Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6117–6121, August 1986
Medical Sciences

Enhanced delivery to target cells by heat-sensitive immunoliposomes

(liposome targeting/controlled release)

SEAN M. SULLIVAN* AND LEAF HUANG†

Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840

Communicated by John D. Baldeschwieler, April 18, 1986

ABSTRACT **Heat-sensitive immunoliposomes are capable of releasing the entrapped content at the target cell surface upon a brief heating to the phase transition temperature of the liposome membrane. In this study we have examined the delivery efficiency of drugs entrapped in heat-sensitive immunoliposomes. Immunoliposomes composed of dipalmitoyl phosphatidylcholine with entrapped [^{3}H]uridine were incubated with target cells at 4°C. The cell–liposome mixture was then heated to 41°C and the uptake of [^{3}H]uridine into the intracellular pool of phosphorylated uridine-containing molecules was measured. The immunoliposomes showed maximal release of the uridine at 41°C, the phase transition temperature of dipalmitoyl phosphatidylcholine liposomes. The largest accumulation of [^{3}H]uridine in the target cells also took place at 41°C. The initial level of uptake of [^{3}H]uridine released from immunoliposomes by heating was greatly enhanced over that observed for free [^{3}H]uridine and [^{3}H]uridine released from liposomes without attached antibody. The nucleoside uptake inhibitors nitrothiobenzylinosine, dipyridamole, and unlabeled uridine were able to inhibit uptake of [^{3}H]uridine released from immunoliposomes. This supports the hypothesis that the enhanced uptake is due to a heat-induced release of [^{3}H]uridine at the cell surface followed by transport and phosphorylation of [^{3}H]uridine by the target cells. These results indicate the feasibility of using the heat-sensitive immunoliposomes as a target-specific drug delivery system.**

Liposomes have been used as carriers for a wide variety of biologically active materials (1–5). Attachment of monoclonal antibodies to the liposome surface has resulted in specific binding of the liposomes to cells expressing a cell surface antigen (for reviews see refs. 6 and 7). Upon binding, the liposomes are internalized and delivered to the lysosomes (8–11). The delivered drug must be able to escape this degrading organelle into the cytosol to exert its therapeutic effect. Although this type of liposome delivery system has been effective in a few cases, it is inefficient for most of the commonly used drugs. To increase the delivery efficiency, site-directed liposomes with special functions have been developed.

pH-sensitive liposomes are one type of special-function liposomes that are capable of fusing with the endosomal membrane upon acidification of the endocytic vacuole, thereby releasing its entrapped contents into the cytoplasm (12–14). However, this mechanism, although quite novel and very successful, is dependent upon endocytosis of the liposomes by the target cells. Efficient delivery of immunoliposome-encapsulated drugs to cells that do not actively endocytose requires an alternative approach. Heat-sensitive liposomes have been shown to potentially fulfill this need (15–18). Attachment of antibody to the heat-sensitive liposomes maintains target cell specificity with respect to binding. Once bound to the target cell, these immunoliposomes are able to release their entrapped contents upon heating to the phase transition temperature of the liposomal lipid. The advantage arises from the formation of a large localized concentration of drug at the target cell surface, which serves to enhance drug uptake over that of drug released into the bulk medium.

In the initial characterization of these immunoliposomes, the fluorescent dye carboxyfluorescein was used to monitor the release properties (19). This dye was not transported by the cells (20) and therefore, the delivery efficiency could not be evaluated. In this report we examine delivery efficiency, using uridine uptake as a model system for delivery of cytotoxic nucleosides. Nucleoside transport was chosen for three reasons. First, nucleoside transport is rapid and therefore able to take advantage of a high localized concentration created at the cell surface. Second, nucleoside metabolism has been well characterized with respect to kinetic parameters and availability of inhibitors. Third, information obtained from uridine delivery can be directly applied to the delivery of chemotherapeutic nucleoside analogues such as 1-β-D-arabinofuranosylcytosine, which has similar uptake characteristics (21). We report here the enhanced uptake of uridine released from immunoliposomes bound to the target cells.

