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SIMULTANEOUS INTERACTION OF MONOCLONAL ANTIBODY-TARGETED LIPOSOMES WITH TWO RECEPTORS ON K562 CELLS

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We have investigated the interaction of targeted liposomes with human erythrocytes, and K562 cells, a human leukemic line which expresses both glycophorin A and Fc receptors. Liposomes conjugated to monoclonal anti-human glycophorin A bind to human erythrocytes in 80-fold greater amounts than liposomes conjugated to a non-specific monoclonal antibody. Binding is inhibited by soluble anti-glycophorin but not by its Fab fragment. In contrast, binding of antibody-conjugated liposomes to K562 cells is very high irrespective of the specificity of the antibody. Liposomes conjugated to a nonspecific monoclonal antibody interact with K562 cells via an Fc receptor, and binding is inhibited by soluble human IgG. Liposomes conjugated to anti-human glycophorin A interact with K562 cells via an Fc receptor and glycophorin A. Binding is not inhibited by either human IgG or anti-glycophorin Fab alone. Binding is only partially inhibited by anti-glycophorin, or by human IgG in the presence of anti-glycophorin Fab, and completely inhibited only by human IgG in the presence of anti-glycophorin. Simultaneous binding of targeted liposomes to two cell membrane antigens is therefore partially resistant to inhibition by single soluble ligands even when they are present in large excess. We conclude that simultaneous binding to more than one receptor may be of considerable advantage for in vivo applications of targeted liposomes.

Introduction

The targeting of liposomes with monoclonal antibodies [1] is a promising method for specific delivery of drugs to tumor cells. Appropriate choice of antibody should permit liposome targeting to a surface antigen expressed only on the target cells. The resultant binding of antibody-targeted liposomes to the cell surface may promote their internalization by triggering endocytosis [2], which should afford efficient delivery of liposome-encapsulated macromolecules or drugs to cells.

In previous studies [1], a 200-fold increase in liposome binding to erythrocytes was observed when the liposomes were covalently conjugated to a polyclonal rabbit anti-human erythrocyte $F(ab')_2$. Moreover, hemagglutination studies suggested that the conjugated liposomes, being a multivalent complex of antibodies, had a higher affinity for the cells than the unconjugated antibody [1].

The multivalent interaction of antibody-targeted liposomes with the cell surface could be important in the in vivo use of targeted liposomes for several

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Abbreviations: Mes, 4-morpholineethanesulphonic acid; Mops, 4-morpholinepropanesulphonic acid.

reasons. The proportion of targeted liposomes which bind to the target cells will be greater than the proportion of unconjugated antibody which would bind to cells under the same conditions, because the affinity of the liposomes for the cells is greater than that of the unconjugated antibody. For the same reason, the binding of targeted liposomes to cells may be partially insensitive to inhibition by the monovalent antigens which are frequently released into extracellular fluids by tumor cells.

It is possible to confer specificity on each liposome for more than one antigen by conjugation to a mixture of monoclonal antibodies. Liposomes capable of binding to several antigens on the cell surface may be valuable for several reasons. Such interactions may be even more insensitive to inhibition by soluble antigens than the interaction of liposomes directed to a single determinant. Moreover, targeting to several specificities on the tumor cell surface may allow liposomes to be targeted to tumors which do not possess a unique antigen, but rather display a unique combination of two or more antigens. The latter possibility may circumvent the central problem in tumor immunology: the lack of tumor specific antigens.

With these possibilities in mind, we have further examined the interaction of targeted liposomes with human erythrocytes, and with K562 cells, a human cell line derived from a patient with chronic myelogenous leukemia in blast transformation [3]. K562 cells have both erythroid and granulocytic characteristics and express both glycophorin A [4] and Fc receptors [5]. Liposomes conjugated to a nonspecific monoclonal antibody bind to K562 cells via an Fc receptor without prior antigen-antibody interaction. Liposomes targeted with monoclonal anti-human glycophorin A bind to K562 cells by interactions with both Fc receptor and glycophorin A. The simultaneous interaction of targeted liposomes with Fc receptors and glycophorin A on K562 cells is less susceptible to soluble ligand inhibition than the interaction of targeted liposomes with a single surface receptor.

