*Proc. Natl. Acad. Sci. USA*
Vol. 87, pp. 5744–5748, August 1990
Medical Sciences

# Lipid composition is important for highly efficient target binding and retention of immunoliposomes

(reticuloendothelial system/targeted drug delivery/gangliosides)

KAZUO MARUYAMA*, STEPHEN J. KENNEL†, AND LEAF HUANG*‡

*Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840; and †Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

*Communicated by John D. Baldeschwieler, May 11, 1990*

ABSTRACT **By taking advantage of a monoclonal IgG antibody, 34A, which is highly specific to pulmonary endothelial cells, we have prepared liposomes containing various amounts of antibody molecules (immunoliposomes). These immunoliposomes accumulate specifically in the lung when injected i.v. Two lipid compositions were used: phosphatidylcholine/cholesterol/phosphatidylserine (PS), 10:5:1 (mol/mol), a composition that allows liposomes to be readily taken up by the reticuloendothelial system (RES) (liver and spleen), and phosphatidylcholine/cholesterol/ganglioside $GM_1$, 10:5:1 (mol/mol), a composition that allows liposomes to avoid or delay the RES uptake (the so-called stealth liposomes). Although an increase in the number of antibody molecules per liposome was accompanied by an increased level of lung binding of the immunoliposomes, differences due to the lipid composition were more profound. For example, stealth immunoliposomes containing an antibody/lipid ratio = 1:37 (wt/wt) accumulated in lung to a level of 60% of the injected dose, whereas PS-containing immunoliposomes with a higher antibody/lipid ratio (1:8) only accumulated 50% of the injected dose in the lung. Conjugation of antibody to the stealth liposome did not increase the rate of liposome uptake by liver; this rate was approximately 10-fold lower than that of the PS-containing liposomes without antibody. Stealth immunoliposomes with high antibody content also showed long retention in the lung. The $t_{1/2}$ of lung residence for the stealth immunoliposomes with an antibody/lipid ratio = 1:11 (wt/wt) was ≈24 hr. The fact that stealth immunoliposomes showed a longer retention time in the lung than the PS-containing immunoliposomes of similar antibody content suggests that macrophages may play a role in the removal of the bound immunoliposomes from the pulmonary endothelium. Alternatively, dissociated stealth immunoliposomes may reenter the circulation and rebind to the lung target, causing an apparent slow overall dissociation rate. These results can be understood on the basis of two competing kinetic processes: lung binding whose rate is directly proportional to the antibody content of the immunoliposomes and uptake by RES whose rate is significantly reduced in the case of the stealth liposomes. Even for a modest level of antibody content, the half-life for target binding of immunoliposomes was significantly shorter than the half-life of liver uptake of the liposomes, resulting in a favorable target binding. Significant immunoliposome binding to the lung is not due to the fact that tail vein-injected liposomes flow through the lung capillary bed before they encounter the liver, because portal vein-injected immunoliposomes showed the same rate and extent of target binding as the tail vein-injected ones.**

The use of liposomes in drug delivery has been extensively studied (for a recent book, see ref. 1). Among their many advantages, liposomes can be readily conjugated with targeting ligands for enhanced target cell binding (2). Liposomes containing monoclonal or polyclonal antibody (immunoliposomes) are effective in specific binding and drug delivery to target cells *in vitro* (for a recent review, see ref. 3). Relatively little work has been done on immunoliposome binding *in vivo* (4). We have recently described a model system using immunoliposomes containing monoclonal antibodies to gp112, a pulmonary endothelium antigen (5). Since gp112 is located exclusively and abundantly on the lumenal surface of the pulmonary capillary vessel wall (6, 7), i.v. injected immunoliposomes gain direct access and bind efficiently to the lung target (5, 8, 9). This convenient model has allowed us to investigate the effect on target binding of some physical parameters of liposomes such as antibody/lipid ratio, liposome size, and injection dose (8). Under optimal conditions, it was found that up to 60% of the injected immunoliposomes were bound to the lung target; the uptake of immunoliposomes by liver and spleen, the major reticuloendothelial system (RES), was only about 12% (9).

