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Targeting of anti-Thy 1.1 monoclonal antibody conjugated liposomes in Thy 1.1 mice after intravenous administration

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^{125}I-labeled liposomes, conjugated to an anti-Thy 1.1 monoclonal antibody (MRCOX7), demonstrated up to 7.4-fold greater lymph node uptake than liposomes conjugated to non-specific monoclonal antibody (R-10) after intravenous injection into Thy 1.1 (AKR-J) mice. Uptake of anti-Thy 1.1-conjugated liposomes by the lymph nodes of AKR-J mice was 3-times greater than their uptake by lymph nodes of Thy 1.2 (AKR-Cu) mice. Lymph node localization of anti-Thy 1.1-liposomes was equal to that of control monoclonal antibody-liposomes in Thy 1.2 mice. Conjugation to either monoclonal antibody substantially increased liposome clearance by the liver, while decreasing liposome uptake in a number of organs outside the reticuloendothelial system. Changes in liposome size and phospholipid composition did not significantly alter these results. Administration of a large predose of unconjugated liposomes prior to injection of MRCOX7-conjugated liposomes increased blood levels and reduced liver uptake of the monoclonal antibody-liposome conjugates, but did not further enhance lymph node uptake. This study demonstrates that targeting of liposomes by conjugation to the appropriate monoclonal antibody, can significantly increase their uptake in lymph nodes which contain high levels of cells expressing the target antigen. However, conjugation to monoclonal antibody also increases clearance of liposomes by the liver. To increase the uptake of monoclonal antibody-conjugated liposomes in target tissue, substantial reduction of their clearance by the reticuloendothelial system will be required.

Introduction

The therapeutic index of most cytotoxic and antimicrobial agents is limited by their lack of target cell specificity. Selective drug delivery to involved tissues by a targeted drug carrier system may both increase drug efficacy and reduce associated toxicity. Ligand-directed therapy via monoclonal antibodies, has increased uptake of chemically attached drugs [1], and radioisotopes [2] within selected target tissues after intravenous (i.v.) injection. Recently, methods for covalently binding monoclonal antibody to the surface of liposomes have been described [3–7]. These monoclonal antibody-liposome complexes have been shown to retain their specificity for cells expressing target antigen in vitro [8–12], and to bind circulating target cells after i.v. injection into mice [13].

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Abbreviations: LUV, large unilamellar vesicles; SUV, small unilamellar vesicles; PC, phosphatidylcholine; MPB-PE, 4-(*p*-maleimidophenyl)butyryl phosphatidylethanolamine.

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Liposomes conjugated to monoclonal antibody offer several advantages over direct monoclonal antibody-drug conjugates. A wide range of drugs and macromolecules can be encapsulated within either the liposome bilayer membrane or the internal aqueous space. Encapsulation does not require linking of the drug to the targeting ligand via chemical bonds, which must be broken down within the target cell for drug activity. A liposome can deliver thousands of drug molecules into an individual target cell [14]. Drug delivery by liposomes can reduce acute systemic side effects and a range of organ toxicities, while preserving or enhancing efficacy of encapsulated antitumor [15] and antimicrobial [16] agents. Further, thousands of monoclonal antibodies of a single or multiple different specificities may be bound by the surface of a single liposome. Such monoclonal antibody-liposome conjugates bind to cells expressing the target antigen by multivalent interactions, and can bind to target cells in the presence of excess soluble antibody [12].

The ability of circulating monoclonal antibody-directed liposomes to reach target cells will depend on both the accessibility of the target to liposomes within the vascular compartment, and the extent of clearance mediated by the reticuloendothelial system (RES). Unconjugated liposomes injected i.v. are taken up largely by the liver and spleen, and demonstrate limited ability to penetrate non-RES vascular endothelial barriers [17]. Thus, antigen bearing cells either within the circulation or located in organs where liposomes readily extravasate represent the most appropriate targets for monoclonal antibody-directed liposomes. We have examined the tissue localization of ^{125}I-labeled liposomes conjugated to an anti-Thy 1.1 monoclonal antibody (MRCOX7), or to a control monoclonal antibody of the same subclass, (R-10), following either i.v. or subcutaneous injection in Thy 1.1 (AKR-J), and Thy 1.2 (AKR-Cu) mice. Thy 1.1 is a murine lymphoid differentiation antigen primarily expressed on T lymphocytes present in blood, thymus, lymph nodes and spleen of Thy 1.1 mice. AKR-Cu (Thy 1.2) mice differ from AKR-J at the Thy 1 locus, and therefore demonstrate the effect of conjugated anti-Thy 1.1 monoclonal antibody on liposome organ uptake in animals which lack the Thy 1.1 antigen [18].