MATERIALS AND METHODS

Reagents. Isolation, iodination, and derivatization of mouse monoclonal anti-H2K^k IgG with the palmitic acid ester of *N*-hydroxysuccinimide have previously been described (8, 19, 22). Monoclonal P3 IgG was purified from ascites fluid of mice bearing intraperitoneal P3-X63-Ag8 cells. This IgG has unknown binding specificity and does not bind with RDM4 cells. It was used as a control IgG. Dipalmitoyl phosphatidylcholine (Pam_2-PtdCho) was purchased from Avanti Polar Lipids and stored in $CHCl_3$ under N_2 at −20°C. Phosphate was determined by the method of Bartlet (23). [^{3}H]Hexadecyl cholestanyl ether was used as a lipid marker (24). Uridine was purchased from Sigma. [^{3}H]Uridine was purchased from ICN. *S*-(4-Nitrobenzyl)-6-thioinosine was purchased from Aldrich. Dipyridamole [2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine] was obtained from Boehringer Mannheim. Deoxycholate was purchased from Calbiochem and recrystallized twice.

Preparation of Immunoliposomes with Entrapped Uridine. Immunoliposomes were prepared as previously described (19). Briefly, small unilamellar liposomes composed of Pam_2-PtdCho were prepared and allowed to fuse at 4°C for 3–30 days. The large liposomes were separated from smaller ones by using a 35-ml preparative 5–20% continuous sucrose

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.

Abbreviation: Pam_2-PtdCho, dipalmitoyl phosphatidylcholine.

*Present address: Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.
†To whom reprint requests should be addressed.

density gradient centrifuged at 80,000 × g for 18 hr. A 27 mM Pam_2-PtdCho liposome suspension was prepared in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline containing 50 mM [^{3}H]uridine, 1 mM EGTA, and 0.02% NaN_3. The [^{3}H]uridine specific activity was 2–4 × 10^5 cpm/nmol. The suspension was placed in a temperature-regulated chamber set at 41°C. A 15-mg/ml solution of palmitoyl-specific antibody in phosphate-buffered saline containing 3.8 mM deoxycholate, pH 8.0, was injected into the liposome suspension at a rate of 0.26 μl/min by using an infusion pump. The suspension was annealed at 43°C for 20 min and equilibrated to room temperature over a period of 20 min. The suspension was dialyzed overnight against phosphate-buffered saline containing 1 mM EGTA and 0.02% NaN_3 to remove untrapped [^{3}H]uridine and residual deoxycholate. Unincorporated palmitoyl antibody was removed by the same centrifugation procedure used to fractionate the stock Pam_2-PtdCho liposomes. The uridine/antibody peak was pooled and dialyzed to remove sucrose. "Bare liposomes" (without attached palmitoyl antibody) were prepared in the same fashion as the immunoliposomes except that only phosphate-buffered saline/3.8 mM deoxycholate was injected into the liposome suspension. Bare liposomes had the same trapping efficiency as immunoliposomes. Prior to cell incubation, immunoliposomes and bare liposomes were dialyzed against culture media without serum to remove NaN_3.