Regular liposomes are rapidly and efficiently taken up by RES cells in the liver and spleen (10–12). Coating liposomes with intact immunoglobulin molecules often leads to enhanced uptake of the immunoliposomes by the RES (13, 14). Highly efficient target binding and a relatively low level of RES uptake of the immunoliposomes described above seemingly violate this rule. Furthermore, it has been suggested (8) that liposomes with a lipid composition that allows them to avoid or delay the uptake by RES [i.e., the so-called "Stealth Liposomes" (Liposome Technology Inc., Menlo Park, CA)] (15, 16) should show more efficient target binding. This hypothesis is based on the unverified assumption that target binding of liposomes is kinetically competitive with the RES uptake of liposomes. Our model system has provided an opportunity to test this hypothesis. We describe here a kinetic study on the target binding and the RES uptake of immunoliposomes, with a special emphasis on examining the role of the stealth lipid composition. The data have revealed some important insights into the mechanism of the highly efficient immunoliposome binding to the target.

## MATERIALS AND METHODS

**Materials.** Egg phosphatidylcholine (PC) and bovine brain phosphatidylserine (PS) were obtained from Avanti Polar Lipids, and cholesterol was from Sigma. Ganglioside $GM_1$ from bovine was obtained from Calbiochem. The stearylamide complex of diethylenetriaminepentaacetic acid (DTPA–SA) was synthesized according to ref. 17, and $^{111}$In-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NGPE, *N*-glutaryl phosphatidylethanolamine; PC, egg phosphatidylcholine; PS, phosphatidylserine; RES, reticuloendothelial system; DPTA–SA, stearylamide complex of diethylenetriaminepentaacetic acid.

‡To whom reprint requests should be addressed.

labeled DTPA–SA ($^{111}$In-DTPA–SA) was prepared as described (9). Briefly, 1 μl of $^{111}InCl_3$ (carrier-free, New England Nuclear) and 600 μl of 1 mM DTPA–SA, which was dissolved in hot (≈65°C) ethanol with brief sonication, were mixed to form the $^{111}$In-DTPA–SA complex. This lipophilic radiolabel is not transferred to the serum components from liposomes (K.M. and L.H., unpublished data), and it is not rapidly metabolized *in vivo* (9). The synthesis of *N*-glutaryl phosphatidylethanolamine (NGPE) has been described (18).

**Antibody.** Rat monoclonal antibody 34A (IgG2a) was purified from *nu/nu* mouse ascites fluid as described (19). 34A was radiolabeled with $^{125}$I, using the Iodo-Gen (Pierce) method, to a specific activity of 2–4 × $10^5$ cpm/μg and conjugated with NGPE as described (9). Briefly, dry NGPE was solubilized with octyl glucoside in Mes buffer (10 mM Mes/150 mM NaCl, pH 5.5) (NGPE/octyl glucoside = 0.06:1, mol/mol). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (12 μmol in Mes buffer) and *N*-hydroxysulfosuccinimide (4.8 μmol in Mes buffer) were added to 224 nmol of NGPE solubilized with octyl glucoside and then incubated for 10 min at room temperature. The mixture was neutralized with 100 mM Hepes buffer (pH 7.5) and 1 M NaOH to pH 7.5. 34A with a trace amount of $^{125}$I-labeled 34A was then added to achieve an antibody/NGPE molar ratio of 1:14 and incubated for 8 hr at 4°C with gentle stirring.

**Preparation of Immunoliposomes.** Immunoliposomes containing 34A were prepared by a detergent dialysis method as described (9). Two lipid compositions were used: PC/cholesterol/$GM_1$, 10:5:1 (mol/mol) and PC/cholesterol/PS, 10:5:1 (mol/mol). Lipid mixtures containing $^{111}$In-DTPA–SA (1%) were dried with $N_2$ gas. The dried lipid was solubilized with 100 mM octyl glucoside in phosphate-buffered saline (PBS; 137 mM NaCl/2.7 mM KCl/1.5 mM $KH_2PO_4$/0.1 mM $Na_2HPO_4$, pH 7.4) (lipid/octyl glucoside = 1:5, mol/mol). The resultant solution was mixed vigorously with 34A conjugated with NGPE (antibody/lipid = 1:4 or 1:16, wt/wt), and then the mixture was dialyzed against PBS for 16–20 hr at 4°C. The immunoliposomes were extruded four times through a stack of two Nuclepore membranes (pore size, 0.4 μm). The average size of the liposomes was measured with a Coulter (Hialeah, FL) N4SD submicron particle size analyzer. The immunoliposomes were separated from the unbound antibody (50–70% of the original amount) on a Bio-Gel A1.5M (Bio-Rad) column. The peak liposome fractions were pooled and diluted to 1 mg of lipid per ml of PBS (pH 7.4). The amount of 34A incorporated into liposomes and the final ratio of antibody/lipid in the immunoliposomes containing 34A were calculated from the radioactivities of $^{111}$In for lipids and $^{125}$I for the antibody.

