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A sensitive method to introduce membrane-bound proteins into recipient cells based on affinity enrichment of lipid vesicles to the recipient cell prior to fusion

Håkan Eriksson [a,c], Bo Baldetorp [b], Bo Mattiasson [a] and Hans-Olov Sjögren [c]

[a] *Department of Pure and Applied Biochemistry, Chemical Center, University of Lund, Box 124, S-221 00 Lund,* [b] *Department of Oncology, University Hospital, S-221 85 Lund, and* [c] *The Wallenberg Laboratory, University of Lund, Box 7031, S-220 07 Lund (Sweden)*

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A sensitive analytical procedure for studying membrane-bound structures has been developed. Membrane glycoproteins inserted into liposomes were transferred to recipient cells by use of a lectin, concanavalin A, bound to the cells as a bridge to generate proximity between the recipient cell and the glycoprotein-containing liposome, prior to exposure to the fusing agent, poly(ethylene glycol). Partially purified histocompatibility antigen from rats was introduced into the membrane of human lymphocytes. After treating the cells with poly(ethylene glycol) under fusion conditions, some of the antigen present in the preparation could not be eluted with α-methyl mannoside and EDTA, indicating that incorporation in the cell membrane had taken place. This antigen remained exposed on the lymphocyte surface for approximately 1 h as demonstrated by sensitivity of the lymphocytes to the lytic effect of an antiserum to the histocompatibility antigen in the presence of complement. Some of the lectin molecules seemed to be internalized in the cells but no induction of cell mitosis was observed. The described method gives an opportunity to work with small amounts of membrane proteins inserted into liposomes, introducing them into recipient cells for analysis of their biological activities.

Introduction

In laboratory work, membrane proteins often present particular problems due to their lability when removed from the environment of phospholipids in the membrane and, also, because of the hydrophobic character of parts of the molecules. These properties have made it difficult to study and/or quantitate membrane proteins in a reliable way. In this context, however, liposome technology seems to offer very promising possibilities.

Membrane preparations can be introduced into foreign cells by fusion of plasma membranes or liposomes with recipient cells without severely affecting the viability of the cells. Poly(ethylene glycol), added to a mixture of cells and plasma membrane vesicles or liposomes, has been reported to cause fusion of cells with the vesicles [1–5]. Fusion of cells and liposomes containing reconstituted membrane proteins has also been reported with the use of Sendai virus glycoproteins [6–8]. These initial and very important papers indicated a possibility to transfer membrane proteins to receptor cells, but they never dealt with transfer of small amounts of membrane proteins, and those experiments were not designed to push the sensitivity of the system. When dealing with enriched preparations of a certain membrane structure, one has a markedly reduced amount available in the fusion

step and the efficiency in the subsequent fusion is consequently put under pressure.

The more purified a membrane preparation to insert is, the higher demands it puts on the fusion process. This paper addresses some of the key questions in this connection.

The present investigation was performed to develop a sensitive procedure by which small amounts of membrane proteins may be transferred to recipient cells. The general principle is to specifically enrich the membrane protein containing lipid vesicles in close proximity to the acceptor cells by use of biospecific interaction with a lectin artificially prelocalized on the cell surface.

As a model system, the introduction of new antigens into human lymphocytes was studied to confirm that they kept their properties after the transfer. As antigen we chose the rat histocompatibility antigen (RT-1) which was partially purified prior to incorporation into lipid vesicles.

Materials

Phosphatidylcholine type V-E, phosphatidylethanolamine type III, dried cells of *Micrococcus lysodeikticus,* α-methyl mannoside grade II, lactoperoxidase 80–100 units/mg, fluorescein isothiocyanate (FITC) isomer I and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-chloride) were obtained from Sigma Chemicals Co., U.S.A.

Ficoll-Paque, lentil lectin Sepharose and Sephadex G-25 (PD-10 columns) were purchased from Pharmacia Fine Chemicals AB, Sweden and Bio-Gel P-4 (medium) and Bio-Gel A-5m (50–100 mesh) from Bio-Rad, Richmond, U.S.A. Concanavalin A 2 × crystallyzed, and bovine serum albumin Cohn fraction V were supplied from Miles Biochemicals, Slough, U.K. and poly(ethylene glycol) 1540, pharmaceutical grade, was from Polyscience Inc., Warrington, U.S.A.

