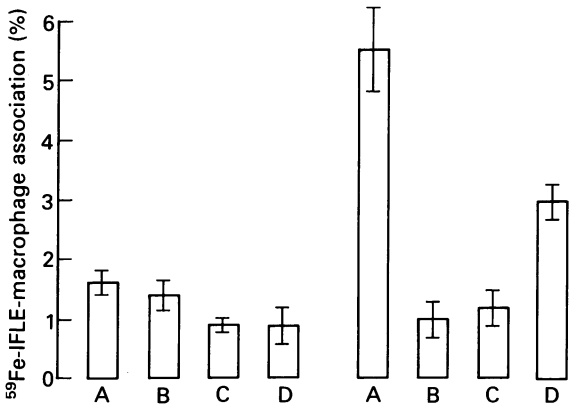

Fig. 1. ^{59}Fe-IFLE-macrophage association in the presence of IgG-depleted HI-NBCS and its stimulation by bovine IgG. The macrophage cultures were seeded with 7×10^5 peritoneal cells and 2×10^5 ^{59}Fe-IFLE were added to each culture in medium containing either 50% HI-NBCS or 50% IgG-depleted HI-NBCS, with or without 13 mg/ml bovine IgG or 13 mg/ml BSA. (*left*) Untreated ^{59}Fe-IFLE ($n=6$); (*right*) neuraminidase-treated ^{59}Fe-IFLE ($n=12$). Neuraminidase treatment of ^{59}Fe-IFLE was performed using 0.2 U neuraminidase (*Cl. perfringens*)/ml. The vertical bars represent ± 1SE. A, 50% HI-NBCS; B, 50% IgG-depleted HI-NBCS; C, 50% IgG-depleted HI-NBCS + 13 mg BSA/ml; D, 50% IgG-depleted HI-NBCS + 13 mg bovine IgG/ml.

tionated serum (result not shown). The interaction of the neuraminidase-treated cells with the macrophages, was reduced to less than 20% of the value found with the unfractionated HI-NBCS ($P<0{\cdot}001$) (Fig. 1). The addition of 13 mg/ml bovine IgG to the IgG-depleted HI-NBCS did not alter the percentage association of untreated ^{59}Fe-IFLE with the phagocytes. However, it markedly stimulated the interaction of the macrophages with neuraminidase-treated ^{59}Fe-IFLE ($P<0{\cdot}001$) which rose to approximately 60% of the value found with the native HI-NBCS (Fig. 1). Supplementation of the IgG-depleted HI-NBCS with the same concentration of BSA failed to significantly alter the association with the phagocytes of either the untreated or enzyme-treated erythroblasts (Fig. 1).

The interaction of trypsin-treated macrophages with ^{59}Fe-IFLE

Mouse peritoneal macrophages express trypsin-sensitive as well as trypsin-resistent receptors for the Fc portion of IgG immunoglobulins (Unkeless *et al.* 1981). Experiments were performed to determine which of these receptors might be involved in erythroblast phagocytosis.

Trypsinization of macrophages with 0.1 and 0.5% (w/v) enzyme caused a progressive reduction in their interaction with both untreated and neuraminidase-treated ^{59}Fe-IFLE. When the enzyme concentration was increased to 1% there was no further decline in erythroblast–macrophage association which remained around 25% of the control value (Table 1, Fig. 2). When macrophages pretreated with 1% trypsin were re-incubated for 24 h in serum-free medium (TC20) and then exposed to the erythroblasts, ^{59}Fe-IFLE-macrophage association approached control values (results not shown). None of the trypsin concentrations employed had any effect on the number of macrophages in the monolayers (Fig. 2).

Does IFLE-macrophage interaction involve antibodies specific for galactose moieties of desialated red cell membrane glycoproteins?