Determination of Uridine Uptake. RDM4 cells were cultured in RPMI 1640 medium supplemented with 1 mM sodium pyruvate and 10% fetal bovine serum. For incubation with liposomes, cells were collected by centrifugation at 500 rpm for 5 min in a desk-top centrifuge. Cells were resuspended in cell culture medium containing 10% dialyzed donor bovine serum to a concentration of 2 × 10^7 cells per ml. Then 0.5 ml of cell suspension was added to a 6-ml culture tube and allowed to equilibrate to 4°C for 10 min. One microgram of immunoliposome-bound palmitoyl antibody, equivalent to 34 μg of Pam_2PtdCho, was added to the RDM4 cells and incubated at 4°C for 1 hr. This was followed by an 18°C incubation for 10 min. The incubation mixture was then diluted to a final volume of 4 ml with culture medium and placed in a 42.5°C water bath. Because it took about 1 min to reach 41°C, free [^{3}H]uridine was added to control cells after the cell suspension was placed in the water bath for 1 min. The amount of free [^{3}H]uridine added was equal to that released from the immunoliposomes, which was previously determined. After heating for the designated time, the samples were placed in ice and centrifuged at 1000 × g for 10 min. The ^{3}H cpm released into supernatant was measured. Pellets were frozen in an ethanol/dry ice bath, thawed and solubilized with 1% Triton X-100 containing 10 mM unlabeled uridine, and applied to DEAE-cellulose filters (Whatman DE-81). The filters were washed four times with 500 ml of 10 mM ammonium formate and twice with 500 ml of water. Filters were incubated overnight with 1 ml of Protosol tissue solubilizer (New England Nuclear) and radioactivity was measured with 10 ml of toluene-based scintillation fluor.

Determination of Temperature-Dependent Uridine Release and Uptake. The protocol was the same as above except that after the 10-min incubation at 18°C, cells were incubated for 5 min at the designated temperature and then placed on ice. Percent release was monitored according to Magin and Morse (25). A 0.2-ml sample of suspension was centrifuged at 200,000 × g for 20 min to pellet the cells and liposomes. The ^{3}H cpm in the supernatant and the pellet were measured to determine the percent [^{3}H]uridine release.

Inhibition of Uridine Uptake by Nucleoside Transport Inhibitors. The incubation conditions were the same as used for uptake measurements with the following modifications. After the 10-min incubation at 18°C, the cell suspension was diluted to 4 ml with cell culture medium containing the inhibitor and incubation at 18°C was continued for another 10 min before heating.

RESULTS

Phosphorylation of uridine occurs only intracellularly and is therefore a good criterion for examining the efficiency of uridine delivery by the immunoliposomes. Uridine transport and metabolism are collectively referred to as "uptake." Specifically, the uptake process involves diffusion of extracellular nucleosides across the plasma membrane via a transport system, followed by the intracellular kinase-catalyzed phosphorylation of transported uridine. An assay was developed that measured all phosphorylated uridine metabolites, including UMP, UDP, UTP, UDP-glucose, and RNA. Phosphorylated uridine metabolites were collected on an anion-exchange filter and unphosphorylated uridine was washed away. This assay and paper chromatographic analysis of perchloric acid-soluble material (26) yielded comparable results (data not shown).

Temperature-Dependent Release of Uridine from Immunoliposomes. Heat-sensitive immunoliposomes were prepared containing 50 mM [^{3}H]uridine. Incubation with 10^7 RDM4 cells yielded 15–20% of palmitoyl antibody bound and 5–8% of [^{3}H]uridine bound. A 50-fold excess of free antibody was able to inhibit the palmitoyl antibody binding and [^{3}H]uridine binding (data not shown). This demonstrated the cell-associated uridine to be the result of immunoliposome binding and not due to uptake of released uridine during the incubation. Release of [^{3}H]uridine from heat-sensitive immunoliposomes bound to RDM4 cells is shown in Fig. 1. Below 41°C, negligible amounts of [^{3}H]uridine had been released from the immunoliposomes. It was only at 41°C or above that the maximal amount of [^{3}H]uridine release was observed. No further increase in percent release was observed above 41°C. These results demonstrate the ability of the heat-sensitive immunoliposomes to release uridine upon heating, and they also closely resemble our results for release of carboxyfluorescein from the same immunoliposome system (19).

