Comparison of Anti-Tac and Anti-Transferrin Receptor-Conjugated Liposomes for Specific Drug Delivery to Adult T-Cell Leukemia

By Kristen M. Hege, David L. Daleke, Thomas A. Waldmann, and Katherine K. Matthay

Adult T-cell leukemia (ATL) is a rapidly progressive and usually fatal malignancy of mature T cells characterized by the expression of large numbers of interleukin-2 (IL-2) receptors on the cell surface. Anti-Tac, a monoclonal antibody directed against the IL-2 receptor, was conjugated to liposomes and compared with anti-transferrin receptor (anti-TFR) conjugates for specific binding, internalization, and intracellular drug delivery to ATL cells. Two independent assays were used: a fluorimetric assay with liposome encapsulated 1-hydroxypyrene-3,6,8-trisulfonic acid, a pH-sensitive fluorescent dye, and a growth inhibition assay using methotrexate-γ-arrestape, a liposome-dependent cytotoxic drug. MT-1 and HUT-102 cell lines derived from patients with ATL were compared with Molt-4, a leukemia cell line that does not express IL-2 receptors in an induced state. Fluorimetric studies showed specific binding and internalization of anti-Tac-conjugated liposomes by HUT-102 and MT-1 but not by the Tac-negative cell line Molt-4, demonstrating the lack of nonspecific or Fc receptor-mediated uptake. Anti-TFR-conjugated liposomes were effectively bound and internalized by all three cell lines and consistently showed the highest degree of cellular liposome uptake. Drug-containing liposomes conjugated to anti-Tac were more than tenfold more effective in causing growth inhibition of ATL cells than the nonspecific control conjugates. Anti-Tac conjugates caused minimal growth inhibition of Molt-4 cells over the concentration range effective against the ATL cells. Anti-TFR-coupled liposomes gave better growth inhibition of HUT-102 and MT-1 cells (40- to 60-fold) than anti-Tac conjugates. Both anti-Tac-directed and anti-TFR-directed liposomes are effective for intracellular drug delivery to ATL cells and may represent a useful method of treatment in this disease.

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appear to be selectively concentrated in coated pits before uptake.11

Two independent assays of liposome endocytosis were used in this investigation: a fluorescence assay using the pH-sensitive dye pyranine, or 1-hydroxypyrene-3,6,8-trisulfonic acid (HPTS),24 which serves as a marker of internalization of liposome encapsulated dye into intracellular low pH compartments,25 and a growth inhibition assay using liposome encapsulated methotrexate-γ-aspartate, a relatively impermeant drug which requires liposome-mediated endocytosis for cellular uptake.26

MATERIALS AND METHODS

Phosphatidycholine and transphosphatidylated phosphatidylethanolamine from egg yolk were obtained from Avanti (Birming-
ham, AL) and used without further purification. N-Succinimidyl-3-(2-pyridyldithio)-propionate and succinimidyl-4-(p-maleimido-phenyl)-butyrate were obtained from Pierce. Cholesterol was obtained from Sigma (St Louis, MO) and recrystallized four times from cold methanol. The 4-(p-maleimido-phenyl)-butyrophosphatidylethanolamine was synthesized from SMPB and PE and purified as described.27 All lipids were stored at -70°C in chloroform solution under argon in sealed ampules until use. Methotrexate-γ-aspartate was synthesized and provided by Dr J.R. Piper, Southern Research Institute, Birmingham, AL. Pyrane (HPTS) was purchased from Molecular Probes (Eugene, OR). All chemicals were reagent grade.

Antibodies. Monoclonal antibodies were prepared and purified as previously described.28 Hybridoma SS.1 (anti-sheep erythrocyte, irrelevant control) was supplied by Dr M. Cohn of the Salk Institute, San Diego, CA. The anti-Tac monoclonal antibody was provided by Dr T. Waldmann. Molt-4 is a human T-cell leukemia line which does not express IL-2 receptors in a noninduced state29 and was provided by Dr M. Cowan at UCSF, San Francisco, CA. All cell lines were grown in RPMI medium supplemented with 2 mmol/L glutamine, 10% fetal bovine serum, and antibiotics with 5% carbon dioxide at 37°C.