Silica gel plates (Kiselgel 60 ohne Fluoreszenzindikator), 0.1% ninhydrin and molybdatophosphoric acid spray reagents came from Merck, Darmstadt, F.R.G. and the dyes acridine orange 'GURR' and ethidium bromide from BDH, Poole, U.K.

Sodium iodine ($Na^{125}I$) for protein ionidation 3.7 GBq/ml (100 mCi/ml) was purchased from the Radiochemical Centre, Amersham, U.K. and [*methyl*-^{3}H]thymidine (2 Ci/mmol) was from New England Nuclear, Boston, U.S.A.

FITC-labeled rabbit anti-rat IgG was obtained from DAKOPATTS a/s, Copenhagen, Denmark and rabbit anti-contanavalin A from E · Y Laboratories Inc., San Mateo, U.S.A. Antiserum against the rat strain Wistar-Furth (WF) was produced by immunization of adult animals of the Brown Norway (BN) strian, with three biweekly injections intraperitoneally of $2 \cdot 10^7$ allogenic spleen and lymphnode cells.

Fresh serum of guinea pigs was frozen and stored at −80°C, thawed and used as the source of complement. RPMI 1640 was supplied from Flow Laboratories, Solna, Sweden.

Phosphate-buffered saline used in the experiments was Dulbecco's phosphate buffer containing no Ca^{2+} or Mg^{2+}.

Methods

Lymphocyte preparation

Human peripheral blood lymphocytes from healthy donors of blood type O, Rh^+, were purified by Ficoll-Hypaque centrifugation [9] and depleted of monocytes on a collagen column [10]. The cells were washed with phosphate-buffered saline and suspended in the buffer to cell concentrations of approximately $(1–2) \cdot 10^8$ cells/ml.

Partial purification of histocompatibility antigen (RT-1) from rat of the inbred strain Wistar-Furth (WF)

Plasma membranes of spleen tissue of WF rats were prepared, solubilized with deoxycholate and affinity-separated on lentil lectin Sepharose as previously described [11,12]. Protein determination was carried out according to Lowry et al. [13], using bovine serum albumin as a standard. After solubilization of the plasma membranes, the protein concentration was 7.2 mg/ml. Eluted glycoproteins were concentrated to 0.54 mg/ml.

Preparation of lipids

Glycolipids were extracted from *Micrococcus lysodeikticus* as previously described [14–16].

Fluorescence labeling of phosphatidylethanolamine was prepared by dissolving 4 mg of NBD-chloride in 500 μl chloroform containing 5

mg phosphatidylethanolamine and 15 μl of triethylamine. The mixture was incubated in the darkness for 8 h at room temperature. The fluorescent phosphatidylethanolamine conjugate was purified twice by thin-layer chromatography on silica plates using the solvent system chloroform/methanol/water (70 : 25 : 4, v/v). After purification, one fluorescent and phosphate-containing spot was observed. The conjugate was finally dissolved in 2.5 ml chloroform/methanol/water (70 : 25 : 4, v/v).

^{125}I-labeling of concanavalin A

The lectin was labeled with Na^{125}I according to the lactoperoxidase method [17]. 25 μl concanavalin A, 2.6 mg/ml in phosphate-buffered saline were added to 10 μl Na^{125}I (37 MBq). 2 μl lactoperoxidase (2 mg/ml) and 10 μl H_2O_2 (0.003%, v/v) were added under magnetic stirring. After 15 s the iodination was stopped by adding 0.4 ml phosphate-buffered saline. The unbound ^{125}I was separated by passing the preparation through a Bio-Gel P-4 column (10 × 1 cm). The fraction eluted in the void volume was collected and stored at 4°C. The lectin had a specific activity of 10 μCi/μg (400 kBq/μg).

Lipid vesicle preparation

Lipid vesicles containing the RT-1 antigen were prepared as previously described [16] by removal of the detergent by gel filtration on Sephadex G-25 (PD-10 columns).