Uptake of Free Uridine and Uridine Released from Immunoliposomes and Bare Liposomes. Shown in Fig. 2 is the uptake of free [^{3}H]uridine and [^{3}H]uridine released from heat-sensitive immunoliposomes or heat-sensitive bare lipo-

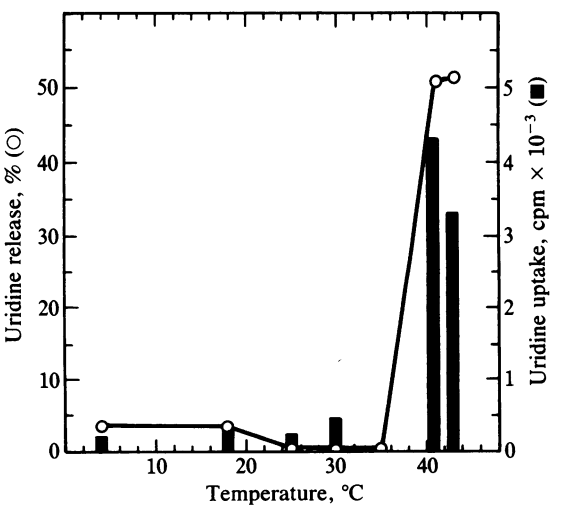

FIG. 1. Temperature-dependent release and uptake of uridine. Cells were incubated with immunoliposomes containing [^{3}H]uridine for 1 hr at 4°C. After 10 min at 18°C, they were heated to the indicated temperature and incubated for 5 min. Aliquots of cell suspension were analyzed for percent uridine release (○) and the remainder of the cells was analyzed for uridine uptake (bars).

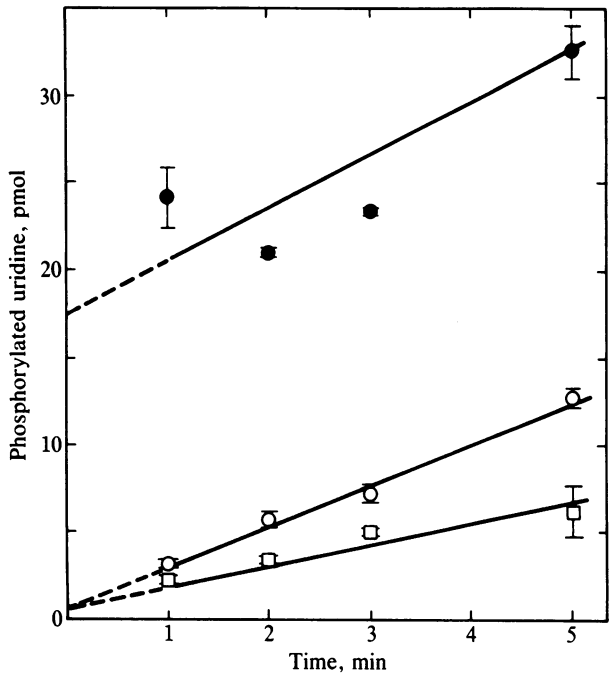

FIG. 2. Kinetics of uptake of free [^{3}H]uridine (□) and [^{3}H]uridine released from immunoliposomes (●) and bare liposomes (○). Zero time was 1 min after the cell suspension was placed in the heating bath. Straight lines are linear regressions of data points (±SD) with r values of 0.83, 0.99, and 0.98 for immunoliposomes, bare liposomes, and free uridine, respectively.

somes as a function of time. When the cell suspension was first placed in the water bath, there was a 1-min ± 10-sec lag period before the suspension reached the release temperature—i.e., 41°C. Since the exact zero time for release was uncertain, we have chosen to indicate the "zero time" in the figure as 1 min after the cell suspension was placed in the water bath. Furthermore, the data for the four time points for each of the three conditions are fitted with straight lines by linear regression and extrapolated to zero time to estimate the accumulation of phosphorylated [^{3}H]uridine at the initial period of release. For release of [^{3}H]uridine from immunoliposomes, 17.5 pmol of phosphorylated uridine per 10^7 cells was observed at the initial time of release. The rate of uptake after 1 min (slope of the regression line) was 3.0 pmol/min. The initial accumulation of the phosphorylated uridine for the bare liposomes and free uridine was negligibly small. The later rate of uptake of [^{3}H]uridine released from bare liposomes was 2.4 pmol/min and the rate of uptake for free [^{3}H]uridine was 1.2 pmol/min. Therefore, a significantly higher initial accumulation of the phosphorylated uridine was observed for immunoliposome-released [^{3}H]uridine as compared with that released from the bare liposomes or the free [^{3}H]uridine. No enhanced intracellular accumulation was observed for free uridine in the presence of empty immunoliposomes or for uridine released from P3 immunoliposomes (results not shown). The subsequent uptake rates were not significantly different from one another. These results clearly illustrate that the delivery of encapsulated contents by the heat-sensitive immunoliposomes is enhanced only in the initial period of heating.