Liposome preparation. A mixture of phosphatidycholine, cholesterol, and MPB-PE (10:5:1 molar ratio) was suspended in a solution of 50 mmol/L methotrexate-γ-aspartate or 50 mmol/L HPTS in isotonic buffer containing 50 mmol/L 2-(N-morpholinoo)ethanesulfonic acid, 50 mmol/L 2-(N-morpholino)propanesulfonic acid (MES/MOPS), and NaCl to 290 mOsm, pH 7.5. Liposomes were prepared by reverse-phase evaporation and were extruded six times through two 0.08-μm pore polycarbonate membranes under sterile conditions with a high-pressure extruder (Lipex Biomembranes, Vancouver, Canada). The reverse-phase evaporation vesicles were then conjugated for 18 hours at 25°C with monoclonal antibody (MoAb) previously thiolated with SPDP.27 The conjugated liposomes were separated from unbound protein by flotation on a metrizamide gradient with modifications previously described.38 Liposomes were then separated from unencapsulated drug and metrizamide with a Sephadex G50 (Pharmacia) column equilibrated with isotonic 50 mmol/L MES/MOPS/NaCl, pH 7.5, and concentrated to 5 mmol/L lipid in an Amicon diaflow filtration device. After sterilization by passage through a 0.2-μm filter, the liposomes were analyzed for lipid,39 protein,40 and drug41 content. Liposome size was estimated by triplicate measurements of laser light scattering on a Coulter model N4 submicron particle analyzer.42 Liposome characteristics for HPTS and methotrexate-γ-aspartate-containing liposomes are shown in Table 1. Mean drug/lipid was 0.038 mol/mol, and mean protein/lipid was 20 g/mol. Mean size before conjugation was 101 nm, which increased to a mean of 159 nm after conjugation to antibody.

Indirect immunofluorescence. For each cell line, 1 × 10^6 cells per sample were washed in growth medium and resuspended in 100 μL growth medium. Antibody (5 μL) (anti-Tac, anti-TFR, anti-sheep erythrocyte (SS.1), and anti-neuroblastoma antibodies (anti-

### Table 1. Liposome Characteristics

<table>
<thead>
<tr>
<th>Drug or HPTS/Lipid (mol/mol)</th>
<th>Protein/Lipid (g/mol)</th>
<th>Liposome Diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Tac</td>
<td>0.040</td>
<td>15</td>
</tr>
<tr>
<td>Anti-TFR</td>
<td>0.041</td>
<td>9</td>
</tr>
<tr>
<td>Control Ab: SS.1*</td>
<td>0.033</td>
<td>25</td>
</tr>
<tr>
<td>HPTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Tac</td>
<td>0.171†</td>
<td>15</td>
</tr>
<tr>
<td>Anti-TFR</td>
<td>0.171</td>
<td>8</td>
</tr>
<tr>
<td>Anti-TFR</td>
<td>0.171</td>
<td>21</td>
</tr>
<tr>
<td>Control Ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsan†</td>
<td>0.171</td>
<td>14</td>
</tr>
<tr>
<td>14.2a</td>
<td>0.171</td>
<td>54</td>
</tr>
<tr>
<td>390</td>
<td>0.171</td>
<td>16</td>
</tr>
</tbody>
</table>

*SS.1, anti-sheep erythrocyte antibody.
†hsan, 14.2a, and 390, monoclonal anti-NB antibodies.
‡Calculated from an average lipid diameter of 100 nm and internal HPTS concentration of 50 mmol/L.
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of the endosomal or lysosomal compartments. Leukemia cells can be viewed by excitation with wide-band violet illumination (350 nm) was added to each sample and incubated for an additional 30 minutes at 0°C. Cells were then washed twice and examined by fluorescence microscopy as described below.

