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The development of IL-2 conjugated liposomes for therapeutic purposes

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Abstract

A unique immunoliposome has been developed as a drug delivery vehicle for immunotherapy. Human recombinant interleukin-2 (IL-2) has been chemically coupled to the external surface of small unilamellar vesicles (SUVs) containing methotrexate as a candidate immunosuppresive agent in order to specifically direct the drug-bearing liposome to activated T-cells expressing the high affinity IL-2 receptor. This drug delivery system is designed to deliver an immunosuppressive agent to those cells that actively participate in disorders such as graft rejection without delivering an effective but potentially toxic drug to all cells of the immune system as well as other healthy tissues. IL-2 was chemically modified with succinimidyl 4-[*p*-maleidophenyl butyrate](SMPB) while the receptor binding domain on IL-2 was protected by monoclonal anti-IL-2 bound to Protein A-Silica Gel. The antibody recognizes the receptor binding domain of the IL-2 molecule. The IL-2 was derivatized with S-succinimidyl-S-thioacetate (SATA) in order to add an acetyl thioester group to the lipid and create the complex. The derivatized lipid (SATA-PE) was then part of the liposome formulation containing DSPC:cholesterol: SATA-PE at a mole ratio of 1.5:1.0:0.26. SMPB-IL-2 was covalently coupled to the external surface of the SUV after deacetylation of the thioester moiety at pH 7.4 in PBS. Liposomes prepared by sonication or extrusion had an average diameter of 46–50 nm. SUV-IL-2 bound to the high affinity IL-2 receptor as measured by competitive binding assays and Scatchard analysis using $^{111}InCl_2$-loaded liposomes The preparation exhibited a binding constant of 30 pM, consistent with values for free IL-2 cited in the literature. SUV IL-2 could be used as the sole source of IL-2 for the murine CTLL-2 T-cell line or for human mitogen-activated PBLs. The presence of IL-2 coupled to the surface was absolutely required for delivery of the drug to the cell. When methotrexate was encapsulated within the internal aqueous space, receptor-mediated endocytosis led to the inhibition of proliferation due to delivery of MTX to the cytoplasm of the cell. More than 90% of the methotrexate was retained within the liposome during storage over a 24-h period at 4°C. This immunoliposome represents a new class of cell specific immunoliposomes whose entry into the cell is controlled by a cell surface receptor. © 1998 Elsevier Science B.V.

Keywords: Liposomes; Drug delivery; Cell receptor; Interleukin-2; Immunotherapy

Abbreviations: SUV, small unilamellar vesicle; SMPB, succinimidyl 4-[*p*-maleidophenyl butyrate]); IL-2, interleukin-2; SATA, (S-succinimidyl-S-thioacetate); PE, phosphatidylethanolamine; MTX, methotrexate

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1. Introduction

A novel immunoliposome has been developed as a vehicle to treat immunological disorders such as graft rejection which are characterized by abnormally high levels of cytolytic T-cell ($CD8^+$) activity. As an example a 10% increase in IL-2 receptors has been found on lymphocytes infiltrating renal allografts after transplantation in cynomolgus monkeys [1]. At present there is no therapeutic mechanism available to direct the delivery of an immunosuppressive agent to this specific T-cell subset involved in the tissue rejection process while bypassing healthy tissues and other cells that do not have an immunological role. The utility of this complex is further demonstrated by using it as a tool to study receptor-mediated endocytosis.

Liposomal IL-2 (SUV-IL-2) is a small unilamellar vesicle (SUV) with human recombinant IL-2 covalently coupled to the external surface and an immunosuppressive agent designed to suppress metabolic and cytolytic activity of $CD8^+$ cells encapsulated within the internal aqueous space. We have taken advantage of the presence of high affinity IL-2 receptors on activated $CD8^+$ cells as a tool to attract IL-2 bearing liposomes to them and thus control the desired cell specificity of immunosuppressive therapy to clonally expanded T-cells while minimizing drug delivery to healthy tissue. Immunotoxins using an antibody to either the IL-2 or T-cell receptor have been studied as cell specific immunotherapeutics [2–5].