Methods

Liposomes were prepared in 50 mM Mes, 50 mM Mops, 40 mM NaCl, 1 mM disodium ethylenediaminetetraacetate (buffer 1) (pH 6.7) adjusted to 290 mOsm with sodium chloride. The vesicles were prepared by the method of Szoka and Papahadjopoulos [6] from phosphatidylcholine : cholesterol : 4-(*p*-maleimidophenyl)butyrylphosphatidylethanolamine (10 : 10 : 1), plus trace [^{3}H]dipalmitoylphosphatidylcholine (spec. act. 500 Ci/mol) to give 2000 cpm/nmol lipid. The liposomes were conjugated to the murine monoclonal anti-human glycophorin A or monoclonal anti-sheep erythrocyte antibody by the method of Martin and Papahadjopoulos [7] with modifications. LICR Lon R10, a hybridoma secreting anti-glycophorin A, IgG_1 was kindly provided by Dr. P.A. Edwards [8]. The S-S.1 hybridoma, secreting anti-sheep erythrocyte antibody, IgG_{2A}, was obtained from Dr. M. Cohn of the Salk Institute. The hybridomas were grown in Balb/c mice, primed intraperitoneally with pristane. The antibodies were purified from the ascitic fluid on a staphylococcal protein A affinity column [9]. The purified antibody was extensively dialyzed with distilled water and lyophilised. Prior to use, antibody was dissolved and centrifuged for $3 \cdot 10^6 \times g$ min to remove aggregates. The intact antibody was thiolated for conjugation [10] at an *N*-succinimidyl 3-(2-pyridyldithio)propionate : protein ratio of 15 : 1 which gave five reactive thiol groups per IgG molecule. After reduction in 25 mM dithiothreitol at pH 4.5, the antibody was purified on a 2×30 cm Sephadex G50 column equilibrated with argon-purged buffer 1 (pH 6.7). The vesicles were added to the antibody in a 10 ml Amicon concentrating chamber under argon. The mixture was reduced to approx. 4 ml, and allowed to react at room temperature for 16 h. All procedures after the reduction of the derivatized antibody were performed under argon. The vesicles were separated from unbound antibody by flotation in metrizamide [11] and analyzed for lipid [12] and protein [13]. The properties of the products are given in the legends to the figures. Human erythrocytes were washed three times in isotonic saline and resuspended in incubation buffer (calcium, magnesium free Dulbecco's phosphate-buffered saline with 1% bovine serum albumin). K562 cells were cultured in RPMI 1640 medium supplemented with 15% (v/v) foetal calf serum, 100 U/ml penicillin and streptomycin sulphate

and grown in 5% CO_2. Cells at 10^6/ml of medium were harvested by centrifugation for 10 min at $1200 \times g$ in a Beckman Model TJ-6 centrifuge. The cell pellet was resuspended in incubation buffer.

For binding studies, 10^7 erythrocytes or $2 \cdot 10^6$ K562 cells were incubated for 1 h at 37°C with varying amounts of targeted or non-targeted liposomes (0–100 nmol lipid) in duplicate, in 0.2. ml incubation buffer. The cells were separated from unbound vesicles by centrifugation through 10% w/v Dextran T40 in incubation buffer. The cell pellet was resuspended and taken up in scintillant for measuring the amount of associated [^{3}H] lipid-labelled vesicles. Hemoglobin caused 15% quenching of counts which was corrected for with standards. In all experiments, the mean of duplicate points are shown. The standard deviation did not exceed 5%.

Three types of antibody inhibition studies were performed. Co-incubation: 20 nmol of targeted liposomes were mixed, in duplicate, with varying amounts of soluble antibody and incubated with 10^7 erythrocytes or $2 \cdot 10^6$ K562 cells for 1 h at 37°C in 0.2 ml incubation buffer. Preincubation: soluble antibody or antibody fragments were incubated with cells for 15 min to 1 h at 37°C, followed by 20 nmol of targeted liposomes for 1 h. Post-incubation: 20 nmol of targeted liposomes were incubated with cells for 1 h at 37°C, followed by soluble antibody for 1 h. The cells with associated targeted vesicles were separated from unbound material by centrifugation through 10% w/v dextran and counted for lipid label as described above. Results are expressed as a percentage of lipid binding to cells in the absence of competing free soluble ligand.