Unless otherwise stated, 1 ml of vesicles were prepared from 313 μg lipids extracted from *Micrococcus lysodeikticus* and 54 μg glycoproteins eluted from lentil lectin Sepharose.

Flow cytometric analysis

FITC-stained cells were analyzed at a concentration of about 10^6 cells/ml, on a Cytofluorograph System 50-H (Ortho Instruments, Westwood, MA) as previously described [16]. With an appropriate setting, only the fluorescence signals from the lymphocyte population were gated into the multichannel distribution analyzer with a setting of 512 channels in resolution. 10000–50000 lymphocytes were analyzed in each experiment and the fluorescence intensity from the mean cell population was determined as the channel number on the multichannel analyzer corresponding to the peak.

Experimental procedure to transfer the RT-1 antigen to lymphocytes

Incubation of cells and concanavalin A to generate a 'receptor' for carbohydrates. Suspended lymphocytes, containing $(1–2) \cdot 10^7$ cells, in a volume of 100 μl were mixed with 40 μl of concanavalin A solution (16 μg/ml) and incubated for 15 min, at 20°C.

Binding of lipid vesicles containing RT-1 antigen to the cells. After dilution with 910 μl phosphate-buffered saline, 1 ml of the cell suspension was added to 0.5 ml lipid vesicles.

Addition of poly(ethylene glycol) to obtain fusion conditions. The cell-vesicle mixture was incubated for 1 h at 37°C on a rocking-table and 1 ml poly(ethylene glycol) 1540 (45% w/w in phosphate-buffered saline) was added to 100 μl of the cell-vesicle mixture with gentle and continuous mixing over a 1 min time period.

Elution of concanavalin A bound to the cells. 30 s later, 5 ml of phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin, 0.1 M α-methyl mannoside and 10 mM EDTA was slowly added over a period of 2 min. The cells were then sedimented by centrifugation for 5 min at 200 × *g* and resuspended in 2 ml phosphate-buffered saline, bovine serum albumin, α-methyl mannoside and EDTA. This washing of the cells was repeated once more with 1 ml phosphate-buffered saline, bovine serum albumin, α-methyl mannoside and EDTA. All washing steps were performed with buffer solutions prewarmed to 37°C.

After washing of the cells, the pellets were incubated 30 min at 4°C with anti-WF serum or normal BN serum diluted 1 : 20 in phosphate-buffered saline containing 10 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and 1% (w/v) bovine serum albumin, pH 7.4 (buffer 1). The cells were washed twice in phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin (buffer 2), stained with FITC-labeled rabbit anti-rat IgG, washed, suspended in 1 ml buffer 1 and the fluorescence from the cells was assayed on a cell flow cytofluorometer.

The proportion of viable cells was recorded after staining with a mixture of the nucleic acid-specific dyes, acridine orange and ethidium bromide [18].

Detection of concanavalin A not eluted from the cells

Lymphocytes (100 μl of $15 \cdot 10^7$ cells/ml) were incubated with 40 μl ^{125}I-labeled concanavalin A (16 μg/ml, 10 μCi/μg) for 15 min at 20°C. The cells were diluted in 910 μl phosphate-buffered saline and added to 500 μl of phosphate-buffered saline instead of lipid vesicles. 1 ml of phosphate-buffered saline or 30% and 45% poly(ethylene glycol) solution, respectively, was added to 100 μl of the cell suspension and the cells were washed three times with either buffer 2 or phosphate-buffered saline, bovine serum albumin, α-methyl mannoside and EDTA. The rest of the cell suspension was incubated for 1 h at 37°C before treatment with poly(ethylene glycol) and washing in the same manner as previously described. The radioactivity of washed cells was measured and normalized as cpm/10^6 cells and the percentage binding sites on the cells still occupied by the lectin was calculated.

Lymphocytes (100 μl of $17 \cdot 10^7$ cells/ml) were incubated with 40 μl concanavalin A (16 μg/ml). The cells were treated as after incubation with ^{125}I-labeled concanavalin A. After washing, the pellets were incubated for 30 min at 4°C with rabbit anti-concanavalin A antibodies diluted 1 : 20 in buffer 1. The cells were washed twice with buffer 2 and stained with FITC-labeled goat anti-rabbit IgG, prepared according to Huang et al. [19]. After washing, the cells were suspended in 1 ml buffer 1 and analyzed on a cell flow cytofluorometer.