Antigen-stimulated T-cells express the high affinity interleukin (IL-2) receptor on their cell surface [6]. This lymphokine is absolutely required for CD8 T-cell development [6] and is produced by T-helper cells ($CD4^+$) cells. The high affinity IL-2 receptor is a trimer, composed of the α(55,000 kDa), β(75,000 kDa) and γ(60,000 kDa) subunits [2,7,8]. The β subunit can independently bind IL-2 and is responsible for signal transduction and proliferation events. The association constant of the high affinity receptor for IL-2 is 10^{-12} M. Non-activated cells fail to express the high affinity IL-2 receptor. High affinity IL-2 receptor have been on B cells and monocytes [6].

The aim of this study was to prepare an immunoliposome with human recombinant IL-2 covalently coupled to the external surface that would be recognized by the IL-2 receptor. The distinguishing feature of this drug delivery vehicle is that the covalently coupled IL-2 would remain bioactive and retain both its receptor binding and growth factor activities in addition to as serving as the directing agent of the complex [2–4,9]. Antiproliferative activity would then be due to the intracytoplasmic delivery of the encapsulated immunosuppressive agent. More importantly, spurious delivery of the drug to cells that do not express the IL-2 receptor would not occur.

In contrast, IL-2 fusion toxins which are hybrid molecules made up of receptor specific ligands or antibodies and a portion of highly toxic plant or bacterial toxins can only deliver one molecule of fusion toxin per receptor rather than many more molecules of drug encapsulated within the liposome. Here we present a description of the preparation and properties of liposomal IL-2 (SUV IL-2).

2. Materials and methods

2.1. Phospholipids and antibodies

Phosphatidylcholine and distearoyl phosphatidyethanolamine were purchased from Avanti Polar Lipids. Protein A silica gel was purchased from Chromato Chem, Missoula, MT. SMPB (Succinimidyl 4-[*p*-maleidophenyl butyrate]) and the BCA Protein assay reagent were from Pierce Chemicals. Cholesterol (99% + pure) and methotrexate (MTX) were from Sigma Chemical. SATA (S-succinimidyl-S-thioacetate) was from Cal Biochem. Human recombinant IL-2 was from Sandoz, Basel. Alkaline phosphatase substrate, AP-coupled goat, anti-rabbit IgG and triethanolamine buffer were from BioRad. 6-carboxyfluorescein was purchased from Kodak. Monoclonal and polyclonal anti-human IL-2 were purchased from Genzyme.

2.2. Cell lines and culture conditions

The murine T-cell line CTLL-2 was obtained from Sandoz, and grown in the presence of human recombinant IL-2 at 25 ng/ml. Human peripheral blood lymphocytes (PBL) were isolated from whole blood using Ficoll–Hypaque, and stimulated for 72 h with 5

μg/ml phytohemagglutinin M, Sigma Chemicals. All cultures were maintained in ISCOVE's modified DMEM, supplemented with 10% fetal calf serum and antibiotics.

2.3. Proliferation and cytotoxicity assays

The response of PBLs or CTLL-2 cells used as IL-2 receptor positive cells to liposomal IL-2 with or without MTX, was assessed after 72 h in culture. Data presented are the average of two replicates per concentration assayed. Concentration ranges of free or encapsulated drug maximally effective were first established prior to expanding the range. A rigorous statistical analysis cannot be done. Liposome preparations were dialysed at 4°C against serum-free media, sterile filtered by passage through 0.22 μm filters, and serially diluted in the wells of 96-well culture plates. Aliquots of cells were then added with antibiotics to a density of 2×10^4 cells/well. Cultures were incubated for a total of 16 h. Prior to termination of the assay, (^{3}H)TdR(0.5 μCi/well) was added and cells incubated for an additional 5 h. The cells were harvested onto glass fiber filters with a cell harvester (Skatron) and counted with Bioflour, New England Nuclear.