Neuraminidase treatment of IFLE renders them agglutinable by the lectin PNA (Wiener

Table 1. The effect of trypsin treatment of macrophages on their subsequent association with ^{59}Fe-IFLE. The macrophage cultures were seeded with 7×10^5 peritoneal cells and treated with 1% (w/v) trypsin; 2×10^5 ^{59}Fe-IFLE were added to each culture in the presence of 50% HI-NBCS. Neuraminidase treatment of ^{59}Fe-IFLE was performed with 0.2 U neuraminidase (*Cl. perfringens*)/ml.

^{59}Fe-IFLE	^{59}Fe-IFLE-macrophage association (mean ± 1SE)		
	Untreated macrophages	Trypsin-treated macrophages	*P*
Untreated	2.7±0.5 (n=6)	0.7±0.2 (n=6)	<0.001
Neuraminidase-treated	8.4±0.9 (n=12)	2.0±0.2 (n=9)	<0.001

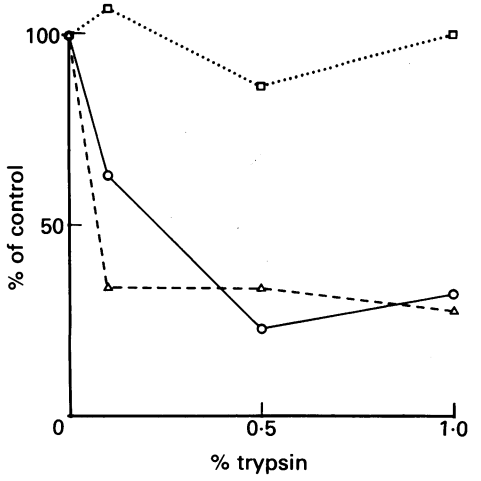

Fig. 2. The effect of trypsin treatment of macrophages on their adherence to culture dishes and subsequent association with ^{59}Fe-IFLE. The macrophage cultures were seeded with 7×10^5 peritoneal cells and 2×10^5 ^{59}Fe-IFLE added to each culture in medium containing 50% HI-NBCS. Neuraminidase treatment of ^{59}Fe-IFLE was performed using 0.2 U neuraminidase (*Cl. perfringens*)/ml. □ No. of macrophages in monolayers; △ association of untreated ^{59}Fe-IFLE with macrophages; ○ association of neuraminidase-treated ^{59}Fe-IFLE with macrophages.

& Wickramasinghe 1983*b*) which recognizes D-galactose moieties (Lis & Sharon 1977). As neuraminidase-treated ^{59}Fe-IFLE require IgG for their association with macrophages it was of interest to test whether galactose-specific antibodies of the HI-NBCS participate in the process. Hence, the interaction of neuraminidase-treated ^{59}Fe-IFLE with macrophages was assessed in the presence of 50% HI-NBCS and 0·075 M D-galactose. Under these conditions, the sugar did not cause any inhibition of ^{59}Fe-IFLE-macrophage association (Table 2). Nor did preincubation of neuraminidase-treated ^{59}Fe-IFLE with PNA (to block any galactose moieties exposed on the cell surface) impair their subsequent association with macrophages in the presence of 50% HI-NBCS (Table 2).

Moreover HI-NBCS absorbed with rabbit red cells (which have galactose-moieties exposed on their surface) (Skutelsky *et al.* 1977) was as effective as HI-NBCS absorbed with human red cells which lack free galactose residues on their surface, in promoting the association of neuraminidase-treated ^{59}Fe-IFLE with macrophages (Table 2).

Does IFLE-macrophage interaction involve antibodies specific for the red cell membrane skeleton protein spectrin?