Assay of mitogenic activity

The RT-1 antigen was transferred to lymphocytes from vesicles prepared from 313 μg extracted lipids and 54 μg eluted glycoproteins. One sample of the cell-vesicle mixture was untreated and another sample was treated with 45% poly(ethylene glycol). The samples were washed with 5, 2 and 1 ml phosphate-buffered saline containing bovine serum albumin, α-methyl mannoside and EDTA and once with 5 ml RPMI 1640 supplemented with 10% fetal calf serum to remove remaining α-methyl mannoside and, finally, were resuspended in the same medium. 200 μl lymphocytes ($1 \cdot 10^6$/ml) were added per well of a microtiter plate and were incubated for 72 h at 37°C in an atmosphere containing 8% CO_2, with or without concanavalin A (8 μg/ml). The cells were pulsed for 8 h with [*methyl*-^{3}H]thymidine (1 μCi/well) and then transferred to filters and were washed and dried before being counted in a liquid scintillation counter. As a comparison, lymphocytes were washed with buffer 2 or phosphate-buffered saline with bovine-serum albumin, α-methyl mannoside and EDTA prior to washing with RPMI 1640 supplemented with 10% fetal calf serum and subsequent incubation in microtiter wells under the conditions previously described.

Complement lysis of the cells

After transfer of RT-1 antigen into lymphocytes, ten preparations of cells with associated RT-1 antigen were pooled and suspended in 1.1 ml RPMI supplemented with 10% fetal calf serum. To 50 μl of the cells were added 50 μl of each dilution of an anti WF-serum serially diluted in RPMI supplemented with 10% fetal calf serum. The cells were incubated for 30 min at 4°C and washed with 1 ml buffer 2. After careful resuspension of the cells, 50 μl active or heat inactivated (30 min, 57°C) guinea pig complement diluted 1 : 1 with buffer 1 were added, and the cells were incubated for 45 min at 37°C. The cells were sedimented and resuspended in 1 ml phosphate-buffered saline containing acridine orange and ethidium bromide (0.5 μg of each dye), and the number of viable cells was analyzed on a cell flow cytofluorometer [18].

Results

Determination of concanavalin A and poly(ethylene glycol) concentrations suitable for transfer of lipid vesicles to the surface membranes of lymphocytes

A 'cell-bound receptor' for carbohydrates was generated by incubating cells and concanavalin A. High concentrations of concanavalin A aggregated the cells and increased the ability of cell-cell fusion after addition of poly(ethylene glycol). The degree of cell aggregation after incubation with various concentrations of concanavalin A was determined as a decrease in the number of cells being able to pass through a column of Bio-Gel A-5m (Fig. 1). In the subsequent assay, 4.6 μg concanavalin A/ml was used. The largest amount of fluorescent vesicles associated with cells without an increased cell death

