LITERATURE CITED

1. B. D. Brondz, Folia Biol. (Prague), 14, 115 (1968).
2. B. D. Brondz and A. E. Snegireva, Immunology, 20, 457 (1971).
3. B. D. Brondz, S. G. Egorova, and I. F. Kotomina, Eur. J. Immunol., 5, 741 (1975).
4. B. D. Brondz, I. K. Egorov, and G. I. Drizlikh, J. Exp. Med., 141, 11 (1975).
5. B. D. Brondz, A. F. Kirkin, and S. Yu. Epikhina, Byull. Éksp. Biol. Med., No. 7, 68 (1975).
6. K. T. Brunner, J. Mauel, H. Rudolf, et al., Immunology, 18, 501 (1970).
7. T. D. Canty, J. R. Wunderlich, and F. Fletcher, Immunology, 106, 200 (1971).
8. P. Golstein, E. A. J. Swedmyr, and H. Blomgren, Eur. J. Immunol., 2, 380 (1972).
9. C. S. Henney, J. Immunol., 107, 1558 (1971).
10. E. Kedar, M. O. de Landazuri, and J. L. Fahey, J. Immunol., 112, 37 (1974).
11. R. D. Stulting and G. Berke, J. Exp. Med., 137, 932 (1973).
12. D. B. Wilson, J. Exp. Med., 122, 143 (1965).

PREPARATION OF LIPOSOMES WITH IMMUNOLOGICAL SPECIFICITY

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Artificial lipid vesicles (liposomes) containing immunoglobulins were obtained. The immunoglobulins contained in the liposomes were shown to preserve their immunological activity: Liposomes containing rabbit anti-mouse immunoglobulin agglutinate under the influence of donkey anti-rabbit immunoglobulin or mouse serum. Liposomes containing inulin-^{3}H, carrying immunoglobulins against antigenic determinants of the cell surface were selectively bound by target cells but not by control cells. Specific binding with cell surface antigenic determinants was also demonstrated for liposomes carrying nonimmune globulins as well as immunoglobulins. It was shown by the direct immunofluorescence method that nonimmune globulins, in the form of complexes with immune liposomes, are selectively bound by target cells. With the aid of such liposomes it is possible to supply substances selectively to certain types of cells and also to "fit" new antigens into the cell membrane.

KEY WORDS: liposomes; immunoglobulins; antigens.

Reports have recently been published on the introduction of substances incorporated in to artificial lipid vesicles (liposomes) into cells in vivo and in vitro [3, 7, 10-12]. However, opportunities for using liposomes as carriers of substances in vivo are limited by the specificity of their distribution among the tissues [7, 9]. For the directional introduction of substances with the aid of liposomes it would be worthwhile to be able to bind liposomes selectively with cells of a particular type.

This paper describes a method of obtaining liposomes with immunological specificity and their selective binding to cells.

EXPERIMENTAL METHOD

Inulin-^{3}H (specific activity 690 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, England. Concanavalin A was kindly provided by V. I. Gel'fand. Rabbit and rat immunoglobulins were obtained

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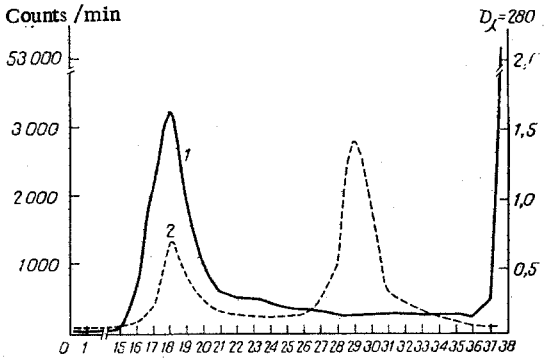

Fig. 1. Separation of liposomes from free immunoglobulin and inulin-^{3}H on Sepharose 6B column. Volume of fractions 1.5 cm^3. 1) Radioactivity (0.15 ml); 2) optical density.