The temperature dependence for this enhanced uptake of immunoliposome-released [^{3}H]uridine is shown in Fig. 1. Below 35°C, negligible amounts of [^{3}H]uridine were released from the immunoliposomes. Consequently, only background levels of [^{3}H]uridine uptake were observed. Above 41°C, maximal [^{3}H]uridine release from the bound immunoliposomes was observed. The largest accumulation of phosphorylated [^{3}H]uridine metabolites was also observed at this temperature range. In a control experiment, free [^{3}H]uridine uptake showed a gradual increase in the uptake rate as a function of temperature, but no abrupt increase was observed at 41°C (data not shown). A decrease in uptake of immunoliposome-released [^{3}H]uridine was observed at 43°C. This decreased rate of uptake was also observed for free [^{3}H]uridine. These results show that the enhanced uridine uptake took place at temperatures at which uridine release from the cell-bound immunoliposomes was maximal. Furthermore, the enhanced uptake was due to [^{3}H]uridine released from the immunoliposomes and not due to temperature-dependent stimulation of the uptake process itself.

Inhibition of Uridine Uptake. The mode of entry for the released [^{3}H]uridine was examined by using three types of nucleoside uptake inhibitors: nitrothiobenzylinosine (K_i = 1 nM), dipyridamole (K_i = 12 μM from the intercept; K_i = 3 μM from the slope) (27, 28), and unlabeled uridine. The results are shown in Fig. 3, in which *A* represents inhibition by unlabeled uridine, *B* represents inhibition by nitrothiobenzylinosine, and *C* represents inhibition by dipyridamole. Inhibitor was added during the dilution of the cell incubation mixture to 4 ml and was allowed to incubate with the cells an additional 10 min at 18°C. The additional incubation time did not affect the percent of immunoliposomes bound to the target cells (data not shown). Estimation of the concentration necessary to produce 50% inhibition of [^{3}H]uridine uptake yielded 35 μM unlabeled uridine for immunoliposomes and 5 μM for free [^{3}H]uridine, 79 nM nitrothiobenzylinosine for immunoliposomes and 5 nM for free [^{3}H]uridine, and 16 μM dipyridamole for immunoliposomes and 2 μM for free [^{3}H]-uridine. In summary, these results show that uptake of uridine released from immunoliposomes can be inhibited by various nucleoside uptake inhibitors. Also, the concentration

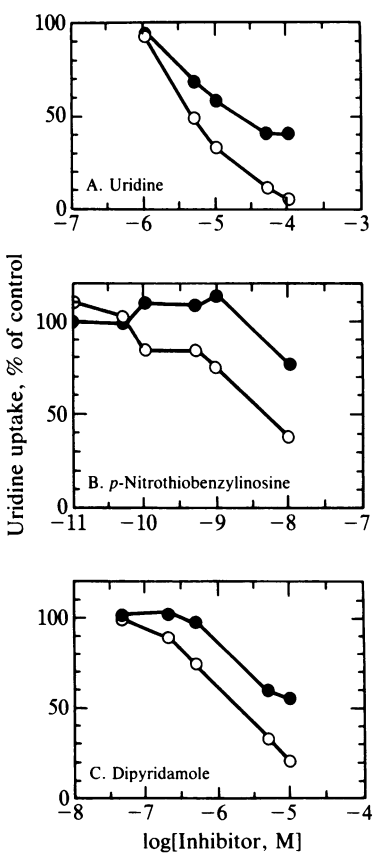

FIG. 3. Inhibition of uptake of immunoliposome-released (●) and free (○) [^{3}H]uridine by nucleoside transport inhibitors. Data are expressed as percent of uptake in control cells that had not been treated with inhibitors.

of inhibitor necessary to produce 50% inhibition was considerably greater for the immunoliposomes than for free [^{3}H]uridine, with nitrothiobenzylinosine exhibiting the largest concentration differential.