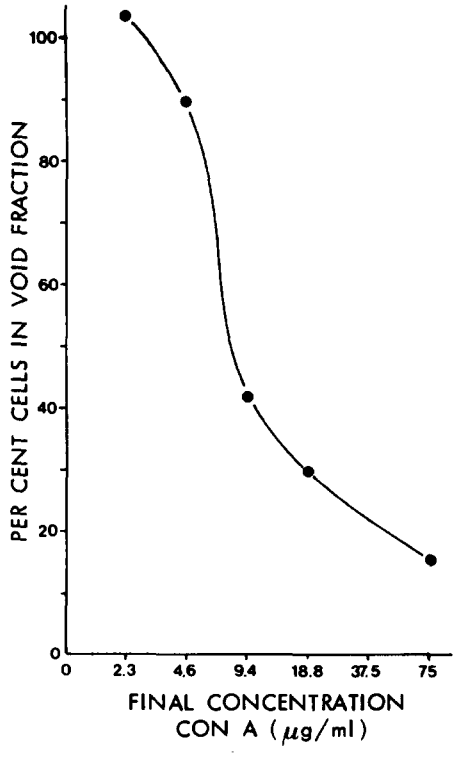

Fig. 1. Incubation of lymphocytes and concanavalin A, con A. 500 μl cell suspension ($18 \cdot 10^7$ cells/ml) were incubated with 200 μl concanavalin A in concentrations varying from 8.2 to 263 μg/ml. After 15 min at 20°C, 500 μl of the incubation mixture were applied to a 10 ml column of Bio-Gel A-5m. Prior to use, the column was equilibrated with phosphate-buffered saline and the cells were eluted with the same buffer. 1-ml fractions were collected. The largest cell number was obtained in fraction four and the number of cells in this fraction was counted and expressed as percentage of cells recovered without incubation with concanavalin A. A new column was used for each concanavalin A concentration.

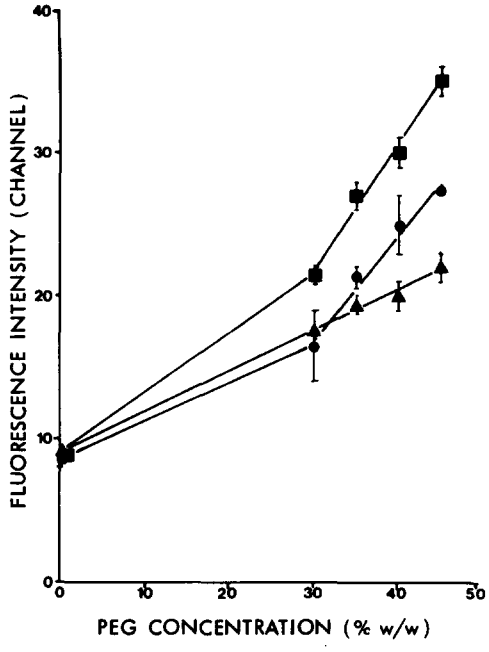

Fig. 2. Fluorescence associated with the cells after incubation with lipid vesicles containing fluorescent lipids. Lymphocytes, 100 μl of a suspension containing $14 \cdot 10^7$ cells/ml were incubated for 15 min at 20°C with 40 μl concanavalin A (16 μg/ml) or phosphate-buffered saline. The cells were diluted with 910 μl phosphate-buffered saline, and 1 ml of the cell suspension was added to 0.5 ml vesicles prepared from 625 μg lipids from *Micrococcus lysodeikticus*, 50 μg phosphatidylethanolamine-NBD conjugate and solubilized plasma membranes corresponding to 180 μg protein. After 60 min at 37°C, 1 ml of phosphate-buffered saline or 1 ml of 30, 35, 40 or 45% (w/w) poly(ethylene glycol) (PEG) was added to 100 μl of the cell-vesicle incubates. Cells incubated with concanavalin A prior to addition of lipid vesicles were either washed with phosphate-buffered saline and 0.5% (w/v) bovine serum albumin (■) or the same buffer containing 0.1 M α-methyl mannoside and 10 mM EDTA (●). Cells not incubated with concanavalin A were washed with phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin (▲).

was obtained after treatment with 45% (w/w) poly(ethylene glycol (Fig. 2). At higher poly(ethylene glycol) concentrations there was a dramatically increased lysis of the cells. Fluorescence of higher intensity was obtained when cells had been preincubated with concanavalin A. Some of the fluorescence was only loosely bound to the cells and could be removed by treatment with a combination of α-methyl mannoside and EDTA (Fig. 2).

Transfer of rat histocompatibility antigen (RT-1) to human lymphocytes

Approximately the same amount of cells was recovered from the transfer procedure with or without addition of poly(ethylene glycol) (85% of the cells). However, after poly(ethylene glycol) treatment 10–15% of the recovered cells were dead. Poly(ethylene glycol) treatment in combination with lipid vesicles increased the percentage of dead cells to 20–25%.

After mixing cells and lipid vesicles with no poly(ethylene glycol) added, none or only a very small amount of RT-1 antigen was detected on the cells (Table I), as reflected by no increased fluores-

TABLE I

For the cell-associated fluorescence, results are given as the channel number corresponding to the mean fluorescence intensity in the multichannel analysis on a cell flow cytofluorometer. PBS, phosphate-buffered saline; BSA, bovine serum albumin; Con A, concanavalin A; PEG, poly(ethylene glycol).