Liposome-cell association assay. The pH-dependent fluorescence dye, HPTS, was encapsulated in the liposomes to monitor their progress from the neutral extracellular compartment to the low pH of the endosomal or lysosomal compartments. Leukemia cells (2 x 10⁶ cells per time point) were washed and incubated in 0.5 mL growth media with the appropriate antibody-conjugated liposomes (10 nmol/10⁶ cells) for 45 minutes at 0°C. Unbound liposomes were removed by centrifugation of cells through a 10% dextran gradient, and the cells were then reincubated at 37°C in growth media. At designated time points, cells were washed with chilled Dulbecco's phosphate-buffered saline and then examined by fluorescence microscopy or fluorometry. In experiments designed to simulate conditions of the growth inhibition studies described below, 1 x 10⁶ cells in growth medium were plated per well in 24-well plates, 1 mL per well. Antibody-conjugated liposomes containing HPTS were added (25 nmol/L) and incubated for 72 hours at 37°C. Replicate samples were pooled, 2 x 10⁶ cells per sample were centrifuged through dextran, and cell associated fluorescence was measured as described below.

Fluorescence microscopy and fluorometry. Fluorescence microscopy was used for visual evaluation of cell association and endocytosis. The relatively pH-independent fluorescence of HPTS can be viewed by excitation with wide-band violet illumination (350 to 410 nm) provided by the Hoechst filter cube, reflected through long-wave-pass dichroic mirror (455 nm) and long-wave-pass barrier filter (445 nm). The second filter cube, FITC, selects narrow blue band (450 to 490 nm) for excitation with long-pass dichroic mirror (455 nm) and long-wave-pass barrier filter (515 nm) so that only the fluorescence of HPTS at neutral or basic pH can be seen. The appearance of bright cellular fluorescence seen with the Hoechst cube but not with the FITC cube reflects HPTS in the intracellular low pH compartment. Photographs of identical fields were taken with phase-contrast, Hoechst, and FITC filters at each time point.

Quantitative measurements of fluorescence emission at 510 nm were made on a SPEX Fluorolog 2 equipped with a photon counting device. Sample temperature was controlled with a circulating water bath at 20°C. Leukemia cells (2 x 10⁶) were suspended in phosphate-buffered saline (2 mL) and gently mixed with a magnetic stirbar in the cuvette. The samples were excited at 403 nm (HPTS at pH 7.4, 413 nm (total HPTS, invariant with pH), and 450 nm (HPTS at neutral or basic pH), and the emission spectra were recorded. After correction for cell autofluorescence, the fraction of HPTS-containing liposomes within the endosomal (low pH) compartment was calculated from the formula: Fraction at low pH = [R_{low pH}/R_{neutral pH} - R_{neutral pH}], where R is the ratio of fluorescence emission at 510 nm after excitation at 450 nm normalized to excitation at 413 nm. The lowest value for 450/413 (R_{neutral pH}) was achieved after incubation of each cell line with anti-TFR–conjugated liposomes (25 mol/L) for 72 hours and was used as a reference for other conjugated liposomes. The value for R_{low pH} was obtained by incubation of cells with free 1 mmol/L HPTS for 24 hours, washing and then measuring the 450/413 emission ratio after cell lysis with 0.1% Triton X-100. R_{low pH} varied with each line as follows: MT-1, 0.13; HUT-102, 0.63; Molt-4, 0.5. According to the known pH dependence of HPTS emission ratios, this corresponds to an average pH of the endosomal compartment for MT-1 of 5.7, HUT-102, 6.6, and Molt-4, 6.5.

Growth inhibition. Leukemia cells were plated in growth medium at 10⁶ per well in 24-well plates. Cells were treated with antibody-conjugated liposomes or free drug (0.003 to 30 μmol/L), incubated 72 hours in 3% carbon dioxide, and counted with a Model Fc Coulter. The percentage of growth was calculated as: ([final cell number - initial cells]/[control cell number - initial cells] x 100), and IC₅₀ was determined from a plot of percentage of growth versus log₁₀ drug concentration.