Materials and Methods

Lipids were obtained or purified as previously described [8]. Monoclonal antibodies MRCOX7 (anti-Thy 1.1) and LICR Lon R10 (anti-glycophorin A) were obtained, grown up and purified as previously described [12]. Both antibodies are of IgG1 subclass.

Liposome preparation. Large unilamellar vesicles (LUV) were prepared by the reverse phase evaporation method of Szoka and Paphadjopoulos [19] in 50 mM 2(-*N*-morpholino)ethanesulfonic acid (Mes)/50 mM 3-(*N*-morpholino)propanesulfonic acid (Mops)/1 mM EDTA/60 mM NaCl (ph 6.7). ^{125}I-labeled *p*-hydroxybenzimidine phosphatidylethanolamine (^{125}I-BPE, spec. act. $1.94 \cdot 10^7$ cpm/μmol), was prepared by the method of Szoka et al. and has been shown by them not to exchange with plasma lipoproteins for at least 6 hours after injection [20]. LUV were prepared from 10 μmoles phosphatidylcholine (PC), with cholesterol and 4-(*p*-maleimidophenyl)butyryl phosphatidylethanolamine (MPB-PE) in a 10 : 10 : 1 molar ratio. OX7-LUV had a specific activity of $1.88 \cdot 10^6$ cpm/μmol phospholipid, and R-10-LUV, $2.05 \cdot 10^6$ cpm/μmol. Small unilamellar vesicles (SUV) were composed of 10 μmoles sphingomyelin with cholesterol and MPB-PE in a 10 : 10 : 1 molar ratio. They were prepared by sonication for 1 h in a bath type sonicator and larger liposomes were removed by centrifugation at 100 000 × *g* for 60 min [9]. OX7-SUV had a specific activity of $4.5 \cdot 10^5$ cpm per μmole phospholipid.

Antibody conjugation. Liposome-monoclonal antibody conjugates were prepared as in Heath et al. [8]. Conjugated LUV were separated from unbound antibody by flotation on metrizamide gradients [8], and analyzed for lipid [21] and protein [22]. Conjugated SUV were separated from unbound antibody by passage over a Sepharose 6B column eluted with Mes/Mops NaCl buffer (pH 6.7) and analyzed for protein and lipid as above.

Cell lines and in vitro studies. The R1.1 cell line, a T-lymphoma from the C58 mouse and the AKR/J SL2 cell line, a T-lymphoma from the AKR mouse, were obtained and grown as previously described [9]. In vitro monoclonal antibody liposome-cell binding studies were also carried out as previously described [9].

Injection of monoclonal antibody-liposomes into mice. Monoclonal antibody-conjugated liposomes (0.4 μmol phospholipid in 0.2 ml buffer) were injected intravenously via the tail vein into 6–8 week old AKR-J (Jackson Labs, Bar Harbor, Maine) or AKR-Cu mice, obtained from Cumberland Farms (Cumberland, TN). To produce a reticuloendothelial system blockade, 0.2 ml of 10 μmol/ml unconjugated liposomes was injected intravenously 1 h prior to the injection of ^{125}I-labeled, MRCOX7-conjugated liposomes. Animals were killed by cervical dislocation 1 or 24 h post injection. Blood, thymus, axillary, inguinal and mesenteric lymph nodes, liver, spleen, gut and kidneys were dissected out, weighed and counted for gamma emission. All counts were then background corrected. Liposome levels in organs are expressed in nanomoles phospholipid per gram of wet tissue. All organ levels are blood corrected according to the method of Mauk et al. [23].

Results

The association of MRCOX7-conjugated liposomes (LUV) with target AKR/J SL2 murine T lymphoma cells in vitro was 5.6 times greater than association with control R1.1 cells, thus confirming the specificity of MRCOX7-liposomes for cells expressing the Thy 1.1 antigen (Table I). A similar increase in the association of liposomes bearing anti-Thy 1.1 monoclonal antibody with Thy 1.1 compared to control cells has been reported previously [13].