2.4. Preparation of monoclonal anti-IL-2 resin

400 μl of Protein A Silica Gel in a siliconized glass column was washed successively at 22°C with 10 ml Dulbecco's Ca^{++}–Mg^{++} free PBS, pH 8.6, PBS +1.0 M NaCl, 0.2 M Na citrate, pH 3.5, and finally PBS until no protein was detectable in the washes. 1–2 mg of monoclonal anti-IL-2 in PBS was added to the resin and allowed to bind for 30 min. The resin-bound antibody was washed with PBS until all non-bound antibody was removed as monitored by the BCA protein assay. The resin was then washed with PBS containing 12.5% gluteraldehyde, pH 7.4 and finally with PBS. All operations were carried out at room temperature.

2.5. Modification of IL-2 with SMPB on Anti-IL-2 resin

Modification of IL-2 to prepare SMPB-IL-2 was carried out on the antibody affinity resin in order to protect the IL-2 receptor binding domain of the protein. IL-2 at 2 mg/ml was dissolved in 0.05% acetic acid. 100–200 μg/ml was then added to the column in PBS, pH 7.4 and incubated 16 h at 4°C.

The resin was washed extensively with buffer to remove non-bound IL-2 The amount of non-bound protein was quantitated by the BCA assay and thus the amount of resin-bound IL-2 determined. SMPB in dioxane was then added to give a five-fold molar excess of reagent/IL-2 and modification carried out for 10 min at 22°C. The reaction was quenched by the addition of 10 mM Tris buffer, pH 8.0. The modified IL-2 was eluted from the resin with 0.05 M Tris, pH 8.0, 2 M K SCN. SMPB IL-2 was stored in the presence of 0.1% BSA, 0.2 mM PMSF at 4°C.

2.6. Preparation of SATA-modified phosphatidylethanolamine

Use of SATA introduces a protected thiol group into proteins [10]. In the course of this study, it was found that this reagent successfully adds a sulfhydryl to the primary amino group of phosphatidylethanolamine as well. The modified phospholipid is called SATA-PE. A five-fold molar excess of SATA was added to DSPE in $CHCl_3$:MeOH(7:3) containing triethanolamine(1/250 volume). Modification was allowed to proceed for 2 h, 22°C under nitrogen. A three-fold volume excess of acetonitrile was added and the material stored at −20°C for 1–2 h. The precipitate containing SATA-PE was collected by filtration and then redissolved by washing with $CHCl_3$. The SATA-PE was purified on preparative TLC in $CHCl_3$:MeOH (7:3) with a yield of >80%. The R_f of SATA-PE was 0.68. The modified phospholipid was stable for over 12 months when stored under nitrogen at −20°C in $CHCl_3$:MeOH(2:1).

2.7. Preparation of small unilamellar vesicles

Small unilamellar vesicles (SUVs) were prepared from DSPC:cholesterol:SATA-PE with a mole ratio of 1.5:1.0:0.26 by hydrating a dried lipid film with PBS, pH 7.4 at 65°C for 60 min. The lipid suspension was sonicated for 10 min at 65°C with a probe sonicator and then annealed for 10 min at the same temperature. Particles of titanium were removed by centrifugation; $12{,}000 \times g$ for 10 min. Attempts were also made to prepare immunoliposomes by extrusion where the resulting diameter of the SUVs was 90 nm.

Use of this approach was abandoned due to the lack of consistency in the handling of the extruder (Avanti). All data are presented using sonicated SUVs.

2.8. Size determination of immunoliposomes

Sizing data were collected on a NICOMP (model 270) at 25°C.

The date were collected in a volume/weight mode and expressed using a Gaussian distribution.