For ligand inhibition studies antibodies were dissolved in phosphate-buffered saline and centrifuged at $100000 \times g$ for 30 min on a Beckman model L3-50 ultracentrifuge to remove aggregated protein. The aggregated human IgG was prepared by heating a protein solution at 63°C for 20 min, followed by cooling overnight at 4°C. Protein clumps were removed by centrifugation at $1200 \times g$ for 10 min, and the supernatant was filtered through a Millipore filter (0.2 μM pore size). Protein concentration was determined by the method of Lowry et al. [13]. Murine monoclonal Fab, anti-human glycophorin A, was prepared by papain (Sigma Biochemicals, St. Louis, MO) digestion of the intact antibody, following the procedure of Dresser [14]. Undigested IgG and free Fc was absorbed on a protein A-Sepharose CL-4B column (Pharmacia) equilibrated with 0.1 m phosphate buffer (pH 8.0) [15]. The absence of undigested IgG in the final preparation was checked by slab gel electrophoresis in 10% (w/v) SDS-polyacrylamide [16].

120 μg of Fab or intact anti-glycophorin A antibody was radio-iodinated at room temperature using 0.05 U lactoperoxidase, 0.01 U glucose oxidase, 0.2% glucose, 1 mCi Na^{125}I and calcium, magnesium-free phosphate-buffered saline for 4 min in a total volume of 0.2 ml. ^{125}I-labelled protein was separated from free Na^{125}I by gel filtration on a Sephadex G-25 column (Pharmacia PD-10) equilibrated with phosphate-buffered saline. The final concentrations and specific activity of the radio-iodinated Fab and intact antibody were 72 μg/ml, $3.685 \cdot 10^6$ cpm/μg and 56 μg/ml, $17.795 \cdot 10^6$ cpm/μg, respectively.

Human erythrocytes membranes, free of hemoglobin, were prepared by the method of Dodge et al. [17]. The red cell membrane components were separated by slab gel electrophoresis in 10% (w/v) SDS-polyacrylamide [16]. 120 μg of protein were added to each of six wells. After fixing the gel in 25% isopropyl alcohol/10% acetic acid, one half of the gel was stained for glycoprotein with periodic acid Schiff reagent and the other half was prepared for autoradiography. The gel was suspended in 40 ml Burridges buffer (50 mM Tris/150 mM NaCl/8 mM NaN_3, pH 7.4) with 3% bovine serum albumin and 250 μl ^{125}I-labelled Fab and left with gentle mixing at room temperature overnight. The gel was washed extensively in Burridges buffer, dried and subjected to autoradiography on Kodak NS-2T X-ray film for 24 h.

For binding studies, 10^7 human erythrocytes (duplicate samples) were incubated for 15 min at room temperature with 0–1.44 μg of ^{125}I-labelled Fab or intact anti-human glycophorin, in 0.21 ml incubation buffer. the cells were separated from unbound protein by centrifugation through 0.8 ml of 1 : 9 Apezion A oil: *n*-butylphthalate (2 min, Beckman Microfuge B). The supernatant was discarded and ^{125}I activity associated with erythro-

cytes counted in a Beckman 300 series gamma counter. Specificity of binding was verified by the inhibition of cell binding with a 100-fold excess (118 μg) of unlabelled intact antibody.

Results

Binding to human erythrocytes

Table I presents the binding of ^{125}I-labelled anti-human glycophorin A antibody or its ^{125}I-labeled Fab fragment to human erythrocytes. Specificity of binding was demonstrated by its inhibition with a 100-fold excess of unlabelled antibody. Prior incubation of red cells with a 10-fold excess of soluble human IgG did not inhibit binding of either Fab or intact IgG. Intact anti-human glycophorin A bound to erythrocytes in 10-fold greater amounts than Fab fragment.