Organ uptake of liposomes conjugated to anti-Thy 1.1-MoAb did not differ significantly from that of liposomes conjugated to control-monoclonal antibody, 1 h after i.v. injection into Thy 1.2 mice. However, compared to unconjugated liposomes of identical lipid composition, both monoclonal antibody-liposome conjugates showed significantly greater uptake by the liver (up to 3-fold), and reduced levels in most other organs. Thus, monoclonal antibody conjugation markedly accelerated clearance of circulating liposomes by the liver and significantly reduced their uptake by non-RES organs 1 h after i.v. injection. Although the amount of R-10 conjugated to LUV liposomes was approx. 3-times higher than that of MRCOX7, no significant differences in organ uptake of liposomes conjugated to either monoclonal antibody were noted (Table II).

Lymph node uptake of liposomes (LUV) conjugated to anti-Thy 1.1-MoAb in Thy 1.1 mice was 7.4-fold greater than that of liposomes conjugated to control-monoclonal antibody at 1 h

TABLE I

ASSOCIATION OF MRCOX7-LIPOSOMES WITH AKR/J SL2 AND R 1.1 CELLS IN VITRO

LUV were prepared and conjugated to monoclonal antibody as described in Materials and Methods. The protein to phospholipid ratio expressed as g/mole was 194. Assuming a 0.6-μm liposome diameter and unilamellar vesicles, this value calculates as approx. 4582 MRCOX7 antibody molecules per liposome. Cell association studies were performed as described in Materials and Methods.

Cell Line	Phospholipid bound (nmol/10^7 cells)	
AKR/J SL2 (Thy 1.1)	6.2 ± 0.13	$P < 0.001$ [a]
R1.1 (control)	1.1 ± 0.04	

[a] MRCOX7 liposome association with target versus control cells was compared by Student's *t*-test.

TABLE II

ORGAN LOCALIZATION OF UNCONJUGATED OR MONOCLONAL ANTIBODY-CONJUGATED LIPOSOMES 1 HOUR AFTER INTRAVENOUS INJECTION INTO AKR-Cu (CONTROL) MICE

The units represent nanomoles phospholipid per gram of wet tissue and are determined by dividing cpm/g tissue by cpm/μmol phospholipid injected. N = four animals per data point. The LUV preparation and monoclonal antibody conjugation techniques are described in Materials and Metods. The protein (antibody) to lipid ratio, expressed as g protein per mole phospholipid were 194 and 581 for MRCOX7 and R-10 LUV, respectively. Assuming a 0.6 μm liposome diameter and unilamellar vesicles, these values calculate as approximately 4582 MRCOX7 molecules and 13722 R-10 molecules per liposome.

Organ	Amount deposited (nmol/g) of liposomes injected		
	MRCOX7-LUV	Unconjugated	R-10-LUV
Lymph nodes	2.4 ± 0.5	3.8 ± 0.7	1.5 ± 0.5
Spleen	50.0 ± 19.0	31.1 ± 7.3	48.8 ± 3.5
Thymus	1.8 ± 0.3	4.0 ± 0.9	1.6 ± 0.5
Liver	192.9 ± 24.9	61.0 ± 8.6	139.3 ± 27.5
Gut	3.2 ± 0.4	23.8 ± 5.7	3.5 ± 1.3
Kidneys	5.5 ± 1.3	14.8 ± 3.2	5.6 ± 2.0
Blood	2.8 ± 0.3	14.4 ± 3.1	3.1 ± 0.9

TABLE III

ORGAN LOCALIZATION OF LIPOSOMES CONJUGATED TO MRCOX7 (ANTI-Thy 1.1) AND TO R-10 (CONTROL) MONOCLONAL ANTIBODY 1 AND 24 HOURS POST INTRAVENOUS ADMINISTRATION IN AKR-J (Thy 1.1) MICE

The units represent nanomoles phospholipid per gram of wet tissue determined by dividing cpm/g tissue by cpm/μmol phospholipid injected. N = four animals per point at 1 h, two animals per point at 24 h. The LUV preparation and monoclonal antibody conjugation techniques are described in Materials and Methods. The protein (antibody) to lipid ratio, expressed as g protein per mole phospholipid were 194 and 581 for MRCOX7 and R-10 LUV, respectively. Assuming a 0.6 μm liposome diameter and unilamellar vesicles, these values calculate as approx. 4582 MRCOX7 molecules and 13722 R-10 molecules per liposome.