**Biodistribution Studies.** Liposomes (200 μg of lipid) were injected into BALB/c mice (6–8 weeks old) by means of the tail vein, and the distribution of the liposomes in major organs and blood was examined as a function of time. At the desired time, mice were lightly anesthetized and bled by eye puncture. Blood was collected and weighed. The mice were sacrificed by cervical dislocation and dissected. Organs were collected, weighed, and analyzed for $^{111}$In radioactivity in a γ counter. The results are presented as the percentage of the total injected dose for each organ. The total radioactivity in the blood was determined by assuming that the total volume of blood was 7.3% of the body weight (20).

For the portal vein injections, mice were anesthetized with ketamine (Parke-Davis) and underwent laparotomy. The needle was kept at the injection site for the desired time to avoid excessive bleeding.

## RESULTS

We have tested six different liposome preparations, which differ in lipid composition and antibody content, for accumulation in lung. The lipid composition of PC/cholesterol/PS, 10:5:1, which is widely used for liposomal drug delivery (1), contains PS, a well-known opsonin that allows elevated uptake of liposomes by the RES (21, 22). Liposomes with this lipid composition are hereafter called PS-containing liposomes. The lipid composition PC/cholesterol/$GM_1$, 10:5:1, contains $GM_1$, which endows the liposomes with low affinity to RES and thus a prolonged residence time in circulation (16, 23–25). These liposomes have been named stealth liposomes (15). We have also varied the amount of antibody 34A conjugated to the liposomes, which is expressed in terms of the protein/lipid ratio (wt/wt). Since all liposome preparations were 250–300 nm in average diameter and largely unilamellar in nature, the average number of antibody molecules per liposome can be calculated directly from the value of the protein/lipid ratio of a given liposome preparation by the method of Enoch and Strittmatter (26). For example, a protein/lipid ratio of 1:11 corresponds to 174 antibody molecules per liposome. This number includes those antibody molecules facing the inside of the liposomes, which would not be available for the target binding.

Liposomes labeled with $^{111}$In-DTPA–SA were injected by means of the tail vein, and the biodistribution of the injected liposomes in various major organs was measured at different time periods. The accumulation of liposomes in the target organ, the lung, is shown in Fig. 1. It is clear that liposomes, stealth or opsonized, without antibody 34A did not accumulate in the lung at all. Lung accumulation of the immunoliposomes was dependent on the number of antibody molecules per liposome. For stealth immunoliposomes with an antibody/lipid ratio of 1:11, lung accumulation was ≈70% of the injected dose, whereas those with a ratio of 1:37 only accumulated ≈60% of the injected dose. For the PS-containing immunoliposomes with an antibody/lipid ratio of 1:8, lung accumulation was ≈50%, and those with a ratio of 1:31 accumulated only about 25% of the injected dose. These results also indicated that the level of target binding of the immunoliposomes strongly depended on the lipid composition. The stealth composition allowed a significantly higher level of binding than the PS-containing composition. Stealth immunoliposomes with a lower antibody content (antibody/lipid ratio = 1:37) bound to the lung target better than the