Fig. 1 shows SDS-polyacrylamide gels of human red cell membranes. The bands are visualized either with periodic acid-Schiff staining of glycoproteins or by autoradiography after exposure to ^{125}I-labeled Fab fragment of the anti-glycophorin A. The monomer and dimer of glycophorin A are clearly visible by Schiff stain and by localization of the iodinated ligand. In addition high molecular weight aggregates of glycophorin A, although only faintly visible on Schiff staining, were clearly visible on the autoradiography. We may therefore confirm [8] that LICR R10 and its Fab fragment interact with human glycophorin A.

TABLE I

BINDING OF ^{125}I-LABELLED Fab MONOMER OR ^{125}I-LABELLED INTACT ANTI-HUMAN GLYCOPHORIN A TO HUMAN ERYTHROCYTES

Varying amounts of antibody or Fab monomer was incubated with 10^7 human erythrocytes in 0.21 ml phosphate-buffered saline. Binding expressed as a percent of antibody added ± 1 S.D.

Protein added (ng)	Binding (percent of added)	
	Fab	Intact IgG
288	2.8 ± 0.4	31.1 ± 0.02
576	3.5 ± 0.9	32.8 ± 0.01
864	3.9 ± 0.1	33.0 ± 9.0
1 152	4.5 ± 0.3	37.4 ± 2.0
1 440	3.5 ± 0.2	32.0 ± 7.9

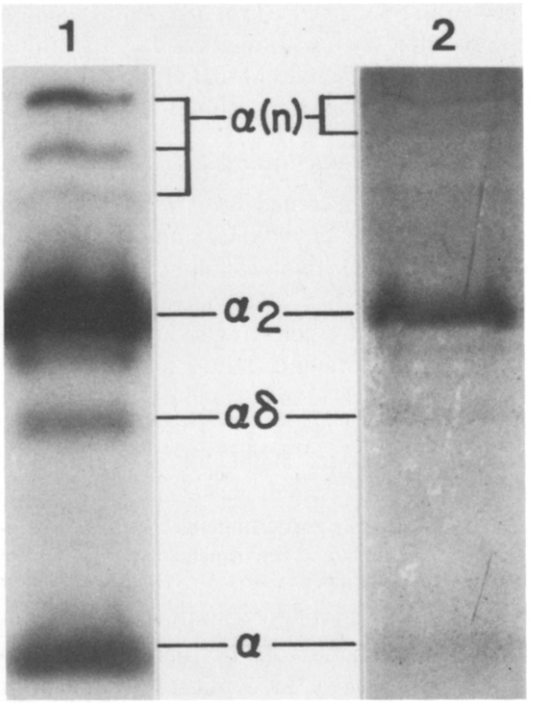

Fig. 1. SDS-polyacrylamide gel electrophoresis of human red cell membranes. Gels were stained with ^{125}I-labelled Fab monomer anti-human glycophorin A (1) or periodic acid schiff (2). Nomenclature after Anstee [23] α(n), macroaggregated glycophorin A; α_2, glycophorin A dimer; α_δ, glycophorins A and B; α, glycophorin A monomer.

Fig. 2 shows the binding of liposomes to human erythrocytes. 60–80% of liposomes conjugated to anti-human glycophorin A bind to the cells when they are incubated with 1–80 nmol lipid. Binding of unconjugated liposomes to erythrocytes is highest at the lowest level of lipid addition and varies between 1 and 3%. The greatest effect of targeting is seen when erythrocytes are incubated with 74 nmol of lipid. At this lipid concentration, targeted liposomes bind in 65-fold greater amounts than unconjugated liposomes. The binding of liposomes conjugated with anti-sheep erythrocyte antibody to human erythrocytes is low and similar to that of unconjugated liposomes (data not shown).