Organ	Amount deposited (nmol/g)			
	OX7-LUV (1 h)	R-10-LUV (1 h)	OX7-LUV (24 h)	R-10-LUV (24 h)
Lymph nodes	7.4± 2.1 $P < 0.005$ [a]	1.0±0.5	4.3±0.4 $P < 0.05$ [a]	1.3±0.5
Spleen	27.8± 7.0	42.4±5.9	38.8±5.0	53.3±4.2
Thymus	2.3± 0.4	1.3±0.3	7.0±0.0	7.0±0
Liver	137.4±52.1	111.8±7.1	78.9±1.2	68.6±4.5
Gut	2.9± 0.6	2.1±0.4	2.8±1.2	2.2±1.1
Kidneys	5.0± 1.5	4.2±1.5	1.7±0.3	1.1±0.4
Blood	2.2± 0.7	3.2±0.7	0.7±0.1	0.6±0.2

[a] Organ levels of targeted monoclonal antibody-liposomes were compared to those of control monoclonal antibody-liposomes by Student's t-test.

post intravenous injection, and remained 3.3-fold higher at 24 h (Table III). (At the 24 h time point, possible exchange of liposome associated ^{125}I-BPE with plasma lipoproteins cannot be excluded). At 1 h, lymph node uptake of MRCOX7-liposomes was 1.9-fold greater than that of unconjugated liposomes ($P < 0.025$ by Student's t-test) and uptake of MRCOX7-liposomes was 3-fold greater in lymph nodes of Thy 1.1 mice than in Thy 1.2 mice ($P < 0.005$). Other than the lymph nodes, no significant differences in organ uptake of MRCOX7 liposomes compared to R-10-liposomes were noted in either AKR-J or AKR-Cu mice. Thus, although enhancemnt of targeted liposome uptake in Thy 1.1 lymph nodes was achieved, no increased uptake in thymus and spleen, which also contain high levels of Thy 1.1 positive cells, was observed.

TABLE IV

ORGAN LOCALIZATION OF MRCOX7 TARGETED LIPOSOMES 1 HOUR AFTER INTRAVENOUS INJECTION

The LUV preparation and monoclonal antibody conjugation techniques are described in Materials and Methods. The protein (antibody) to lipid ratio, expressed as g protein per mole phospholipid was 160. Assuming a 0.6 μm liposome diameter and unilamellar vesicles, this value calculates as approx. 3779 MRCOX7 antibody molecules per liposome. The units represent nanomoles phospholipid per gram of wet tissue, determined by dividing cpm/g tissue by cpm/μmol phospholipid injected. N = two animals per point. Animals received a 2 μmol unconjugated LUV dose 1 h prior to injection of MRCOX7-LUV liposomes.

Organ	Amount deposited (nmol/g) in AKR-J mice which received a 'blocking' pre-dose
Lymph nodes	7.8± 1.0
Spleen	34.8± 1.6
Thymus	2.9± 0.2
Liver	84.4±21.6
Gut	2.4± 0.2
Kidneys	6.2± 1.3
Blood	3.5± 0.3

A predose of unconjugated, unlabeled LUV altered the organ distribution of anti-Thy 1.1 conjugated LUV, which were administered 1 h later in Thy 1.1 mice (Table IV). A predose of 2 μmole of unconjugated LUV liposomes reduced liver uptake and increased blood levels of MRCOX7-liposomes approx. 2-fold above the level achieved by the same liposomes, administered without a predose (Table III). Earlier studies have demonstrated that pre-dosing reduces the reticuloendothelial system clearance and increases the circulation time of unconjugated liposomes [24–26]. The present studies indicate that this also is true for monoclonal antibody-conjugated liposomes. However, our results also show that predosing did not enhance the uptake of liposomes conjugated to MRCOX7 by lymph nodes, spleen, or thymus.

TABLE V

ORGAN LOCALIZATION OF UNCONJUGATED OR MRCOX7 CONJUGATED SUV 1 HOUR AFTER INTRAVENOUS INJECTION INTO AKR-Cu AND AKR-J MICE

The units represent nanomoles phospholipid per gram of wet tissue, determined by dividing cpm/g tissue by cpm/μmol phospholipid injected. N = three animals per point. SUV were prepared and conjugated to antibodies as described in Materials and Methods. Protein (antibody) to lipid ratio, expressed as g protein to mole phospholipid was 29. Assuming a 0.075 μm liposome diameter and unilamellar vesicles, this value calculations as approx. 8 MRCOX7 antibody molecules per liposome.