DISCUSSION

Previously, heat-sensitive liposomes have been used to deliver chemotherapeutic drugs to implanted tumors *in vivo* (17, 18). Localized heating of the tumor produced a significant increase in the accumulation of drug released from the heat-sensitive immunoliposomes over that of the controls (16, 17). However, only temporary inhibition of tumor growth was observed (17, 18). It is obvious that by improving the delivery efficiency, the therapeutic index should be increased and the subsequent deleterious effects should be diminished. The main thrust of this research was to show that enhanced cellular uptake of uridine can be achieved by release of uridine at the cell surface as opposed to release at a point distant from the cell surface or simply free in solution. This point was strongly made by the design of the experiment in that the majority (95%) of immunoliposomes were unbound and not removed from the cell suspension prior to heating. Both unbound and bound immunoliposomes have the same release properties and therefore, upon heating, most of the uridine was released into the bulk medium from the unbound immunoliposomes. However, a large accumulation of phosphorylated uridine was observed during the initial period of release from the immunoliposomes, whereas little or no accumulation was observed for [^{3}H]uridine released from bare liposomes or for free [^{3}H]uridine. These results show a local high concentration of uridine released from a small number of bound immunoliposomes made a significant improvement in cellular uptake of uridine.

The mode of the enhanced uptake was examined by using the uridine uptake inhibitors nitrothiobenzylinosine, dipyridamole, and unlabeled uridine. Nitrothiobenzylinosine is an inosine analog and a potent competitive inhibitor of nucleoside transport (20, 27–29). Dipyridamole, sometimes referred to as Persantine, is a noncompetitive inhibitor that yields mixed type inhibition for uridine transport (21). Addition of unlabeled uridine inhibited the uptake by decreasing the specific activity of the entrapped radiolabeled uridine upon release from the immunoliposomes. The results shown in Fig. 3 indicate that approximately an 8-fold greater concentration was required for unlabeled uridine or dipyridamole to inhibit the uptake of immunoliposome released [^{3}H]uridine. A 16-fold concentration difference was obtained for nitrothiobenzylinosine. Release of uridine at the cell surface would create a higher concentration at the cell surface compared to the medium and would therefore require a higher concentration of inhibitor for a similar level of inhibition. These results support the premise that enhancement of uptake of drug delivered by heat-sensitive immunoliposomes was due to release of uridine at the cell surface followed by transport and phosphorylation inside the target cell.

It is important to understand the controlling factors that ultimately determine the concentration and the duration of time that this transient concentration remains at the cell surface. These factors are (*i*) the rate of diffusion of the drug away from the cell surface, (*ii*) the rate of release of the drug from the liposomes, and (*iii*) the rate of heating. A mathematical model was designed to probe the influence each of these factors has on the transiently localized cell surface concentration. The model describes the release of uridine from bound immunoliposomes in terms of a concentration pulse. The rate of uridine release must be faster than the rate of diffusion from the cell surface in order for a transient localized high concentration to exist at the cell surface. Once the release rate becomes slower than the diffusion rate due to dissipation of the concentration gradient across the liposomal membrane, the transient concentration is rapidly diluted. For mathematical simplicity, this model makes the following approximations: (*i*) all the uridine released during the duration of the pulse is released at a constant rate; and (*ii*) phosphorylation and not transport is the rate-limiting step for the observed uptake. The concentration, C, or uridine at the cell surface at time t can be calculated by using the following integrated rate equation for spherically symmetrical diffusion (30):

$$C(t) = \frac{i}{4\pi Dr}\,\mathrm{erfc}\left[\frac{r}{(4Dt)^{1/2}}\right],$$

in which t is less than or equal to t_o, the duration of the pulse in sec, i is the rate of release of uridine from the bound immunoliposomes, D is the diffusion coefficient (10^{-5} cm^2/sec), and r is the distance separating the liposome surface from the site of uptake (5 nm). erfc is the complement of the error function, whose numerical values can be obtained from a standard mathematical table.