2.9. Encapsulation of methotrexate or 6-carboxyfluorescein

Methotrexate in PBS, pH 7.4, was added to the hydrated lipid film prior to sonication. The lipid micelles and drug were sonicated together and non-encapsulated drug was removed by chromatography on Sephadex G 50–80 in PBS. (^{3}H)Methotrexate was added to quantitate the degree of encapsulation and to monitor any drug leakage. Leakage was followed by incubating SUV-MTX at 4°C or 37°C for 72 h in sterile buffer or serum-containing media. Aliquots were centrifuged ($2–3000 \times g$) for 10 min in Centrifree filter units (Amicon). Aliquots of the filtrate were counted in Bioflour. Liposomes containing 6-CF were also prepared by co-sonication and chromatographed as described. Starting concentrations of MTX or 6-CF were 200 μg/ml and an encapsulation efficiency of approximately 5% was achieved.

2.10. Covalent attachment of SMPB-IL-2 to SATA-PE SUVs

All steps in the following reaction sequence were carried out under nitrogen. Pre-formed SATA-PE SUVs were deacetylated by the addition of freshly prepared 0.5 M hydroxylamine in HEPES buffer (pH 8.0) and agitated for 30 min, 22°C. SMPB-IL-2 was added and crosslinking allowed to proceed for 2 h. N-ethylmaleimide was added to a final concentration of 20 mM to block any unreacted sulfhydryl groups on the liposome surface. We had empirically found by us that in order to remove any unconjugated IL-2 the pH was raised to 9.2 and SUV-IL-2 was recovered by passage over CM-Sepharose in 0.01 M Tris buffer, pH 9.2.

2.11. Quantitation of modified IL-2

A sandwich ELISA (Genzyme) was used to quantitate the amount of IL-2 as SMPB-IL-2 or SUV-IL-2 using human recombinant IL-2 as a standard. The presence of lipid did not interfere with the assay. It was observed that once IL-2 was coupled to SMPB, conventional colorimetric assays to determine protein concentration could not be used due to interference by SMPB.

2.12. IL-2 receptor binding assays

The murine T-cell line HT-2 was used as an IL-2 receptor expressing cell. Cells were resuspended at 10^7 cells/ml on 0.4 ml Eppendorf tubes. (^{125}I)IL-2 (NEN, 2–50 μCi/μg) at 0.1, 1.0 and 10 ng/ml was added as the competing ligand along with dilutions of SMPB-IL-2, SUV-SMPB-IL-2, or unmodified IL-2 (25 ng/ml). Samples were incubated and then layered onto Silicon Oil (SF 1250), centrifuged at $12,000 \times g$, 10 min and frozen in a dry ice bath. The tubes were cut to separate cell pellets from supernatants. Pellets and supernatants were counted in a gamma counter (Beckman). The '% bound' was calculated from [cpm pellets/cpm pellet + cpm supernatant] $\times$ 100. Liposomes with encapsulated $^{111}InCl_3$ were used to identify classes of high, intermediate and low affinity IL-2 receptors according to Duncan et al. [11] and Robb et al. [7] using human mitogen-stimulated PBL as IL-2 receptor positive cells.

3. Results

3.1. Chemical modification of interleukin-2 and attachment to liposomes

Fig. 1 illustrates a scheme that produces a liposome with IL-2 covalently attached to its external surface. In order to retain biological activity of the targeting molecule to achieve successful drug delivery, chemical modification of human recombinant IL-2 was designed to add linker groups to lysine residues on IL-2 that were not located within the domain of the protein. This would not interfere with the recognition of and binding to the high affinity IL-2 receptor [7,8,12]. In addition, the cross-linking