RESULTS

Reactivity of leukemia cell lines with monoclonal antibodies by indirect immunofluorescence. Indirect immunofluorescent labeling was performed on each T-cell line to determine the presence or absence of cell surface receptors for the various MoAbs to be used in subsequent studies. Cells were incubated with the described primary MoAbs (anti-Tac, anti-TFR, anti-NB, and SS.1) followed by incubation with FITC-labeled anti-mouse IgG. HUT-102 and MT-1 cells showed fluorescent labeling with anti-Tac and anti-TFR antibodies, whereas Molt-4 cells showed staining with anti-TFR only (Table 2). Staining with anti-Tac was most intense on the MT-1 cells. SS.1 and anti-NB showed no fluorescent labeling with any cell line and were used in subsequent experiments as irrelevant controls.

Cell association and internalization of antibody-targeted liposomes by fluorescence microscopy. To follow the uptake of liposomes initially bound, cells were incubated with antibody-conjugated liposomes at 0°C and unbound liposomes were then removed by centrifugation through a Dextran gradient. Cells were viewed under the fluorescence microscope after designated periods of incubation at 37°C. Endocytosis was indicated by the appearance of bright intracellular vesicles which were observed with the Hoechst filter (reflecting HPTS at all pH ranges), but which were less intense with the FITC filter (reflecting HPTS at neutral and high pH only).

Photomicrographs of MT-1 cells after incubation with both anti-Tac–conjugated and anti-TFR–conjugated liposomes containing HPTS are shown at 0 and 4 hours (Fig 1). Because the patterns of HPTS fluorescence were similar with both MT-1 and HUT-102 cells, only photomicrographs of MT-1 cells, which gave the brightest fluorescence, are shown. Cells incubated with anti-Tac–conjugated liposomes showed little evidence of liposome internalization during the 4-hour period (Fig 1). Initially, anti-Tac–conjugated liposomes appeared to be associated predominantly with the cell surface with some degree of clustering. This pattern of fluorescence remained virtually unchanged at 4 hours except for an occasional cell demonstrating an internal patch of low pH fluorescence. There was little evidence of a general

Table 2. Indirect Immunofluorescence of Human T-Cell Lines With MoAbs

<table>
<thead>
<tr>
<th>Antibody</th>
<th>MT-1</th>
<th>HUT-102</th>
<th>MOLT-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Tac</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-TFR</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Control Ab*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*These include SS.1 and anti-NB (hsan, 14.2a, and 390).
Conjugation of liposomes to the irrelevant control antibody showed minimal fluorescence emission was measured at 510 nm after excitation at 450-490 nm (FITC) and 350-410 nm (Hoechst). Phase fluorescence was measured at 350-410 nm. Anti-Tac and Anti-Transferrin liposomes showed distinct fluorescence patterns.

Progression from cell-surface staining to cytoplasmic fluorescence to suggest active endocytosis of these anti-Tac-conjugated liposomes. A progressive decrease in total cell-associated fluorescence was noted during the 4-hour time course.

In contrast, MT-I cells treated with anti-TFR-conjugated liposomes showed a progressive accumulation of HPTS at low pH in 4 hours (Fig 1). Initially, the cells exhibited a ringlike surface fluorescence similar to that noted with the anti-Tac liposomes. After 4 hours, most of the cell-surface fluorescence had disappeared and bright intracytoplasmic clusters of HPTS were apparent. The bright spots were diminished under FITC, suggesting accumulation of HPTS into low-pH compartments. HPTS-encapsulated liposomes conjugated to the irrelevant control antibody showed minimal cell-associated fluorescence at all time points (photomicrographs not shown).

HUT-102 cells showed lower total cell-associated HPTS fluorescence with the anti-Tac-conjugated liposomes than did MT-I cells, and there was considerable loss of total fluorescence in 4 hours. As with the MT-1 cells, there was little change in the initial ringlike pattern of surface fluorescence (photomicrographs not shown).

Quantitative fluorimetric assay of liposome binding and internalization. The total amount of cell-associated liposomes and the proportion internalized over time into low-pH intracellular compartments were quantitated in a fluorimetric assay. Cells were incubated with antibody-conjugated fluorescent liposomes and prepared as described. Fluorescence emission was measured at 510 nm after excitation at 450-490 nm.