TABLE 1. Immunoagglutination of Liposomes

Protein added	Immune liposomes sonicated with RIG	Ninimmune liposomes incubated with RIG	Nonimmune liposomes (control)
MS	—	—	—
DG	—	+	—
NRG	—	—	—

Legend. RIG) Rabbit anti-mouse immunoglobulin; MS) mouse serum; DG) donkey anti-rabbit immunoglobulin; NRG) nonimmune rabbit γ-globulin. Agglutination of liposomes carried out in drop on coverslip and assessed by 5-point system (++++ very strong agglutination, — no agglutination).

on DEAE-Sephadex A-50 [5]. Phosphatidylcholine was generously provided by L. I. Barsukov. The purity of the lipid was tested by thin-layer chromatography on silica-gel plates. To obtain liposomes, 20 μmoles phosphatidylcholine in an organic solvent was poured into a round-bottomed flask and evaporated to dryness on a rotary vaporizer. To the film of lipids thus obtained 1 ml buffered salt solution (BSS) containing the dissolved immunoglobulin (20 mg/ml) was added. In some experiments the solution also contained inulin-^{3}H (250 μCi/ml). The flask was shaken and the suspension sonicated on the UZDN-1 ultrasonic disintegrator for 20 min at 4°C with a frequency of 22 kHz. Liposomes were separated from the free immunoglobulin on a column (420 × 18 mm) with Sepharose 6B at a rate of elution of 12-15 ml/h. The yield of liposomes was monitored spectrophotometrically on the basis of optical density (λ = 280 nm) and of captured radioactivity.

Mouse spleen cells adsorbed on a Formvar film [2] and secondary cultures of mouse embryonic fibroblasts, grown on coverslips in penicillin flasks [1] were used. The cells were treated on the coverslips. The cells were incubated for 15 min in BSS with concanavalin A (150 μg/ml). After three washes with BSS the cultures were then incubated for 15 min in a solution of liposomes. The cells were then washed three times again with BSS and treated with fluorescent antisera (obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology). In some experiments, samples were prepared from these cultures for counting the label [5] on a Mark II (Nuclear Chicago) scintillation counter.

EXPERIMENTAL RESULTS

Preparation of Immunoglobulin-Containing Liposomes. The liposomes were separated from free immunoglobulin by gel filtration: The liposomes migrated into the outer space of the column, the free immunoglobulin into the inner space. If the solution in which the liposomes were sonicated contained inulin-^{3}H, a peak of radioactivity appeared during gel filtration which coincided with the peak of liposomes determined spectrophotometrically (Fig. 1). Electron microscopy confirmed the presence of liposomes in the test fraction. The liposomes were not fractionated by size.

The presence of immunoglobulins thus did not prevent the formation of liposomes capable of retaining inulin.