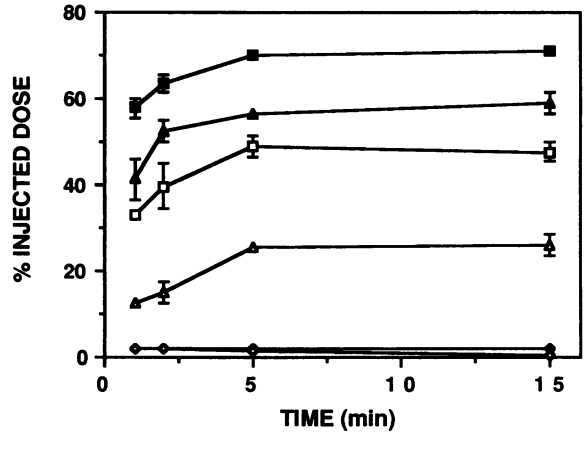


FIG. 1. Lung uptake of stealth liposomes or PS-containing liposomes. Liposomes (200 μg of lipid) were injected by means of the tail vein. Bars represent SD ($n$ = 3). ■, Stealth immunoliposomes with an antibody/lipid ratio = 1:11 and an average diameter = 297 nm. ▲, Stealth immunoliposomes with an antibody/lipid ratio = 1:37 and an average diameter = 292 nm. ◆, Stealth liposomes without antibody and with an average diameter = 268 nm. □, PS-containing immunoliposomes with an antibody/lipid ratio = 1:8 and an average diameter = 253 nm. △, PS-containing immunoliposomes with an antibody/lipid ratio = 1:31 and an average diameter = 255 nm. ◇, PS-containing liposomes without antibody and with an average diameter = 235 nm.

PS-containing immunoliposomes containing more than 4-fold more antibody molecules per liposome (ratio = 1:8). Thus, although the antibody content of the immunoliposome is an important parameter for target binding as previously concluded (8), the lipid composition of the immunoliposome plays an even more important role. Immunoliposome binding to lung was relatively rapid; it reached steady state within 5 min after injection (Fig. 1).

The major organ for nonspecific uptake of liposomes is the liver. Fig. 2 shows the kinetics of liver uptake of immunoliposomes. For liposomes without antibody, PS-containing liposomes were taken up by the liver at a relatively high rate. Uptake increased almost linearly with time, reaching ≈70% of the injected dose at 15 min. Stealth liposomes, on the other hand, showed a relatively low rate of liver uptake; only about 16% of the injected dose accumulated in the liver at 15 min. Thus, the inclusion of $GM_1$ in the liposome significantly reduces the liposome's affinity to the RES. This observation is consistent with those of previous reports (15, 16, 23–25, 27). The PS-containing immunoliposomes showed approximately the same initial rate of liver uptake, but the subsequent rate of liver uptake decreased with increasing level of antibody content of the immunoliposomes. This is probably due to the fact that most of the immunoliposomes were already taken up by the lung at the later time periods. However, the level of liver uptake of the PS-containing immunoliposomes containing a large amount of antibody (antibody/lipid ratio = 1:8) was still significantly higher than that of stealth liposomes containing no antibody (Fig. 2). This result indicates the significance of the lipid composition in controlling the RES uptake of liposomes. Increasing the antibody content of stealth liposomes did not further reduce the rate of liver uptake. For all stealth liposomes examined in this study, the liver uptake rate was ≈3–4% of the injected dose per 10 min, which was ≈10-fold lower than the rate of liver uptake for the PS-containing liposomes (Fig. 2).

Immunoliposomes containing antibody 34A may bind to the lung efficiently because tail vein-injected liposomes must first pass through the lung capillary system before they encounter liver cells. Although this possibility is not likely in view of the rapid blood circulation time (≈12 sec) in mouse (28, 29), we nevertheless performed the portal vein injection experiment. PS-containing immunoliposomes with an antibody/lipid ratio of 1:7 were injected into either the tail vein or the portal vein, and the biodistribution of the immunoliposomes was examined at different periods of time up to 5 min after injection. Data shown in Fig. 3 clearly indicate that the kinetics of liposome uptake by lung and liver and the clearance from blood were indistinguishable for the two routes of injection. The same conclusion was also obtained