Fig. 3 shows the inhibitory effect of soluble anti-human glycophorin A on the binding of 20 nmol of targeted (anti-glycophorin conjugated) liposomes to human erythrocytes. In the co-incubation experiment, there is strong inhibition of

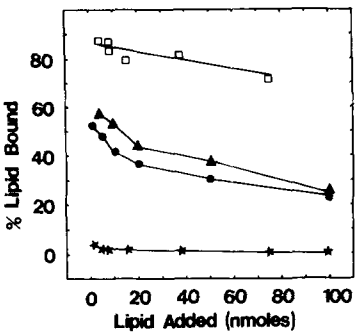

Fig. 2. The association of targeted liposomes with cells. □, 10^7 human erythrocytes incubated with liposomes conjugated to anti-human glycophorin A. ▲, $2 \cdot 10^6$ K562 cells incubated with liposomes conjugated to anti-sheep erythrocyte antibody. ●, $2 \cdot 10^6$ K562 cells incubated with liposomes conjugated to anti-human glycophorin A. ★ 10^7 human erythrocytes incubated with liposomes without conjugated antibody. A similar experiment with $2 \cdot 10^6$ K562 cells showed similar levels of binding of uncoated liposomes (data not shown). Anti-glycophorin coated liposomes contained 225 g antibody per mol lipid, and $4.69 \cdot 10^{12}$ cpm/mol lipid. Anti-sheep erythrocyte-coated liposomes contained 140 g antibody per mol lipid and $3.88 \cdot 10^{12}$ cpm per mol lipid. Uncoated lipsomes contained $4.02 \cdot 10^{12}$ cpm per mol lipid.

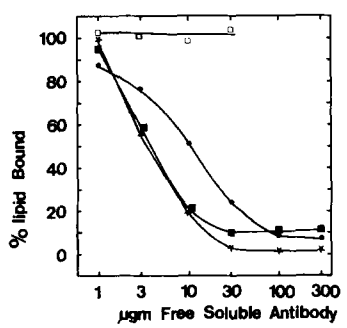

Fig. 3. The inhibition of binding of anti-human glycophorin A targeted liposomes to human erythrocytes by soluble ligands. 10^7 human erythrocytes were incubated with 20 nmol liposomes conjugated to anti-human glycophorin A (272 g protein per mol lipid; $1.37 \cdot 10^{12}$ cpm per mol lipid) in the presence of varying amounts of anti-human glycophorin A, or its Fab fragment. Binding is expressed as a percentage of the binding which occurs in the absence of the competing ligands. ■, Cells were co-incubated with antibody and liposomes. ●, Cells were incubated with antibody 1 h after liposome addition (post-incubation). ★, Cells were incubated with antibody for 1 h prior to addition of liposomes (pre-incubation). □, Cells were incubated with Fab monomer for 10 min prior to liposome addition.

binding by increasing amounts of soluble anti-glycophorin. However, addition of 30–300 μg of free antibody does not inhibit more than 90% of lipid binding. In the pre-incubation and co-incubation experiments, addition of 1–30 μg antibody has very similar effects. However, pre-incubation with 100–300 μg of free antibody inhibits 98% of the binding of targeted liposomes. Post-incubation with 1–30 μg antibody inhibits liposome binding less than co- or pre-incubation with the same amount of antibody. Post-incubation with free antibody, therefore, displaces the vesicles less effectively than co- or pre-incubation. Pre-incubation with up to 30 μg Fab fragment of anti-glycophorin A had no significant effect on liposome binding. The Fab fragment is, therefore, at least 30-times less effective than intact antibody in displacing liposome binding. Neither albumin or human IgG inhibit binding of targeted liposomes to erythrocytes (data not shown). Therefore, inhibition of liposome binding occurs only if the competing ligand interacts with glycophorin A.

Binding to K562 cells

The binding of liposomes conjugated with either monoclonal anti-sheep erythrocyte or anti-human glycophorin A to K562 cells is presented in Fig. 2. The greatest amount of vesicle-cell association was seen in the low range of lipid addition. At 1 nmol of lipid added, 58 and 52%, respectively, of the liposomes conjugated with anti-sheep erythrocyte and anti-glycophorin A antibody were bound to cells. At 100 nmol of lipid added, 25% of both types of antibody-conjugated liposomes were bound to the cells. Binding of unconjugated liposomes to K562 cells was between 2 and 3% when the cells were incubated with 1–100 nmol lipid. Therefore, high levels of liposome binding to K562 cells is dependent on the presence of antibody on the liposome surface.