Fig. 2 shows the accumulation of phosphorylated uridine as a function of time. Extrapolation to zero time for immunoliposomes yields approximately 17.5 pmol of phosphorylated uridine accumulated within a very short period of time. Furthermore, the extrapolated line has a slope similar to that of free uridine uptake. This same result can be obtained by calculating the cell surface concentration using a pulse time of 5 sec and a rate of release from immunoliposomes of 4×10^{-18} mol/sec followed by determination of the accumulation of phosphorylated uridine by Michaelis–Menten kinetics using a K_m of 1.5×10^{-5} M and a V_{max} of 2.7×10^{-12} mol/sec per 10^7 cells (21). These calculations show that diffusion away from the cell surface is too rapid for uridine to accumulate at the cell surface for any period of time after the completion of uridine release from the bound immunoliposomes. This period is most likely determined by the rate of release from the liposomes and the rate of heating. The 5-sec pulse used to make this calculation is not unreasonable because carboxyfluorescein release from the same immunoliposome system bound to the same RDM4 cells was complete within 5 sec (unpublished result).

A mathematical model was developed by Blumenthal *et al.* (20) to describe the effect of the unstirred layer on the diffusion properties of released solute from heat-sensitive liposomes. The model was developed to explain the potential mechanism by which carboxyfluorescein was transported into the cell cytoplasm. This study correctly concluded that diffusion of carboxyfluorescein away from the cell surface is much more rapid than diffusion of the dye across the cell membrane. This is mainly because there is no transport system for carboxyfluorescein in the cell membrane and the permeability of the membrane to the dye is very low. In contrast, we have chosen in our model study an efficient transport system—i.e., nucleoside transport—and conclude that, if the rate of release from the liposome is comparable to the transport rate, increased delivery efficiency can be achieved.

Successful application of this type of controlled drug delivery system will be dependent upon the accessibility of the target cell or tissue and the susceptibility of the target area to localized heating. Presently, the most direct application is for treatment of tissue or cells outside of the body, such as in autologous transplantation after immunotherapy. Alternative administration routes such as subcutaneous and topical administrations may facilitate the liposomal accessibility to the target cells. Subcutaneous administration of liposomes results in rapid clearance from the initial injection site and accumulation in the regional lymph nodes (31), which are amenable to localized heating. Thus, the heat-sensitive im-

munoliposomes may provide potentially effective delivery of drugs for cells localized in the lymph nodes. Topical application of liposomes has been shown to increase the retention of localized drug concentration and minimize systemic absorption (32). Heat-sensitive immunoliposomes could serve to fine tune the steady-state localized concentration by regulating release of the drug through temperature control.

In conclusion, our results demonstrate the feasibility of using heat-sensitive immunoliposomes as a controlled release system. Furthermore, it demonstrates the advantage of this type of drug delivery over that of nontargeted liposomes and free drug, thus increasing the potential of liposomes as a targeted drug delivery system.

We thank Dr. R. Wohlhueter for his helpful discussions concerning nucleoside uptake properties. This work was supported by National Institutes of Health Grant CA 24553. L.H. is a National Institutes of Health Research Career Development Awardee (CA 00718).