Organ	Amount deposited (nmol/g)		
Liposomes	MRCOX7-SUV	MRCOX7-SUV	Unconjugated SUV
Mouse	AKR-CU	AKR-J	AKR-J mice
Lymph nodes	4.2± 6 $P < 0.025$ [a]	7.8± 2.0 $P < 0.025$ [a]	3.5± 1.0
Spleen	48.3±11.7	39.3± 8.7	86.1±17.2
Thymus	2.2± 1.0	2.4± 0.9	9.9± 1.4
Liver	193.4±70.7	190.1±37.2	38.4± 5.4
Gut	4.7± 1.2	5.3± 1.2	14.6± 2.1
Kidneys	7.0± 1.2	7.4± 2.1	9.9± 2.3
Blood	5.3± 0.1	6.2± 0.6	15.4± 3.5

[a] Organ levels of targeted monoclonal antibody-SUV were compared to both unconjugated, and control monoclonal antibody-conjugated SUV by Student's t-test.

Earlier studies have shown that unconjugated SUV injected intravenously demonstrate increased circulation time and reduced hepatic clearance when compared to larger vesicles [27]. In addition, liposomes containing a high molar percentage of sphingomyelin remain in the circulation significantly longer than liposomes composed of phosphatidylcholine [28,29]. We therefore examined the behavior of conjugated SUV, mean diameter 0.075 μm [9], containing sphingomyelin in an attempt to retard the liver clearance and improve the monoclonal antibody-directed targeting obtained with conjugated LUV, 0.6 μm mean diameter [19].

Conjugation of monoclonal antibody onto SUV significantly increased their hepatic uptake, and reduced non-RES uptake after i.v. administration (Table V), similar to the monoclonal antibody-LUV discussed above. Uptake of MRCOX7-SUV by lymph nodes of target AKR-J mice was 1.9 times greater ($P < 0.025$) than observed in AKR-Cu mice. No significant differences in MRCOX7-SUV uptake in other organs were observed for AKR-J compared to AKR-Cu mice. Blood levels of MRCOX7-SUV at 1 h, were 1.9- and 2.8-times greater than that of MRCOX7-LUV in AKR-Cu and AKR-J mice, respectively. Otherwise, organ levels of MRCOX7-LUV and MRCOX7-SUV did not differ significantly in either target or control mice at 1 h. These results indicate that targeted-SUV were no more successful than targeted-LUV in reaching target organs, or avoiding uptake via the reticuloendothelial system in Thy 1.1 mice.

Discussion

We have shown that the presence of an anti-Thy 1.1 monoclonal antibody (MRCOX7) conjugated to the surface of liposomes (LUV and SUV) significantly increased their uptake by the lymph nodes of Thy 1.1 mice after i.v. injection. Increased uptake was observed when the level of MRCOX7-liposomes in AKR-J lymph nodes was compared to that achieved by unconjugated liposomes or by liposomes conjugated to control-monoclonal antibody (R-10) in AKR-J mice; as well as to unconjugated liposomes, and liposomes conjugated to either MRCOX7 or R-10, in the lymph nodes of AKR-Cu (control) mice. No further enhancement in lymph node localization of MRCOX7-liposomes was noted after the administration of a large pre-dose of unconjugated liposomes, which both reduced liver uptake and in-

creased blood levels of the subsequently injected targeted liposomes.

Circulating MRCOX7-liposomes may associate with target cells in lymph nodes either via direct binding to Thy 1.1 cells resident within the lymph nodes, or by binding to circulating Thy 1.1 cells, which subsequently home to lymph nodes [30]. The latter possibility is supported both by the demonstration that anti-Thy 1.1 monoclonal antibody liposomes selectively bind to Thy 1.1 cells within the circulation [13], and that enhanced uptake of MRCOX7-liposomes occurs selectively in lymph nodes, where lymphocytes have been shown to preferentially recirculate via interaction with specific surface receptors on the endothelium of post-capillary venules. Lymphocytes bound to the luminal surface of these high endothelial venules (HEV) subsequently extravasate into the surrounding lymphoid tissue [31]. Within 2 h after their i.v. injection, Thy 1.1 lymphocytes localize in the peripheral lymph nodes of AKR-J mice. These lymphocytes are taken up by lymph nodes in significantly greater amounts than by the spleen or Peyer's patches [32]. Thus, the selective enhancement of MRCOX7-liposome uptake in the lymph nodes observed after i.v. injection into AKR-J mice may be explained by binding to, and subsequent delivery via circulating Thy 1.1 lymphocytes which extravasate through HEV.