Cells exposed to vesicles containing the antigen		Cell-associated fluorescence	
Further treatment	Washing	Anti-WF serum	BN-normal serum
–	PBS (0.5% BSA)	17	12
Pretreatment with Con A	α-methyl-mannoside + EDTA	21	13
Subsequent PEG incubation	PBS (0.5% BSA)	22	14
Pretreatment with Con A and subsequent PEG incubation	α-methylmannoside + EDTA	27	15

cence above the normal background difference of four channels between antiserum and normal BN rat serum. Association of antigen with the cells was detected when the mixture of cells and lipid vesicles was treated with poly(ethylene glycol) under fusion conditions. Incubation of the cells with concanavalin A prior to the treatment with lipid vesicles and poly(ethylene glycol) resulted in a larger amount of antigen associated with the cells. Omitting the poly(ethylene glycol) treatment reduced the amount of detectable antigen.

Amount of concanavalin A remaining on the lymphocytes after washing

^{125}I-labeled concanavalin A or an antiserum against the lectin was used to investigate whether or not concanavalin A was still present on the cells after washing with α-methyl mannoside and EDTA (Table II). Both methods showed that the amount of lectin that could be removed from the cells decreased after incubation at 37°C. This effect was most pronounced when antibodies were used to detect remaining lectins. Treatment of the cells with higher concentrations of poly(ethylene glycol) increased the amount of lectin which became associated with the cells.

A Steck-Wallach plot [20] was used to determine the maximal amount of labeled lectin that can be bound to the cells. Analysis of three different cell preparations showed that the maximal amount of concanavalin A that could be bound to the cells was 485 047 ± 22 679 cpm per 10^6 cells.

TABLE II

CONCANAVALIN A REMAINING ON HUMAN LYMPHOCYTES AFTER FUSION TREATMENT. DETECTION WITH ANTIBODIES AGAINST CONCANAVALIN A OR WITH ^{125}I-LABELED CONCANAVALIN A

Cells were treated as described in Methods under 'Detection of concanavalin A not eluted from cells'. The results for the cell-associated fluorescence (A) are given as the channel number, in the multichannel analysis on a cell flow cytofluorometer, corresponding to the mean fluorescence intensity. Cells incubated with antibodies but not with concanavalin A were registrated in channel 5. The percentages of binding sites occupied (B) were calculated from the binding of ^{125}I-labeled concanavalin A. PEG, poly(ethylene glycol).

PEG concn. (%, w/w)	Incubation for 15 min at 20°C, washing conditions		Subsequent 60 min at 37°C, washing conditions	
	Eluting	Non-eluting	Eluting	Non-eluting
A. Cell-associated fluorescence				
0	6	60	9	34
45	9	88	13	39
B. Percentage of binding sites occupied				
0	1.7	7.3	4.6	6.3
45	1.9	8.8	4.7	7.1

This value was used to calculate the percentage binding sites on cells still occupied by lectin after washing under eluting or non-eluting conditions (Table II).

Since concanavalin A bound to the cells could not be completely eluted, a study of whether or not this polyclonal T-cell activator [21] might induce cell mitosis under the conditions of these experiments was undertaken. There was no augmented incorporation of [^{3}H]thymidine into the cells caused by remaining lectin. Less than 1000 dpm of [^{3}H]thymidine was incorporated into the cells after incubation for 72 h followed by pulsing for 8 h with 1 μCi [^{3}H]thymidine. The optimal concentration of this lectin preparation for stimulating the incorporation of [^{3}H]thymidine into cells was found to be 8 μg/ml. This concentration of concanavalin A induced incorporation of 65 309 $\pm$ 3 806 dpm into untreated cells. Cells washed with α-methyl mannoside and EDTA, and cells incubated with concanavalin A and lipid vesicles also washed with α-methyl mannoside and EDTA showed almost the same amount of incorporation of [^{3}H]thymidine as untreated cells: 70 537 $\pm$ 3510 and 79 993 $\pm$ 10 342 dpm, respectively. However, when the cells were treated with lipid vesicles under fusion conditions, 33 607 $\pm$ 3220 dpm were incorporated into the cells after restimulation with concanavalin A.