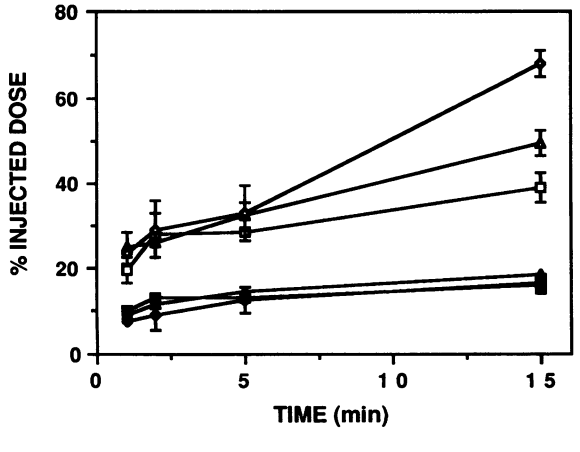


FIG. 2. Liver uptake of stealth liposomes or PS-containing liposomes. The symbols are as indicated in Fig. 1.

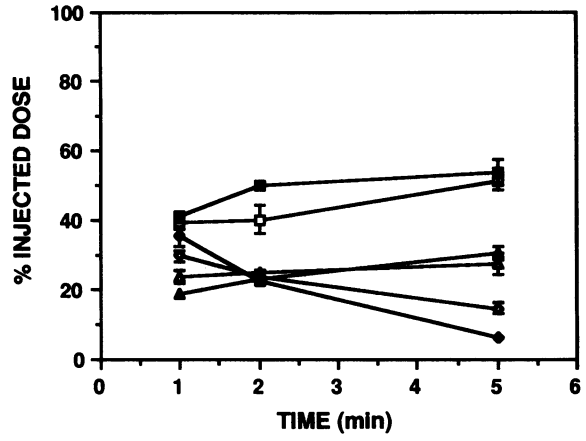


FIG. 3. Comparison between tail-vein injection and portal-vein injection for biodistribution in lung, liver, and blood of PS-containing immunoliposomes (antibody/lipid ratio = 1:7 and average diameter = 230 nm). Bars represent SD ($n$ = 3). □, Lung; △, liver; ◇, blood. Closed symbols indicate tail-vein injection and open symbols indicate portal-vein injection.

for immunoliposomes of the stealth composition (data not shown). Therefore, the accumulation of immunoliposomes in the lung was not due to the first passage effect, but rather to the immunospecific binding of the immunoliposomes.

We have examined the residence time of immunoliposomes at the target site. This aspect of liposome targeting could not be rigorously evaluated with our previously used liposome markers, which showed degradation and metabolism by liver and lung (8). $^{111}$In-DTPA–SA is a marker that is eliminated at a very slow rate ($t_{1/2}$ ≈ 21 days) (9). We could thus measure the immunoliposome retention in the lung using this marker. Four different preparations of immunoliposomes were injected: PS-containing immunoliposomes with a high and low level of antibody content (antibody/lipid ratio = 1:8 and 1:31, respectively) and stealth immunoliposomes with a high and low level of antibody content (ratio = 1:11 and 1:37, respectively). The amount of liposomes retained in the lung was measured up to 8 hr after injection. It is clear from the data shown in Fig. 4 that stealth immunoliposomes are generally retained at a higher level than the PS-containing immunoli-

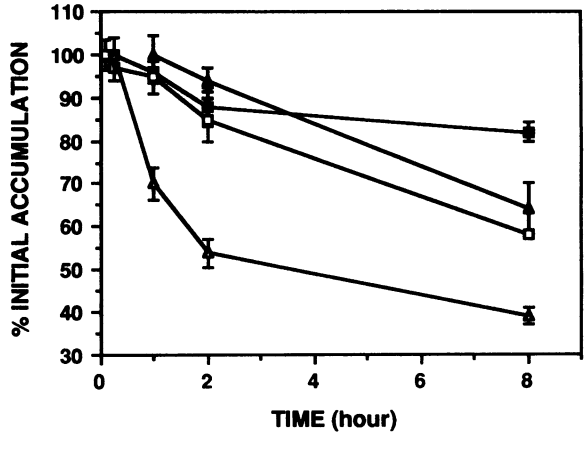


FIG. 4. Retention of immunoliposomes in lung. Immunoliposomes (200 μg of lipid) labeled with $^{111}$In-DTPA–SA were injected i.v. The percent initial accumulation in lung was calculated at the indicated time intervals. Bars represent SD ($n$ = 3). ■, Stealth immunoliposomes with an antibody/lipid ratio = 1:11 and an average diameter = 297 nm. ▲, Stealth immunoliposomes with an antibody/lipid ratio = 1:37 and an average diameter = 292 nm. □, PS-containing immunoliposomes with an antibody/lipid ratio = 1:8 and an average diameter = 253 nm. △, PS-containing immunoliposomes with an antibody/lipid ratio = 1:31 and an average diameter = 255 nm.