Fig. 4 shows the effect of soluble or aggregated human IgG on the binding to K562 cells of liposomes conjugated with non-specific (anti-sheep erythrocyte) antibody. 10 μg aggregated or 30 μg soluble human IgG inhibits 90% of the liposome binding, which suggests that the interaction of nonspecific antibody-coated liposomes with K562 cells is mediated by an Fc receptor. In the presence of a marked excess of IgG, antibody-linked

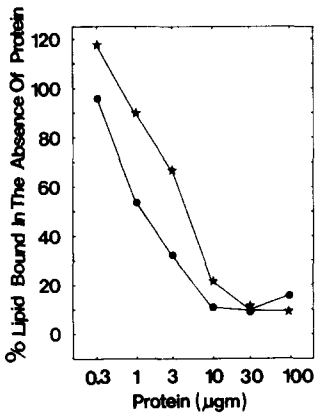

Fig. 4. Ligand inhibition of Fc mediated binding to K562. 20 nmol of lipid attached to anti-sheep erythrocyte antibody, (140 g antibody/mol, $3.88 \cdot 10^{12}$ cpm) was incubated with $2 \cdot 10^6$ K562 cells after prior incubation at 37°C, for 15 min with varying amounts of soluble (★) or aggregated (●) human IgG. Binding is expressed as a percentage of lipid binding to cells in the absence of free ligand.

liposomes bound in 5-fold greater amounts than vesicles without associated protein. Moreover, the aggregated antibody inhibits liposome binding more actively than soluble antibody. This suggests that multivalency of the antibody-linked liposomes is important in Fc receptor-mediated binding.

Table II examines the ability of soluble immunoglobulins from various species to inhibit binding of nonspecific (anti-sheep erythrocyte) antibody-conjugated liposomes to K562 cells. Normal mouse IgG, bovine IgG, rabbit Fab'_2 and mouse anti-sheep erythrocyte monoclonal were ineffective in displacing antibody-conjugated liposomes. However, mouse anti-human glycophorin A produced 53% inhibition of binding, while soluble human and rabbit IgG inhibited liposome binding by 93%.

TABLE II

EFFECTS OF VARIOUS IMMUNOGLOBULINS ON THE BINDING OF MONOCLONAL ANTI-SHEEP ERYTHROCYTE ANTIBODY-LINKED LIPOSOMES TO K562 CELLS

$2 \cdot 10^6$ K562 cells were incubated in 0.2 ml with 20 mol liposomes and 100 μg soluble antibody or fragment. Lipid binding expressed as the percent of lipid which binds in the absence of inhibiting ligand ± 1 S.D.

Immunoglobulin	Lipid binding
Normal rabbit IgG	7 ± 3
Normal mouse IgG	100 ± 15
Normal bovine IgG	99 ± 12
Monoclonal mouse anti-sheep erythrocyte IgG_{2A}	102 ± 12
Normal rabbit $F(ab')_2$	115 ± 12
Monoclonal mouse anti-human glycophorin A, IgG_1	47 ± 5
Normal human IgG	6 ± 1

The time course of the interaction of nonspecific antibody-conjugated vesicles with K562 cells is shown in Fig. 5. Binding of 20 nmol of lipid to the cells was examined over 0–120 min. The cells were pre-incubated for 45 min in the presence or absence of 5 mM azide and 50 mM 2-deoxyglucose before addition of liposomes. Binding of antibody-conjugated liposomes was complete in the first 5 min, and was unaffected by the presence of metabolic inhibitors. Therefore, binding of antibody-conjugated liposomes to the Fc receptor of K562 cells would appear not to be dependent on metabolic energy and is very rapid.