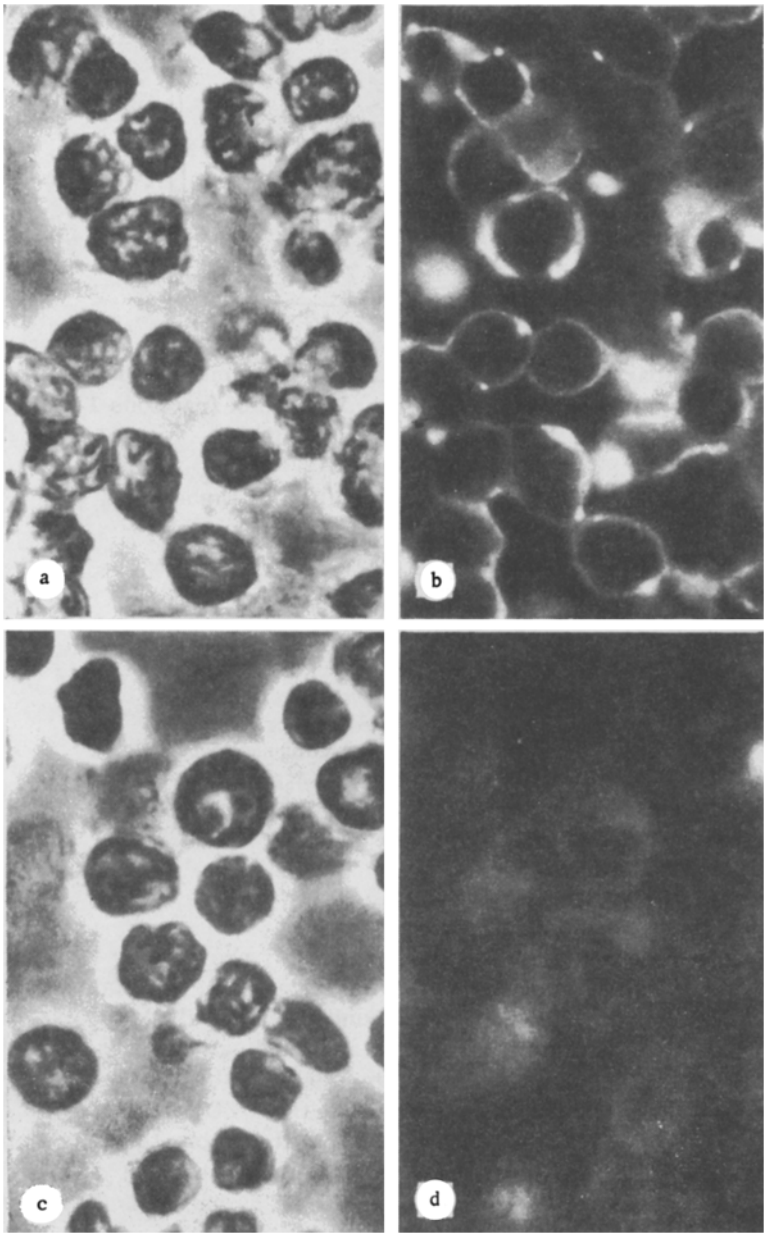

Fig. 2. Immunofluorescence of spleen cells treated with immune anti-concanavalin liposomes: a and b) cells treated beforehand with concanavalin A (a – phase-contrast microscopy, b – immunofluorescence); c and d) control cells not treated with concanavalin A (c – phase-contrast microscopy, d – immunofluorescence). Magnification 1600×. Fluorescence under the influence of anti-rabbit serum.

To test whether the liposomes contained immunoglobulins, immunoagglutination of the liposomes was investigated. If mouse serum or donkey anti-rabbit immunoglobulin was added to the liposomes sonicated in a solution of rabbit anti-mouse immunoglobulin (after separation from the free immunoglobulin), the liposomes agglutinated. Addition of the control solution containing nonimmune γ globulin to the liposomes did not cause agglutination. For immunoagglutination of the liposomes it was essential that sonication of the lipid emulsion during preparation of the liposomes took place in a solution of immunoglobulin. Incubation of liposomes sonicated in BSS with immunoglobulin, followed by separation of the free immunoglobulin by gel filtration, led to the formation of liposomes giving only weak agglutination in the system described (Table 1).