Fig 1. Cell association and endocytosis of antibody-conjugated liposomes with MT-1 adult T-cell leukemia. Cells (2 × 10⁶) were incubated for 45 minutes at 0°C with anti-Tac-conjugated (top) and anti-TFR-conjugated (bottom) liposomes containing HPTS. Unbound liposomes were removed on a dextran gradient, and the cells were incubated for an additional 4-hour period at 37°C. HPTS fluorescence was recorded with the Hoechst filter cube (total HPTS, 350 to 410 nm) and the FITC filter cube (HPTS at neutral pH, 450 to 490 nm) and photographed at the times indicated. Internalization is shown by an intracellular pattern of fluorescence evident at 350 to 410 nm but diminished at 450 to 490 nm.
403, 413, and 450 nm. A decrease in the 450/413 ratio over time represents a transfer of fluorescent liposomes from the neutral pH of the cell surface into low-pH intracellular vesicles. This serves as a direct marker of internalization and enables calculation of the quantity of cell-associated liposomes located at low pH.

Figures 2 through 4 show results of experiments with MT-1, HUT-102, and Molt-4 cells. The relative degree of internalization of anti-Tac, anti-TFR, and the irrelevant control liposome conjugates over time is shown directly by the decrease in the 450/413 ratio. Quantitation of total cell-associated liposomes and the amount internalized into low-pH compartments are shown in the stack graphs. MT-1 cells incubated with anti-Tac-conjugated liposomes gave the highest level of total cell-associated fluorescence of any cell–liposome combination (Fig 2). Despite greater binding, the MT-1 cells internalized a lower proportion of the anti-Tac-conjugated liposomes than the anti-TFR liposome conjugates, as shown by the smaller decrease in 450/413 and the lower percentage of internalization at 30 minutes and 4 hours (Fig 2). However, the absolute amount of internalized HPTS at 4 hours is similar with both conjugated liposomes (anti-Tac 0.052 nmol, anti-TFR 0.063 nmol). Both anti-Tac-conjugated and anti-TFR–conjugated liposomes showed 20-fold better binding and internalization than the irrelevant control conjugate at 4 hours (Fig 2).

The HUT-102 cells showed a lower level of total cell-
with the irrelevant control at all time points and 100-fold lower than that observed with the anti-TFR conjugates, demonstrating the lack of nonspecific binding of the anti-Tac-conjugated liposomes to this Tac-negative line. There was marked binding and internalization of anti-TFR-conjugated liposomes, comparable with that observed with the ATL cell lines.

**Quantitative cell association and uptake after 72-hour continuous incubation with fluorescent liposomes.** To correlate fluorimetry results with those of subsequent growth inhibition studies, cells were incubated continuously with antibody-conjugated liposomes for 72 hours at 37°C. After separation from unbound liposomes on a Dextran gradient, fluorescence emission was measured as described. Because relative proportions of both total cell-associated and internalized liposomes were similar at 2.5 and 25 μmol/L concentrations of phospholipid, only results from the higher concentration are described.

Table 3 shows the amount of liposome-encapsulated HPTS internalized into low-pH compartments for each cell line/antibody-conjugated liposome combination. In all three cell lines, nearly 100% of the cell-associated anti-TFR-conjugated liposomes was located within low-pH compartments at 72 hours (data not shown), yielding 0.12 to 0.24 nmol HPTS at low pH. The total cell-associated and low pH fluorescence with anti-Tac-conjugated liposomes was greater with MT-I cells than HUT-102 cells. However, a higher proportion of the total fluorescence was internalized with the HUT-102 cells (62% v 30%) (data not shown). In both ATL cell lines, anti-Tac and anti-TFR-conjugated liposomes showed a 6- to 143-fold greater amount of HPTS at low pH than the irrelevant control conjugate. Molt-4, the Tac-negative cell line, showed minimal binding and internalization of anti-Tac-conjugated liposomes, yielding an amount of HPTS at low pH comparable to the irrelevant control.