The association of untreated and neuraminidase-treated ^{59}Fe-IFLE with macrophages was determined in the presence of HI-NBCS and different concentrations of spectrin. Fig. 3 shows that this membrane protein caused a dose-related inhibition of the interaction with macrophages of untreated as well as neuraminidase-treated ^{59}Fe-IFLE. When 30

Table 2. Results of experiments designed to test whether galactose-specific antibodies play a role in the association with macrophages of neuraminidase-treated ^{59}Fe-IFLE

^{59}Fe-IFLE	Assay medium	^{59}Fe-IFLE-macrophage association (%)
Neuraminidase-treated	50% 199—50% HI-NBCS containing 0.075 M D-galactose	12.6*
	50% 199—50% HI-NBCS	12.6
Neuraminidase-treated	50% 199—50% HI-NBCS absorbed with rabbit red cells	4.4†
	50% 199—50% HI-NBCS absorbed with human red cells	3.9
Neuraminidase + PNA-treated	50% 199—50% HI-NBCS	3.1†
Neuraminidase-treated	50% 199—50% HI-NBCS	3.0

* 10^6 ^{59}Fe-IFLE were added to macrophage cultures initially seeded with 2×10^6 PC.
† 2×10^5 ^{59}Fe-IFLE were added to macrophage cultures initially seeded with 7×10^5 PC.

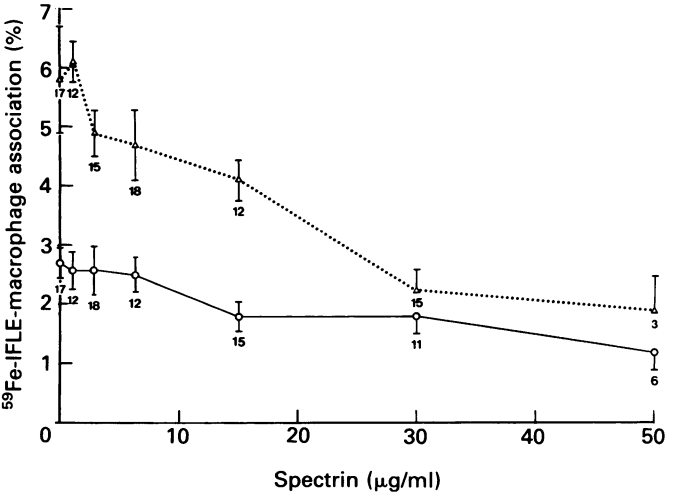

Fig. 3. The effect of spectrin on ^{59}Fe-IFLE-macrophage association. The macrophage cultures were seeded with 7×10^5 peritoneal cells, and 2×10^5 ^{59}Fe-IFLE were added to each culture in medium containing 50% HI-NBCS, Neuraminidase treatment of ^{59}Fe-IFLE was performed using 0.2 U neuraminidase (*Cl. perfringens*)/ml. The vertical bars represent ± 1SE. ○ Untreated ^{59}Fe-IFLE; △ neuraminidase-treated ^{59}Fe-IFLE.

μg/ml spectrin was added to the assay medium the residual binding activity for the control and enzyme-treated erythroblasts amounted to 67% and 38%, respectively, of the values in the absence of spectrin ($P<0.05$). In the presence of 50 μg spectrin/ml the depression of ^{59}Fe-IFLE-macrophage association was even more pronounced (44% and 33% respectively; $P<0.05$).

Fig. 4 gives the results of studies of the association of neuraminidase-treated ^{59}Fe-