Transfer of rat histocompatibility antigen (RT-1) results in sensitivity to complement-dependent cytolysis by anti-RT-1 antibodies

After transfer of the RT-1 antigen to human lymphocytes these cells became susceptible to lysis by anti-WF serum in the presence of complement. Fig. 3 shows the percentage of dead cells after incubation with various dilutions of the anti-WF serum. Lysis of the cells due to antiserum and complement rapidly decreased at antiserum dilutions higher than 1 : 10, but was still detectable at a dilution of 1 : 80.

These results were obtained with cells tested immediately after they had been treated with poly(ethylene glycol) and washed with α-methyl mannoside and EDTA. In contrast, no significant cell lysis was obtained when the cells were stored in RPMI 1640 supplemented with 10% fetal calf serum at 4°C for 60 min prior to exposure to

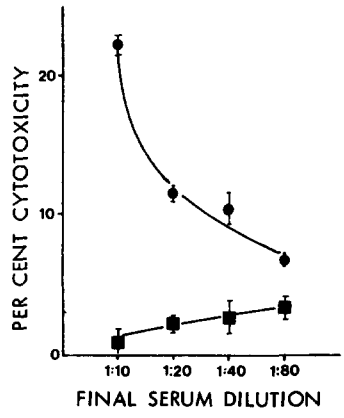

Fig. 3. Complement lysis of cells after transfer of RT-1 antigen. After poly(ethylene glycol) treatment and washing, the cells were incubated with varied dilutions of anti-WF serum and active complement (●) or heat-inactivated complement (■). Percent cytotoxicity of the RT-1 serum is calculated as: $100\times(C-CS)/C$ (C = % viable cells with complement alone, CS = % viable cells with serum and complement). Percent viable cells with complement alone was 62%.

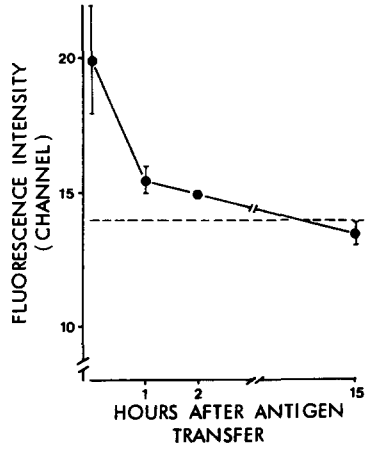

Fig. 4. Elimination of the RT-1 antigen after transfer to the cells. The RT-1 antigen was attached to lymphocytes as described in methods. Ten samples of cells with transferred RT-1 antigen were pooled and suspended in 1 ml RPMI 1640 supplemented with 10% fetal calf serum ($10.2\cdot10^6$ cells/ml) and stored at 4°C. At varying times after suspending the cells, 50 μl of the cells were incubated anti WF serum. The cells were washed and stained with FITC-rabbit anti-rat IgG and analysed on a cell flow cytometer. The channel on the multichannel analyzer corresponding to the fluorescence intensity of the mean cell population is presented as a measure of the amount of remaining antigen. Immediately after suspending the cells in RPMI 1640 supplemented with 10% fetal calf serum, cells incubated with phosphate-buffered saline instead of liposomes had a fluorescence intensity that corresponded to channel 14 (— — —).

antiserum and complement. A more sensitive way to investigate the disappearance of the antigen from the cell surface was to incubate the cells with an antiserum and a FITC-labeled second antibody. This method revealed that the antigen disappeared from the cell surface within few hours (Fig. 4).

Discussion

Poly(ethylene glycol) concentrations of 40% or higher are required for the fusion of cells [22]. These concentrations have also been used to fuse cells and membrane vesicles [1–3]. Increasing amounts of fluorescent liposomes were associated with the cells after adding increasing concentrations of poly(ethylene glycol) to the cell-vesicle mixtures (Fig. 2). The yield of the fusion process continued to increase when 50% poly(ethylene glycol) was used (not shown) but this treatment was very toxic to the cells.