Although enhanced uptake of i.v. injected MRCOX7-liposomes was demonstrated in the lymph nodes of AKR-J mice, increased uptake in the thymus and spleen, organs which also contain high levels of Thy 1.1 cells was not observed. Recent in vivo studies indicate that i.v. doses of less than 10 mg of unconjugated anti-Thy 1.1 monoclonal antibody do not demonstrate selective uptake in the thymus of Thy 1.1 mice [18]. The total amount of liposome-bound MRCOX7 injected per animal was substantially less than 10 mg; therefore failure to achieve thymic targeting was not unexpected. However, enhanced splenic uptake of unconjugated anti-Thy 1.1 monoclonal antibody has been observed at low levels of monoclonal antibody injection [18]. The presence of an attached liposome may limit MRCOX7 access to Thy 1.1 cells within the spleen. However, the high background level of liposome uptake, mediated by splenic macrophages could mask enhanced uptake of MRCOX7-liposomes by Thy 1.1 cells within the spleen.

Liposomes conjugated to monoclonal antibody demonstrated significantly higher liver uptake and reduced levels in blood and non-RES organs, when compared to unconjugated liposomes of idential lipid composition. The mechanism by which the conjugated antibody enhances hepatic clearance is unknown. The presence of IgG_1 molecules on the liposome surface may increase hepatic clearance by several different pathways, including Fc or C3b receptor mediated interactions or via plasma opsonins.

The role of Fc receptor mediated uptake is uncertain. The interaction of the Fc region of IgG_1 antibodies with Fc receptors ordinarily requires that antibody be bound to its target antigen [33]. Therefore, in the absence of soluble circulating target antigen, monoclonal antibody-conjugated liposomes may not induce Fc receptor binding. Even albumin-liposome conjugates are cleared much more rapidly than unconjugated liposomes (Abai, A., Heath, T. and Papahadjopoulos, D., unpublished data, suggesting that the role of Fc mediated uptake may be limited.) The ability of such antibody-liposome conjugates to activate the complement system in vivo also is unclear. Several non-immune macrophage plasma membrane receptors, including those for mannose, lactoferrin, fibronectin and α-macroglobulin have been shown to promote uptake via the reticuloendothelial system of circulating particles [34]. The latter two substances adhere to the surface of unconjugated liposomes incubated in plasma [35,36], and may contribute to hepatic clearance of monoclonal antibody-liposome conjugates.

Significant reticuloendothelial system-mediated clearance of direct monoclonal antibody-radioisotope [2,37] and toxin conjugates [38] has also been reported. Although the increased uptake of liposomes by lymph nodes of Thy 1.1 mice is relatively modest, a similar increase in uptake of ^{125}I-labeled anti-Thy 1.1 monoclonal antibody by subcutaneous tumor implants expressing Thy 1.1 substantially improved isotope-mediated antitumor effects [37]. In addition, actinomycin C-loaded, monoclonal antibody-targeted liposomes injected i.v. have significantly improved antitumor

response when compared to either non-targeted liposomes containing actinomycin D, or to the free drug alone [39].

In summary, we have demonstrated enhanced delivery of liposomes to lymph nodes in Thy 1.1 mice by the attachment of anti-Thy 1.1 monoclonal antibody to the liposome surface. These results suggest that the increased cytotoxicity of drug-loaded anti-Thy 1.1-liposomes against tumor cells bearing the Thy 1.1 antigen in vitro [9], may be applicable to in vivo tumor models. However, like immunotoxins and antibody isotope conjugates [37,38], the great majority of monoclonal antibody targeted-liposomes are cleared from the circulation by the reticuloendothelial system. We are currently investigating several strategies to enhance delivery of monoclonal antibody-directed agents to target cells outside the reticuloendothelial system. Reduction of reticuloendothelial system-mediated liposome clearance may be facilitated by one or more of the following maneuvers: the use of immunoglobulin fragments and non antibody molecules as targeting ligands [5], temporary suppression of reticuloendothelial system function by a variety of maneuvers [23,40], depletion of potentially opsonizing serum factors [41], masking of exposed immunogenic surface molecules, or the inclusion of substances which may reduce reticuloendothelial system recognition of circulating foreign particles. Alternatively, monoclonal antibody-directed liposome therapy of diseases involving the liver may prove useful, either via the non-specific enhancement of hepatic uptake produced by surface bound antibody, or by targeting to specific antigen-bearing cells within the reticuloendothelial system.

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