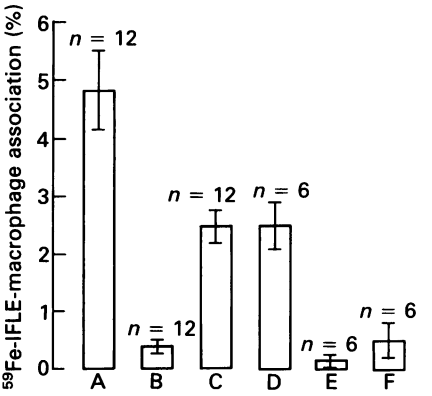

Fig. 4. The effect of mouse monoclonal antispectrin antibodies on the association of neuraminidase-treated ^{59}Fe-IFLE with macrophages. The macrophage cultures were seeded with 7×10^5 peritoneal cells, and 2×10^5 ^{59}Fe-IFLE added to each culture in medium containing 50% HI-NBCS or 50% IgG-depleted HI-NBCS, with or without mouse monoclonal antispectrin or anti-epidermal growth factor receptor (EGR) antibodies in various dilutions. Neuraminidase treatment of ^{59}Fe-IFLE was performed with 0.2 U neuraminidase (*Cl. perfringens*)/ml. The vertical bars represent ± 1SE. A, 50% HI-NBCS; B, 50% IgG-depleted HI-NBCS; C, 50% IgG-depleted HI-NBCS + antispectrin 1:50; D, 50% IgG-depleted HI-NBCS + antispectrin 1:20; E, 50% IgG-depleted HI-NBCS- + anti EGR 1:50; F, 50% IgG-depleted HI-NBCS- + anti EGR 1:20.

IFLE with macrophages in the presence of IgG-depleted HI-NBCS and two concentrations of monoclonal antibody against spectrin. It is evident that ascites fluid containing monoclonal antispectrin in a dilution of 1/50 and 1/20 markedly stimulated ^{59}Fe-IFLE-macrophage interaction ($P < 0.001$) which was restored to about 50% of the value obtained with unmodified HI-NBCS. Higher dilution of the antibody had no effect on the association of the erythroblasts with the phagocytes (results not shown). The addition of ascitic fluid containing monoclonal antispectrin in a dilution of 1/50 to IgG depleted HI-NBCS containing 13 mg/ml bovine IgG, did not increase the association of the erythroblasts with the phagocytes above the value obtained in the presence of the IgG-enriched IgG-depleted HI-NBCS (results not shown). A control incubation with ascites fluid containing a monoclonal antibody against EGR showed no change in the association of neuraminidase-treated ^{59}Fe-IFLE with the macrophages in the presence of IgG-depleted Hi-NBCS.

Antispectrin antibodies in mouse serum

We have previously shown that mouse serum supports IFLE-macrophage interaction as does HI-NBCS (Wiener & Wickramasinghe 1983*b*). Two different batches of mouse serum were tested for their content of antispectrin antibodies. Both were found to contain IgG antibodies against spectrin in titres of at least 1:16.

Discussion

In the presence of IgG-depleted HI-NBCS the interaction with macrophages of neuraminidase-treated erythroblasts was similar to that of neuraminidase-untreated erythroblasts. However, the interaction of neuraminidase-treated but not untreated erythroblasts increased significantly upon the addition of IgG at a physiological concentration. These results imply that neuraminidase treatment of IFLE had revealed antigenic sites which required IgG antibodies for their recognition by the phagocytes. The association of untreated ^{59}Fe-IFLE with macrophages was similar in the presence of unmodified or IgG-depleted HI-NBCS. It is likely that the recognition of the untreated erythroblasts by the macrophage was mediated by immunoglobulins in the HI-NBCS which bound to their surface during their DMSO-induced differentiation in culture. This explanation is supported by the observation that untreated erythroblasts which had been cultured in the absence of serum during the 12-h period before their harvest became partially IgG-dependent for their association with the phagocytes.

Trypsinization of the macrophages caused partial inhibition of their subsequent associ-

ation with untreated or neuraminidase-treated ^{59}Fe-IFLE in the presence of HI-NBCS. This suggests that trypsin-sensitive as well as trypsin-resistent sites on the macrophage surface are involved in the recognition of either untreated or neuraminidase-treated erythroblasts. In view of the present demonstration of the IgG dependence of the interaction with macrophages of neuraminidase-treated ^{59}Fe-IFLE, these membrane sites are likely to include the trypsin-sensitive and trypsin-resistent IgG Fe receptors of mouse peritoneal macrophages defined by others (Unkeless *et al.* 1981). When trypsin-treated macrophages were re-incubated in a serum-free culture medium, their ability to interact with erythroblasts was restored, thereby excluding the possibility that trypsin-sensitive cytophilic antibodies bound to macrophages play any role in their interaction with the erythroblasts.