posomes. Furthermore, within the same lipid composition, retention was dependent on the antibody content of the immunoliposomes. Stealth immunoliposomes with high antibody content showed only about 15% dissociation of the bound immunoliposomes for 8 hr, whereas immunoliposomes of low antibody content showed about 35% loss of the bound immunoliposomes for the same time period. The most drastic dissociation of the bound immunoliposomes was in the case of the PS-containing immunoliposomes with low antibody content. A rapid loss of liposomes occurred within 2 hr of injection, followed by a slower phase of further loss, with only about 35% of the originally bound immunoliposomes remaining in the lung at 8 hr. Thus, immunoliposome retention at the target site is a function of the lipid composition as well as the antibody content of the immunoliposomes.

## DISCUSSION

Data presented in Figs. 1 and 2 can be explained on the basis of two competing processes: binding of immunoliposomes to the lung target and uptake by RES. Due to the rapid blood circulation of mice, injected immunoliposomes should be evenly distributed in the vascular space within the first minute after injection. Rates of target binding and RES uptake should be controlled by the factors concerning the properties of the immunoliposomes and their interactions with the blood components. We have demonstrated previously that the surface density of antibody on liposomes is, among other parameters, an important factor determining the level of target binding of immunoliposomes (8). Data in Fig. 1 now show that a high antibody/lipid ratio of immunoliposomes leads to a high rate of accumulation in the lung. Although we could not collect data at time points shorter than 1 min, it is clear from the data in Fig. 1 that increasing the antibody content of the immunoliposomes resulted in faster accumulation in lung, regardless of the lipid composition. For example, the PS-containing immunoliposomes with an antibody/lipid ratio of 1:31 reached half of the steady-state binding level at about 1 min after injection, whereas the binding of immunoliposomes with an antibody/lipid ratio of 1:8 had already reached 67% of the steady-state level at 1 min (Fig. 1). Thus, the rate of immunoliposome binding to the lung target is very fast, and increasing the antibody content of liposomes makes the rate even higher. This situation is quite different from immunoliposome binding to target cells *in vitro*, where the "on-rate" is not enhanced significantly with increasing antibody density (K. Houck and L.H., unpublished data). This is because the on-rate of binding in a static, nonflow condition is largely limited by the encounter frequency of immunoliposomes with the target, which is diffusion limited. Immunoliposome binding *in vivo*, on the other hand, is not likely limited by the encounter frequency because the rapid blood flow results in frequent collisions of the immunoliposomes with the lung target. The on-rate is likely limited by the probability of immune complex formation when an immunoliposome encounters its target and hence is directly proportional to the antibody density on the immunoliposomes.

The major competing process for immunoliposome target binding is uptake by the RES. Data shown in Fig. 2 show that the stealth lipid composition has produced approximately a 10-fold reduction in the RES uptake rate of liposomes without antibodies. Although the mechanism of the stealth activity of $GM_1$ is not clearly understood (24), attachment of 34A antibody molecules on the liposome surface does not significantly alter this activity. Observations by other investigators indicate that the RES uptake of the IgG antibody-conjugated, $GM_1$-containing liposomes is only slightly increased over that of the antibody-free liposomes (25). However, other studies have shown that a coating of intact IgG molecules on liposome surfaces enhances RES uptake by a Fc receptor-mediated mechanism (13, 14). Either the stealth mechanism of $GM_1$ has somehow masked the Fc receptor recognition of the bound IgG or the IgG is attached in such a way that the Fc portion is not available for binding. The latter mechanism seems more plausible because we have previously shown that IgG molecules covalently modified with fatty acid are not available for anti-Fc antibody binding (30). This is because the activated fatty acid, a hydrophobic reagent, preferentially reacts with the relatively hydrophobic Fc (31). The hydrophobic reagent NGPE used in the present study may also be preferentially attached to the Fc portion of the antibody molecules, rendering them unavailable for Fc receptor binding.