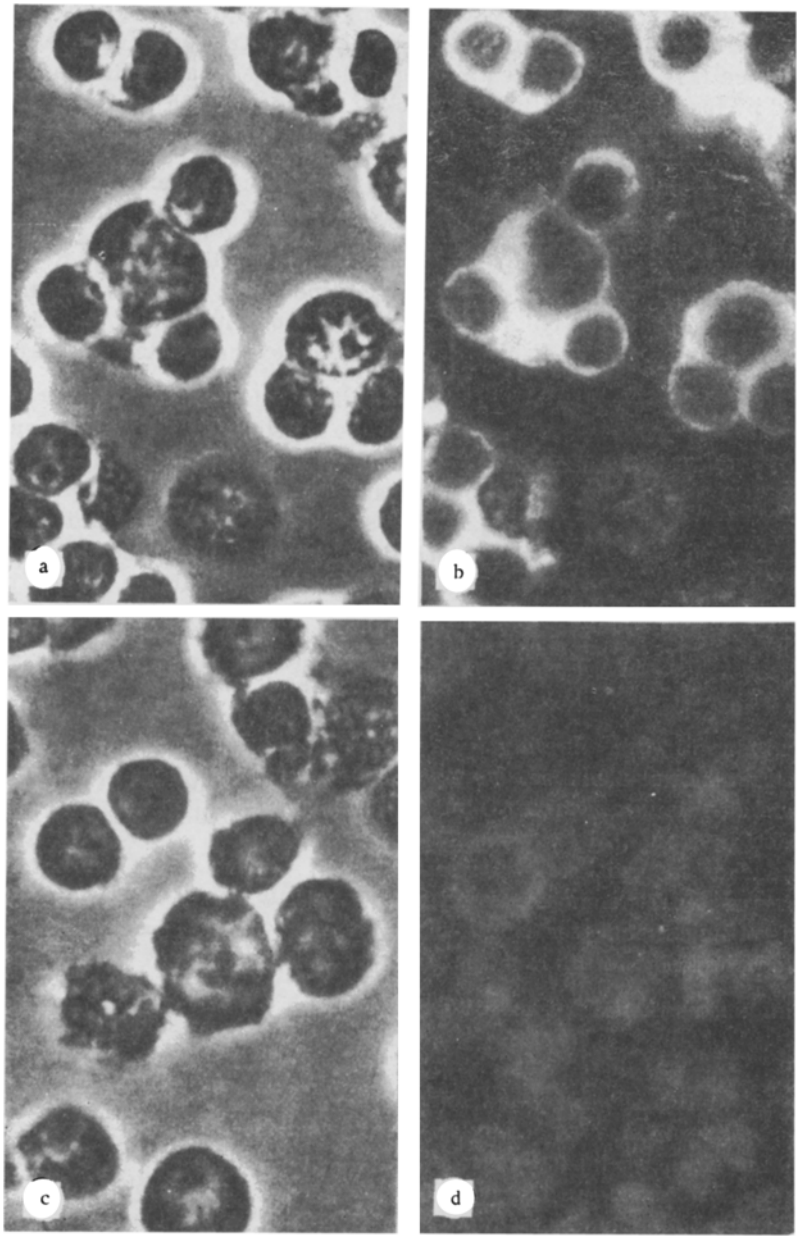

Fig. 3. Immunofluorescence of spleen cells treated with immune anti-concanavalin liposomes containing nonimmune rabbit γ globulin. a and b) Cells treated beforehand with concanavalin A (a – phase-contrast microscopy, b – immunofluorescence); c and d) control cells not treated with concanavalin A (c – phase-contrast microscopy, d – immunofluorescence). Magnification 1600×. Fluorescence under influence of anti-rat serum.

The liposomes obtained thus contained immunoglobulin which preserved its immunological activity while in the liposomes.

Binding of Immune Liposomes to the Cells. The selectivity of binding of the liposomes to cells was investigated in a model system in which antigenic differences of the surface of the cells were obtained by treating some of the cells with concanavalin A. Immune liposomes were obtained as described above by combined sonication of a phospholipid film with rabbit immunoglobulin against concanavalin A. Binding of the immunoglobulin-containing liposomes to the cell surface was determined by the indirect immunofluorescence method with labeled donkey anti-rabbit serum.

After treatment of the cells with immune liposomes for 15 min appreciable fluorescence of the cultures previously treated with concanavalin A was observed (Fig. 2a, b). Control cells not treated with concanavalin A gave virtually no fluorescence (Fig. 2c, d). Treatment of the cells incubated with concanavalin with nonimmune liposomes, followed by incubation with labeled donkey antiserum, did not lead to the appearance of fluorescent cells in the culture.