There is evidence that antibodies against galactose residues of desialated red cell membrane play a role in the elimination of senescent (Alderman *et al.* 1981) and thalassaemic (Galili *et al.* 1983) erythrocytes by the mononuclear phagocyte system *in vivo*. In the present study the association with macrophages of neuraminidase-treated ^{59}Fe-IFLE was not inhibited by the presence of D-galactose in the assay medium or by pre-treatment of the erythroblasts by PNA which binds to galactose residues on the red cell membrane. Nor did absorption of HI-NBCS by rabbit erythrocytes, which have galactose molecules on their surface, decrease its activity to promote the interaction between macrophages and neuraminidase-treated ^{59}Fe-IFLE. These results make it unlikely that antibodies against galactose residues of desialated red cell membrane glycoproteins play any important role in the IFLE-macrophage interaction.

In the presence of relatively low concentrations of human spectrin and 50% HI-NBCS the association with macrophages of untreated as well as neuraminidase-treated ^{59}Fe-IFLE was significantly inhibited. Moreover a mouse monoclonal antibody against human spectrin was found to promote the interaction with macrophages of neuraminidase-treated ^{59}Fe-IFLE, when IgG-depleted HI-NBCS was employed in the assay medium. In addition, two different batches of mouse serum were found to contain antibodies against spectrin. These results suggest that the cytoskeletal protein, spectrin, of neuraminidase-treated IFLE may have become accessible to spectrin-specific antibodies and that these antibodies could play a role in the interaction with macrophages. Spectrin added to the assay medium would then inhibit this process by competing for these antibodies with spectrin on the erythroblast membrane. The association of neuraminidase-treated ^{59}Fe-IFLE with the phagocytes in the presence of IgG-depleted HI-NBCS and monoclonal anti-spectrin antibodies was considerably lower than that found in the presence of unmodified HI-NBCS. This might be due to low activity of the monoclonal antispectrin antibody to mediate phagocytosis compared to that of the naturally occurring polyclonal antibodies. Alternatively, the HI-NBCS could contain antibodies against other membrane proteins or glycoproteins of IFLE which also participate in their interaction with macrophages. Our finding that human spectrin and anti-human spectrin antibodies effect the association with macrophages of mouse erythroblasts is in keeping with the recent observation that human and mouse spectrin are structurally similar (Whitfield *et al.* 1983).

Spectrin, which is located on the inner aspect of the red cell membrane is thought to be linked indirectly to outer membrane glycoproteins and lipids (for review see Cohen 1983). It is not clear if the inferred accessibility of the spectrin of IFLE to specific antibodies is compatible with cell viability or a feature of cell death. Thermodynamical considerations indicate that it is unlikely that spectrin of viable erythroblasts is accessible to specific antibodies which are unlikely to be able to cross the intact phospholipid bilayer of the membrane. It therefore seems possible

that antispectrin is involved in the elimination by macrophages of non-viable rather than viable erythroblasts as the IFLE preparations contain a low percentage (< 10%) of dead cells (Wiener & Wickramasinghe 1982). However, the possibility that spectrin of defective but live erythroblasts becomes exposed to specific immunoglobulins by as yet unknown mechanisms cannot be ruled out.

It has recently been demonstrated that healthy blood donors have auto-antibodies against spectrin in their plasma (Lutz & Whipf 1982). The present data show that mouse serum also contains anti-spectrin antibodies. The role of these naturally occurring antibodies in humans and mice is unknown. In view of the present results it seems possible that anti-spectrin antibodies mediate the elimination of defective erythroblasts by bone marrow macrophages in the course of the minor degree of ineffective erythropoiesis which occurs in the healthy state.

Acknowledgements

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