Data in Fig. 2 show that the RES uptake rate is significantly reduced with stealth immunoliposomes relative to PS-containing immunoliposomes. The net result is that the steady-state level of the target binding of these liposomes is significantly higher than that of the PS-containing immunoliposomes. Although the stealth activity of liposomes is largely contributed by the presence of $GM_1$, the absence of PS in the lipid composition also partially contributes to the activity. This is because the RES uptake of 34A-containing liposomes composed of PC/cholesterol is somewhat lower than that of the 34A-containing liposomes composed of PC/cholesterol/PS (K.M. and L.H., unpublished data). The combined results shown in Figs. 1 and 2 indicate the importance of the stealth activity, generated in this case by manipulation of the lipid composition, in achieving a highly efficient target binding of immunoliposomes. The combination of using the stealth lipid composition to suppress the RES uptake rate on one hand and attaching a large number of antibody molecules to liposomes to enhance the target binding rate on the other produced an unprecedented high level of target binding of liposomes. This observation is completely opposite to the usual behavior of liposomes *in vivo* (i.e., nearly quantitative uptake by the RES).

Data presented in Fig. 3 are consistent with the above conclusions. Portal vein-injected immunoliposomes, even those containing PS, are taken up by the liver at a rate slower than that of the target binding, even though they encounter the liver cells first. No matter which route was used to inject the liposomes, the blood concentration of liposomes came to a steady-state level much earlier than did the level in the liver. It is clear that the high level of immunoliposome binding to lung is not due to the first passage effect.

The observation that stealth immunoliposomes showed a longer retention time in the lung than the PS-containing immunoliposomes (Fig. 4) is a surprising result. Preliminary results have shown that the bound immunoliposomes are not rapidly endocytosed during the initial few hours of binding (L.H., unpublished observations). Therefore, the disappearance of the bound immunoliposomes from the lung is likely due to the dissociation of the liposome from the capillary endothelial cell surface and reentry into the circulation. A slow dissociation rate is expected if liposomes are bound to the cell surface by means of multiple antibody–antigen bonds (32). This is probably why immunoliposomes with a higher antibody/lipid ratio showed a longer retention time in the lung than those with a lower ratio (Fig. 4). The observation that immunoliposomes with the stealth lipid composition showed longer retention times than the PS-containing immunoliposomes suggests that the dissociation of the surface-bound immunoliposomes may involve macrophages, such that the PS-containing liposomes are removed from the target site at a faster rate due to the known opsonin activity of PS (21, 22). Alternatively, it is more likely that the dissociated immunoliposomes can rebind to the lung target as long as they are not removed from circulation by RES. Conse-

quently, the apparent residence time in the lung is prolonged for the stealth immunoliposomes because they stand a better chance of staying in the circulation and hence rebinding to the target.

While these mechanistic speculations will have to be tested experimentally, the observation that stealth lipid composition provides an excellent target binding and retention of immunoliposomes deserves serious attention in terms of its potential as a targeted drug delivery vehicle. Stealth immunoliposomes such as the ones described here should be able to provide a high local concentration of the encapsulated drug at the target site for a prolonged period of time. Unwanted toxic effects of the drug in other tissues and cells may also be reduced. This is particularly important for many highly toxic antitumor drugs for cancer chemotherapy (25).

We thank Carolyn Drake for expert word processing. This work was supported by National Institutes of Health Grants CA 24553 and AI 25834. S.J.K. is supported by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with the Martin Marietta Energy Systems, Inc.