**Growth inhibition of ATL cells after 72-hour continuous incubation with liposomes containing cytotoxic drug.** Growth inhibition experiments using liposome encapsulated methotrexate-γ-aspartate were conducted to determine the cytotoxic efficacy of anti-Tac and anti-TFR-conjugated liposomes in the two Tac-positive cell lines (MT-I, HUT-102) and the Tac-negative cell line Molt-4 (Fig 5). The results paralleled those observed in the 72-hour fluorimetry experiments. Table 3 shows the IC_{50} achieved with each cell line in growth inhibition as compared with the total quantity of liposome-encapsulated dye at low pH as determined from the 72-hour fluorimetry data.

With the HUT-102 cells, anti-Tac-conjugated liposomes containing drug were approximately threefold more effective than free drug and more than tenfold more effective than the irrelevant control MoAb liposomes in causing growth inhibition. The anti-Tac-conjugated liposomes were somewhat less effective with the MT-I cells, in which they caused approximately twofold greater growth inhibition than free drug and more than fivefold greater inhibition than the irrelevant control antibody conjugates. In contrast, the anti-Tac-conjugated liposomes were fourfold less effective than free drug in the Tac-negative cell line Molt-4. Anti-TFR-conjugated liposomes
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Table 3. Correlation of 72-Hour Growth Inhibition and 72-Hour Quantitative Endocytosis of Fluorescent Liposomes

<table>
<thead>
<tr>
<th></th>
<th>MT-1</th>
<th>HUT-102</th>
<th>MOLT-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-REV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-TFR</td>
<td>0.01</td>
<td>0.143</td>
<td>0.05</td>
</tr>
<tr>
<td>Anti-Tac</td>
<td>0.40</td>
<td>0.039</td>
<td>1.2</td>
</tr>
<tr>
<td>Control Ab</td>
<td>&gt;1</td>
<td>0.001</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Free Drug</td>
<td>0.60</td>
<td>0.40</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*IC_{so} is the concentration of free or liposome-encapsulated methotrexate-γ-aspartate required to achieve a 50% reduction in cell growth as compared with control.
†HPTS at low pH is the quantity of dye located within low-pH intracellular compartments after 72-hour incubation of cells with antibody-conjugated liposomes.

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uptake assays. This may have resulted from liposome leakage of their HPTS contents at the cell surface, capping and shedding of fluorescent liposomes from the cell surface, or cell leakage of HPTS after endocytosis.

We also demonstrate selective growth inhibition of ATL cells by anti-Tac-directed liposomes. At concentrations of drug-filled liposomes causing greater than 50% reduction in cell growth of MT-1 and HUT-102, no effect on Molt-4 cells was noted. Liposomes coupled to irrelevant control antibodies failed to cause any growth inhibition of the ATL cells, further demonstrating a lack of nonspecific liposome-associated cellular injury. Anti-Tac-conjugated liposomes are more effective in causing growth inhibition of HUT-102 than of MT-1 cells. This is consistent with the fact that HUT-102 cells express both high-affinity and low-affinity IL-2 receptors, whereas MT-1 cells express only low-affinity receptors. The high-affinity receptor is composed of at least two polypeptide chains (p55 and p75) each of which can bind IL-2 independently.4 p55 is the well-characterized 55-Kd Tac protein which binds the MoAb anti-Tac.11 p75 is a newly discovered 70- to 75-Kd glycoprotein which does not bind anti-Tac.15,43 Low-affinity receptors are composed of either isolated chain.15 Rapid endocytosis of IL-2 is mediated by both the p75 component alone and the two-chain complex but not by the isolated p55 protein (Tac antigen), suggesting that the p75 chain is responsible for triggering ligand internalization.4 HUT-102 cells which express the high-affinity, rapidly internalized receptor complex (p55 and p75) might, therefore take up anti-Tac-conjugated liposomes more readily than MT-1 cells that express only the low-affinity, poorly internalized p55 Tac antigen. This hypothesis is supported by the results of our 72-hour fluorimetry assay, in which a higher proportion of bound anti-Tac-coupled liposomes are internalized by HUT-102 than MT-1 cells.