1. Nicolau, C. & Paraf, A., eds. (1981) *Liposomes, Drugs, and Immunocompetent Cell Functions* (Academic, New York).
2. Ostro, M., ed. (1983) *The Liposomes* (Dekker, New York).
3. Knight, C. G., ed. (1981) *Liposomes: From Physical Structure to Therapeutic Applications* (Elsevier, New York).
4. Gregoriadis, G. & Allison, A. C., eds. (1980) *Liposomes in Biological Systems* (Wiley, New York).
5. Papahadjopoulos, D., ed. (1978) *Ann. N.Y. Acad. Sci.* **308**.
6. Gregoriadis, G., ed. (1985) in *Liposome Technology: Targeted Drug Delivery and Biological Interactions* (CRC, Boca Raton, FL), Vol. 3, pp. 29–75.
7. Connor, J., Sullivan, S. & Huang, L. (1985) *Pharmacol. Ther.* **28**, 341–365.
8. Huang, A., Kennel, S. J. & Huang, L. (1983) *J. Biol. Chem.* **258**, 14034–14040.
9. Machy, P., Pierres, M., Barbet, J. & Leserman, L. D. (1982) *J. Immunol.* **129**, 2098–2102.
10. Heath, T. D., Montgomery, J. A., Piper, J. R. & Papahadjopoulos, D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1377–1381.
11. Dijkstra, J., Van Galen, M. & Scherphof, G. (1985) *Biochim. Biophys. Acta* **845**, 34–42.
12. Connor, J. & Huang, L. (1985) *J. Cell Biol.* **101**, 582–589.
13. Duzgunes, N., Straubinger, R. M., Baldwin, P., Friend, D. S. & Papahadjopoulos, D. (1985) *Biochemistry* **24**, 3091–3098.
14. Ellens, H., Bentz, J. & Szoka, F. C. (1984) *Biochemistry* **23**, 1532–1538.
15. Yatvin, M., Weinstein, J. N., Dennis, W. H. & Blumenthal, R. (1978) *Science* **202**, 1290–1293.
16. Weinstein, J. N., Magin, R. L., Yatvin, M. & Zaharko, D. S. (1979) *Science* **204**, 188–191.
17. Weinstein, J. N., Magin, R. L. & Zharko, D. S. (1980) *Cancer Res.* **40**, 1388–1395.
18. Yatvin, M., Muhlensiepen, H., Porschen, W., Weinstein, J. N. & Feinendegen, L. E. (1981) *Cancer Res.* **41**, 1602–1607.
19. Sullivan, S. M. & Huang, L. (1985) *Biochim. Biophys. Acta* **812**, 116–126.
20. Blumenthal, R., Ralston, E., Dragsten, P., Leserman, L. D. & Weinstein, J. N. (1982) *Membr. Biochem.* **4**, 283–303.
21. Plagemann, P. G. & Wohlhueter, R. M. (1980) *Curr. Top. Membr. Transp.* **14**, 226–330.
22. Huang, A., Tsao, T. S., Kennel, S. J. & Huang, L. (1982) *Biochim. Biophys. Acta* **716**, 140–150.
23. Bartlet, G. R. (1959) *J. Biol. Chem.* **234**, 466–468.
24. Pool, G. L., French, M. E., Edwards, R. A., Huang, L. & Lumb, R. H. (1982) *Lipids* **17**, 448–452.
25. Magin, R. L. & Morse, P. D. (1983) *Biochim. Biophys. Acta* **760**, 357–362.
26. Heichal, O., Ish-Shalom, D., Koren, R. & Stein, W. (1979) *Biochim. Biophys. Acta* **551**, 169–187.
27. Schwenk, M., Hegazy, E. & Lopez del Pino, V. (1984) *Biochim. Biophys. Acta* **805**, 370–374.
28. Plagemann, P. G. W., Marz, R. & Wohlhueter, R. M. (1978) *Cancer Res.* **38**, 978–989.
29. Brajeswar, P., Chen, M. F. & Paterson, A. R. P. (1975) *J. Med. Chem.* **18**, 968–973.
30. Berg, H. C. (1983) *Random Walks in Biology* (Princeton Univ. Press, Princeton, NJ).
31. Tumer, A., Kirby, C., Senior, J. & Gregoriadis, G. (1983) *Biochim. Biophys. Acta* **760**, 119–125.
32. Mezei, M. & Gulasedharam, V. (1980) *Life Sci.* **26**, 1473–1477.