1. Gregoriadis, G. (1988) *Liposomes as Drug Carriers* (Wiley, New York).
2. Torchilin, V. P. (1985) *CRC Crit. Rev. Ther. Drug Carrier Syst.* **2**, 65–115.
3. Wright, S. & Huang, L. (1989) *Adv. Drug Delivery Rev.* **3**, 343–389.
4. Peeters, P. A. M., Storm, G. & Crommelin, D. J. A. (1987) *Adv. Drug Delivery Rev.* **1**, 249–266.
5. Hughes, B. J., Kennel, S., Lee, R. & Huang, L. (1989) *Cancer Res.* **49**, 6214–6220.
6. Kennel, S. J., Lankford, T., Hughes, B. & Hotchkiss, J. (1988) *Lab. Invest.* **59**, 692–701.
7. Rorvik, M. C., Allison, D. P., Hotchkiss, J. A., Witchi, H. P. & Kennel, S. J. (1988) *J. Histochem. Cytochem.* **36**, 741–749.
8. Maruyama, K., Holmberg, E., Kennel, S. J., Klibanov, A., Torchilin, V. P. & Huang, L. (1990) *J. Pharm. Sci.*, in press.
9. Holmberg, E., Maruyama, K., Litzinger, D. C., Wright, S., Davis, M., Kabalka, G. W., Kennel, S. J. & Huang, L. (1989) *Biochem. Biophys. Res. Commun.* **165**, 1272–1278.
10. Poste, G., Bucana, C., Raz, A., Bugelski, P., Kirsh, R. & Fidler, I. J. (1982) *Cancer Res.* **42**, 1412–1422.
11. Gregoriadis, G. & Senior, J. (1986) in *Targeting of Drugs with Synthetic Systems*, eds. Gregoriadis, G., Senior, J. & Poste, G. (Plenum, New York), pp. 183–192.
12. Senior, J. H. (1987) *CRC Crit. Rev. Ther. Drug Carrier Syst.* **3**, 123–193.
13. Aragnol, D. & Leserman, L. D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2699–2703.
14. Derksen, J. T. P., Marselt, H. W. M. & Scherphof, G. L. (1988) *Biochim. Biophys. Acta* **971**, 127–136.
15. Allen, T. M. (1989) in *Liposomes in the Therapy of Infectious Diseases and Cancer*, eds. Berestein, G. L. & Fidler, I. J. (Liss, New York), pp. 405–415.
16. Gabizon, A. & Papahadjopoulos, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6949–6953.
17. Kabalka, G. W., Buonocore, E., Hubner, K., Moss, T., Norley, N. & Huang, L. (1987) *Radiology* **163**, 255–258.
18. Weissig, V., Lasch, J., Klibanov, A. L. & Torchilin, V. P. (1986) *FEBS Lett.* **202**, 86–90.
19. Kennel, S. J., Foote, L. J. & Lankford, P. K. (1981) *Cancer Res.* **41**, 707–712.
20. Wu, M. S., Robbins, J. C., Bugianesi, R. L., Ponpipom, M. M. & Shen, T. Y. (1981) *Biochim. Biophys. Acta* **674**, 255–258.
21. Raz, A., Bucana, C., Fogler, W. E., Poste, G. & Fidler, I. J. (1981) *Cancer Res.* **41**, 487–494.
22. Allen, T. M., Williamson, P. & Schlegel, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8067–8071.
23. Allen, T. M. & Chonn, A. (1987) *FEBS Lett.* **223**, 42–46.
24. Allen, T. M., Hansen, C. & Rutledge, J. (1989) *Biochim. Biophys. Acta* **981**, 27–35.
25. Papahadjopoulos, D. & Gabizon, A. (1987) *Ann. N.Y. Acad. Sci.* **507**, 64–74.
26. Enoch, H. G. & Strittmatter, P. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 145–149.
27. Liu, D. & Huang, L. (1990) *Biochim. Biophys. Acta* **1022**, 348–354.
28. Bell, G. H., Emsline-Smith, D. & Paterson, C. R. (1976) *Textbook of Physiology and Biochemistry* (Livingstone, Edinburgh), 9th Ed., Chap. 28, pp. 371–392.
29. Kaplan, H. M., Brewer, N. R. & Blair, W. H. (1983) in *The Mouse in Biomedical Research*, eds. Foster, H. L., Small, J. D. & Fox, J. G. (Academic, New York), Vol. 3, Chap. 11, pp. 248–292.
30. Connor, J., Sullivan, S. & Huang, L. (1985) *Pharmacol. Ther.* **28**, 341–365.
31. Ho, R. J. Y., Rouse, B. T. & Huang, L. (1986) *Biochemistry* **25**, 5500–5506.
32. Bell, G. I. (1978) *Science* **200**, 618–627.