The liposomes contained a dimannosyldiacylglycerol extracted from *Micrococcus lysodeikticus* [14], which has been reported to bind to concanavalin A [15]. However, binding of vesicles to the cells was probably due to glycoproteins in the lipid vesicles, since the same amount of fluorescence was associated with the cells after addition of varying concentrations of poly(ethylene glycol) to cell-vesicle mixtures where the glycolipid had been omitted in the preparation of lipid vesicles. This indicates that, under the conditions used here, i.e., the use of uncharged lipid vesicles, the association of vesicles to the cells depends on interactions between concanavalin A and glycoproteins [16].

Significant amounts of non-elutable RT-1 antigen were detected on the cells after addition of poly(ethylene glycol) to cells incubated with concanavalin A and lipid vesicles (Table I). The antigen concentration on the cells was approximately 10% of the amount present on a WF lymphocyte. This was calculated from the fluorescence obtained after incubating WF lymphocytes with anti-WF serum and the FITC-labeled antibody under the same conditions as for the modified cells.

The binding of the lectin to the cells and its subsequent elution were investigated both with ^{125}I-labeled concanavalin A and with antibodies against the lectin (Table II). The analysis using ^{125}I-labeled lectin demonstrated the total amount of concanavalin A associated with the cells, while the antibodies selectively detected the lectin exposed on the cell surface. The cells were not incubated with lipid vesicles in these experiments, since they might have sterically hindered the binding of the antibody. Some of the membrane components to which the lectins were bound appear to be shed, since the amount of lectin decreased with prolonged incubation. The decrease was more pronounced when studied by the antibody method. This indicates that some of the lectin molecules were either deeply bound into the cell membrane or capped and internalized. 'Cementing effects' of poly(ethylene glycol) [2] and multi-point attachment of the lectin decrease the amount of lectin that can be eluted after treatment with higher concentrations of poly(ethylene glycol) or after prolonged incubation periods.

The cells were incubated with concanavalin A for 15 min to generate a 'cell-bound receptor' for carbohydrates. During this incubation the concentration of concanavalin A was approx. 5 μg/ml which is close to the optimal concentration for mitogenic stimulation (8 μg/ml) and should have induced mitosis if this process had been allowed to continue.

After dilution of the cell suspension and addition of lipid vesicles, the concentration of the lectin was reduced to about 0.4 μg/ml. However, the incubation time was not sufficient to induce cell mitosis. The poly(ethylene glycol) treatment, which induced fusion between cells and lipid vesicles, reduced the concanavalin A-stimulated incorporation of [^{3}H]thymidine by approx. 50%, as compared to untreated cells. A major part of the reduction could be due to the fact that a proportion of the cells is damaged by the poly(ethylene glycol) treatment. The population of damaged cells includes dead cells (21%) and partially inactivated cells. In addition, the release of lectin-binding glycoproteins from damaged cells would tend to inhibit the mitogenic effect of the lectin [23]. A more speculative explanation would be that the stimulation was affected by the incorporation of glycoproteins and glycolipids in the cell membrane, which has been reported by Jakobovits et al. [8].

It is noteworthy that the cells did become sensi-

tive to lysis by anti-RT-1 serum in the presence of complement. Most of the RT-1 antigen remained detectable on the cells for less than 1 h. The elimination of the WF antigen fits well with the report that, after transferring the receptor for the thyrotropic hormone, the cells could only be stimulated by the hormone during 150 min after fusion [3]. In contrast, a 50 h half-life for some transferred membrane proteins has been reported using radioactively labeled membrane proteins [4]. In the present investigation and also in Ref. 3, the half-life of membrane-incorporated, biologically accessible antigen was in the order of 1–2 h. However, using radioisotope-labeled proteins, internalized proteins are also measured, and thus, these can account for a longer half life.

In conclusion, the described method involving the creation of cell-bound concanavalin A receptors for carbohydrates to optimize attachment of liposomes, and subsequent fusion by poly(ethylene glycol) treatment provides an opportunity to insert purified membrane glycoproteins into liposomes and subsequently introduce them into recipient cells. Such a technique has great potential, since it provides new opportunities of investigating the functions of the cell membrane. Such functions would include induction of proliferative responses, receptor-ligand interactions, cell-cell interactions and signal transfer through the cell membrane. Elimination of the introduced component is an obvious limitation and ways to prolong the half-life must be further investigated.

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