It follows from these experiments that on incubation of immune liposomes with target cells, binding of immunoglobulins of the liposomes with antigenic determinants of the cell surface takes place.

However, the selectivity of binding of the liposomes themselves still remained to be proved, for the fluorescence which was observed could have arisen through escape of the immunoglobulins from the liposomes into the solution (for example, as a result of lysis of the liposomes on interaction with the cell). To prove the selectivity of binding of the liposomes themselves the following experiments were carried out.

Immune liposomes were obtained in a solution of inulin-^{3}H, so that after separation of the liposomes from the free label they contained not only immunoglobulins, but also labeled inulin. After incubation of these liposomes with the cells and after rinsing to remove the unbound label, the radioactivity of the cells was measured. Much more of the label (2850 counts/min/10^5 cells) was bound with cells previously treated with concanavalin A than with the control, untreated cells (220 counts/min/10^5 cells). The addition of unlabeled immune liposomes and free inulin-^{3}H (in the same concentration as in the liposomes) to cells treated with concanavalin A did not increase the degree of binding of the label compared with the control cultures, untreated with concanavalin.

Further evidence of the selectivity of binding of the immune liposomes with the target cells was given by the next experiment: Cells were incubated with immune liposomes carrying not only immune rabbit anti-concanavalin immunoglobulins, but also normal nonimmune rat immunoglobulins. In this experiment adsorption of liposomes was detected from fluorescence of the cultures after treatment of the cells with fluorescent rabbit antiserum against rat globulins. Normal globulin was bound in this experiment only to cells treated beforehand with concanavalin A (Fig. 3a and b). Binding of normal rat globulin by the cells in the normal cultures was negligible (Fig. 3c and d).

Immune liposomes are thus selectively bound with the antigenic determinants of the cell surface. This last experiment also showed that foreign proteins can be delivered to the cell surface with the aid of immune liposomes.

When this investigation was finished, we learned of a paper by Gregoriadis and Neerunjun [8], who succeeded in delivering labeled bleomycin selectively to certain types of cells with the aid of immune liposomes. Other approaches to the problem of increasing the affinity of therapeutic preparations for particular organs with the aid of antibodies have also been suggested [4].

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LITERATURE CITED

1. Yu. M. Vasil'ev, I. M. Gel'fand, L. V. Domnina, et al., Tsitologiya, No. 1, 80 (1972).
2. N. A. Dorfman, in: 7th International Congress of Electron Microscopy. Proceedings, Grenoble (1970), p. 563.
3. L. B. Margolis and L. D. Bergel'son, Dokl. Akad. Nauk SSSR, 225, 941 (1975).
4. L. B. Mekler, S. I. Gorodetskii, I. V. Osechinskii, et al., Dokl. Akad. Nauk SSSR, 185, 1359 (1969).
5. V. A. Rozenblat, A. S. Serpinskaya, and A. A. Stavrovskaya, Dokl. Akad. Nauk SSSR, 211, 1460 (1973).
6. I. S. Baumstark, R. J. Loffin, and W. A. Bardwil, Arch. Biochem., 108, 514 (1964).
7. G. Gregoriadis, FEBS Lett., 36, 292 (1973).
8. G. Gregoriadis and E. D. Neerunjun, Biochem. Biophys. Res. Commun., 65, 537 (1975).
9. M. M. Jonah, E. A. Cerny, and Y. E. Rahman, Biochim. Biophys. Acta, 401, 336 (1975).
10. R. E. Pagano, L. Huang, and C. Way, Nature, 252, 166 (1974).
11. D. Papahadjopoulos, E. Mayhew, G. Poste, et al., Biochim. Biophys. Acta, 323, 23 (1973).
12. Y. E. Rahman and B. J. Wright, J. Cell Biol., 65, 112 (1975).