Fig. 6 shows the ligand inhibition of K562 cell interactions for liposomes conjugated with monoclonal anti-human glycophorin A. 100 μg soluble human IgG produced only 10% inhibition of binding of targeted liposomes, while 100 μg anti-human glycophorin A inhibited liposome binding by 50%. This probably reflects the interaction of the soluble anti-glycophorin A with both glycophorin

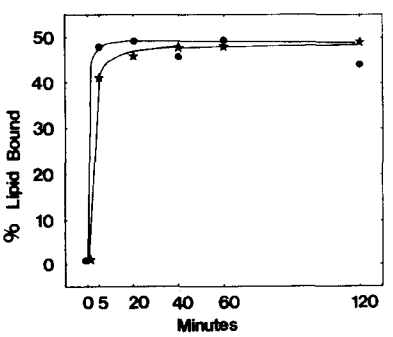

Fig. 5. The time course of liposome binding to K562 cells. 20 nmol lipid conjugated to anti-sheep erythrocyte antibody (140 g antibody/mol, $3.88 \cdot 10^{12}$ cpm/mol) was incubated with $2 \cdot 10^6$ K562 cells in the presence (★) or absence (●) of metabolic inhibitors. Binding is expressed as a percentage of the total amount of lipid added.

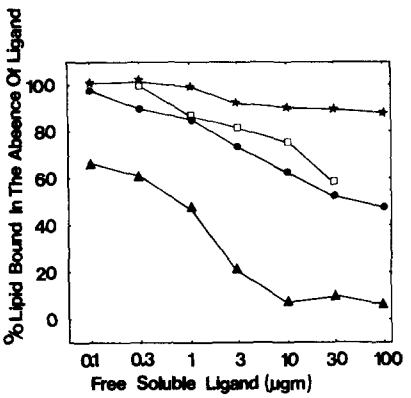

Fig. 6. Inhibition of binding of targeted liposomes to $2 \cdot 10^6$ K562 cells by soluble ligands. Cell were pre-incubated for 15 min at 37°C with varying amounts of intact free antibody or the Fab fragment of anti-human glycophorin A. Cells were incubated with liposomes for 60 min at 37°C. Data is expressed as for Fig. 5. Competing ligands were: ●, Anti-human glycophorin A; ★, soluble human IgG; □, Fab fragment of anti-human glycophorin A + 100 μg soluble human IgG; ▲, Anti-human glycophorin A + 100 μg soluble human IgG.

A and the Fc receptor on the surface of K562 cells. The greatest inhibition of vesicle binding occurred when both soluble anti-glycophorin A and 100 μg of human soluble IgG were added. Incubation with 10 μg anti-glycophorin A and 100 μg human IgG inhibited 90% of liposome binding. Studies (data not shown) with lower concentrations of lipid (1–20 nmol per 0.2 ml) showed a similar pattern for inhibition of liposome binding by soluble ligands. Prior incubation of K562 cells with the Fab fragment of anti-human glycophorin A did not inhibit the binding of anti-glycophorin A targeted vesicles (data not shown). However, in the presence of 100 μg of human IgG, Fab fragment inhibited liposome binding to K562 cells by up to 35% (Fig. 6).

Discussion

The interaction of erythrocytes with liposomes conjugated to anti-glycophorin involves a specific interaction with glycophorin A. Glycophorin A is known to be the only component of the red cell membrane with which this antibody interacts [8]. Liposome binding is inhibited by soluble anti-glycophorin A, but not by bovine serum albumin, bovine IgG, or murine IgG. The Fab fragment of anti-glycophorin A failed to inhibit liposome binding at the concentrations used, and labelled Fab monomer bound to red cells in smaller amounts than labelled intact antibody. These differences most probably occur because the divalent antibody is more effective than the monovalent Fab in binding to the cells. This implies that the antibody is able to undergo a divalent interaction with the erythrocyte membrane. It is also likely that liposome binding may involve a multivalent interaction with the erythrocyte membrane, which may arise from the binding of several antibodies on the liposome surface to the cell membrane. The following calculation demonstrates the opportunity for a multivalent interaction of targeted liposomes with the erythrocyte. We may assume that there are $5 \cdot 10^5$ glycophorin A molecules per red cell [18], the surface area of an erythrocyte is 140 μM^2 [19] and the mean diameter of the vesicles is 0.4 μM [20]. From the cross-sectional area of the liposome (mean: $1.6 \cdot 10^{-11}$ m^2), a bound vesicle would occupy a portion of membrane which contains 450 glycophorin molecules. Although the liposome may not be in contact with all of this membrane area, it will clearly be able to interact with a large number of glycophorin molecules. The extent of the interaction will also depend on the flexibility and mobility of the liposome-bound antibody molecules. We hope, in future work, to analyze the multivalent interaction of liposomes with cells in greater detail.