Our results parallel those of other experiments in which anti-Tac–coupled immunotoxins were used for in vitro therapy of ATL. Kronke et al13 showed that anti-Tac coupled to the A chain of the toxin ricin effectively inhibits protein synthesis in HUT-102B2 cells, whereas irrelevant control antibody conjugates of ricin A do not. Fitzgerald et al11 demonstrated the efficacy of _Pseudomonas_ exotoxin conjugates of anti-Tac for inhibition of protein synthesis in HUT-102B2 cells. These anti-Tac immunotoxins have no effect on the Tac-negative cell line Molt-4. To circumvent the requirement for immunotoxin internalization before expression of its inhibitory effect, Kozak et al13 designed a radionuclide anti-Tac conjugate that is effective when bound to the surface of IL-2 receptor-bearing cells. Protein synthesis of HUT-102B2 cells is inhibited by this radioimmunotoxin, whereas IL-2 receptor-negative cell lines are not. We showed that anti-Tac–conjugated liposomes containing drug cause specific growth inhibition of IL-2 receptor-positive cells. This approach offers the advantage over direct immunotoxins of delivering thousands of drug or toxin molecules to a single target cell as well as enabling encapsulation of different agents within a specific antibody-directed liposome.

In the present study, anti-TFR–conjugated liposomes were consistently more effective than anti-Tac conjugates in causing growth inhibition of ATL cells. This growth inhibition results from internalization of drug-filled liposomes rather than a surface effect secondary to antibody-receptor binding. Our fluorimetry results suggest that anti-TFR conjugates are more rapidly and completely internalized than anti-Tac–coupled liposomes when bound to their respective receptors. A similar result was reported by Fitzgerald et al,11 who compared anti-Tac and anti-TFR conjugates of _Pseudomonas_ exotoxin for their ability to inhibit protein synthesis of HUT-102B2 cells. Electron microscopy of antibodies conjugated to horseradish peroxidase has shown that receptor-bound anti-TFR is selectively concentrated into coated pits on the cell surface before endocytosis. Anti-Tac also enters cells through coated pits and receptors, but unlike anti-TFR it is not selectively concentrated into coated pits before uptake. This may explain in part the more efficient internalization of anti-TFR–coupled liposomes in the present study. Furthermore, Duprez and Dautry-Varsat46 showed the absence of IL-2 receptor recycling to the cell surface after endocytosis of bound IL-2. The TFR, on the other hand, rapidly recycles to the cell surface after endocytosis of ligand.45,46 A potential problem with clinical use of anti-TFR–directed liposomes, however, in ATL therapy is their lack of specificity for the given cell population. However, evidence shows that TFR expression is lower on normal cells than on rapidly dividing malignant cells.47,48 and several studies have demonstrated the in vitro and in vivo efficacy of anti-TFR immunotoxins in therapy of some human cancers.49,50 Thus, anti-TFR–conjugated liposomes might be effective in treatment of ATL.

Although we demonstrated specific and extensive binding of anti-Tac–conjugated liposomes to ATL cells and effective inhibition of growth, internalization of bound liposomes appears to be slow and incomplete. Several possibilities exist for enhancing the activity of antibody-conjugated liposomes directed against the IL-2 receptor in ATL. Production of a new MoAb directed against the p75 component of the IL-2 receptor rather than the p55 Tac antigen might stimulate more effective internalization of the IL-2 receptor. Studies suggest that the p75 protein and not p55 is responsible for triggering endocytosis of bound IL-2. Other antibodies against the IL-2 receptor might also induce receptor clustering in coated pits and thereby increase internalization of conjugated liposomes.

In summary, we demonstrated specific binding and effective intracellular drug delivery to ATL cells using both anti-Tac and anti-TFR–conjugated liposomes. Given the specificity of anti-Tac for these IL-2 receptor-bearing cells and the marked effect of anti-TFR–coupled liposomes in rapidly growing leukemia cells, both antibody conjugates might prove efficacious in treatment of ATL.

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