Several observations described in this report suggest that liposomes conjugated to anti-sheep erythrocyte antibody interact with K562 cells via an Fc receptor. First this antibody has no known specificity for K562 cells, which rules out an interaction with a cell surface antigenic determinant. Second, the interaction is inhibited by normal human IgG, an observation which usually indicates an Fc interaction. Third, the interaction is partially inhibited by nonspecific rabbit IgG, but not by rabbit $F(ab')_2$, which directly suggests a role for the Fc portion of the antibody. The Fc receptor in question appears not to require activation of the Fc region by antigen binding since, as previously noted, the conjugated anti-sheep erythrocyte antibody is not bound to its antigen. Moreover, interaction with K562 cells is inhibited

by both soluble and aggregated human IgG, respectively. It is possible that conjugation to liposomes may fortuitously activate the Fc region of the antibody, since a 30-fold excess of soluble anti-sheep erythrocyte antibody does not inhibit liposome binding. However, we believe the most likely conclusion to be that the Fc receptor in question interacts weakly with all but human IgG, and that the interaction of anti-sheep erythrocyte antibody-coated liposomes with K562 cells may be a striking example of the effect of multivalency on a low-affinity interaction.

Fc receptors capable of interacting with soluble or non-antigen bound antibody have been described. Mouse monocytes are known to have a trypsin-sensitive Fc receptor which binds murine IgG_{2A} that is not bound to its antigen [21]. More recently, Fc receptors on human monocytes have been described which interact with soluble human IgG_1 [22]. The authors [22] also observed an Fc receptor of the same type on HL60 cells, a human leukemic cell line of granulocytic origin. K562 cells, being also of granulocytic origin, may share some characteristics of HL60 cells, including the Fc receptor. Our observations provide a strong indication that K562 cells do possess the same Fc receptor as monocytes and HL60 cells.

Monoclonal anti-glycophorin A was the only murine antibody tested which inhibited the Fc-mediated interaction of liposomes with K562 cells. This inhibition was probably due to the simultaneous interaction of this antibody with glycophorin A and the Fc receptor. The specificity of the antibody for glycophorin A would be expected to enhance the weak interaction with the Fc receptor by concentrating the antibody at the cell surface. The simultaneous interaction of the anti-glycophorin A with the two receptors is strongly suggested by the binding properties of liposomes conjugated to this antibody. The interaction of liposomes conjugated to anti-glycophorin A with K562 cells is not inhibited by either human IgG or the Fab fragment of anti-glycophorin if used alone. However, if used together, they cause a 40% inhibition of liposome-cell interaction. In addition, intact anti-glycophorin A partially inhibits vesicle-cell binding, and its inhibition is considerably enhanced by human IgG. These results confirm that these vesicles interact with both Fc receptors and glycophorin A. Moreover, all cell-associated vesicles must interact with both ligands, since competition of binding only occurs if both ligands are blocked.

We have discussed the implications of liposome interactions with more than one target antigen for drug delivery to specific targets. The experiments described here demonstrate the potential of multiple receptor interactions, which are less susceptible to inhibition by soluble ligands. Preliminary experiments show that encapsulated methotrexate-γ-aspartate delivered to K562 cells by antiglycophorin A targeted liposomes, is 10-times more toxic than the free drug (Bragman, K.S., Heath, T.D., and Papahadjopoulos, D., unpublished data). Moreover, inhibition of drug delivery by targeted liposomes is best achieved by both human IgG and anti-human glycophorin A. With the increasing availability of monoclonal antibodies, the selective delivery of drugs or macromolecules via liposomes to specific cell types may become an important therapeutic tool.

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