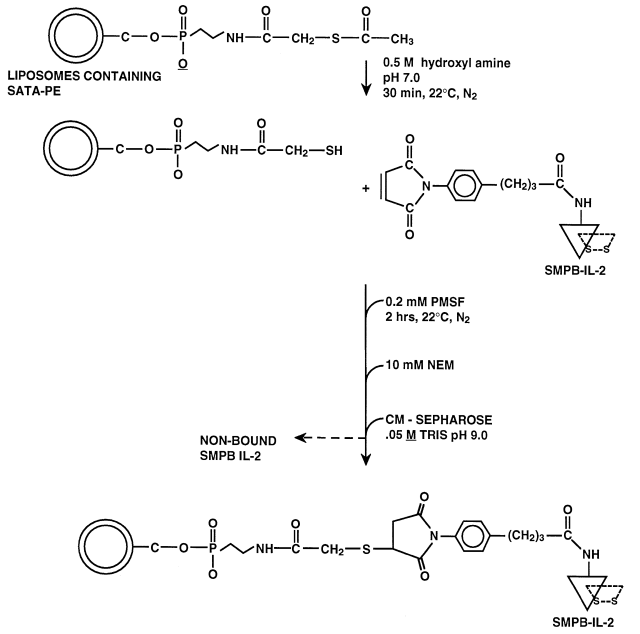

Fig. 1. The structure of liposomal IL-2 is represented along with the scheme for the preparation and covalent attachment of SMPB-modified human recombinant IL-2 to the external surface of SUVs. IL-2 represented by the triangle and parallelogram, is covalently attached to the primary amino group of phosphatidylethanolamine. SATA-modified PE was stored at −20°C in $CHCl_3$:MeOH and SMPB-IL-2 is stored in PBS, pH 7.4 until used in the reactions described.

reaction scheme was specifically chosen to avoid inactivating the protein by disruption of the disulfide group at residues 58–105 on the IL-2 molecule [13]. Consequently, sulfhydryl-specific reagents were not used. The amino-specific reagent SMPB was chosen to react with the lysine residues of the protein at pH 7.4.

This reagent contains a maleimide which is available for coupling with the sulfhydryl that is exposed after deacetylation of SATA-PE. This restriction of modification was accomplished by deriving the protein with SMPB while simultaneously protecting the binding domain with the use of a monoclonal antibody-affinity resin. The resin was composed of silica gel-protein A and anti-human IL-2 whose epitope was the receptor binding domain of IL-2. The interaction of IL-2 and antibody served to hide the binding domain from chemical modification.

The preparation of SATA-PE, a derivatized form of phosphatidylethanolamine containing a blocked sulfhydryl group available for coupling after deacetylation, has not been previously described in the literature. SATA has previously been used only as a modifying and cross-linking reagent for proteins by attaching to the primary amino groups [7,8,12]. SATA-PE has been found to be extremely stable. It can be prepared in large enough quantities and stored in solution at −20°C with complete integrity of the thioester moiety. The resin was stable for 6–8 months if stored in the presence of 0.2 mM PMSF AT 4°C.

Liposomes containing SATA-PE were deacetylated to generate a free sulfhydryl which would react with the maleimide of SMPB addition of N-ethylmaleimide was added to block reactive sulfhydryl groups appearing after deacetylation of SATA-PE on the liposomal surface which could produce aggregates joined by disulfide bonds.

3.2. Physical characterization of SUV-IL-2

Preparations were characterized in terms of size, receptor binding activity, stability and the retention of the encapsulated drug. Sonicated empty liposomes, SUV-IL-2 or liposomes with encapsulated MTX were consistently 48–50 nm in diameter. The particle size of SUV-IL-2, SUV-MTX-IL-2, and SUV-MTX, as determined by laser light scattering analysis (NICOMP), were 46.1, 47.2 and 47.7 nm respectively. The presence of IL-2 bound to the external surface did not alter the diameter of the vesicle.

3.3. Receptor binding studies

The capacity of SUV-IL-2 or SMPB-IL-2 to bind the IL-2 receptor on murine T-cells was followed over a fourteen day period with a series of competition binding assays in the presence of (^{125}I)IL-2 as indicated in Fig. 2. Data expressed as '% bound' versus the concentration of the competing form of IL-2 demonstrate consistent behavior in the ability of either SMPB-IL-2 or SUV-IL-2 to compete with (^{125}I) IL-2 for occupancy on the receptors of HT-2 cells. The concentrations of competitors used were at saturating levels in each study. Liposome-coupled IL-2 bound with less affinity than SMPB-IL-2 to the receptor at each concentration and at each time point examined. Even though the native form of IL-2 was the most effective competitor for occupancy on the

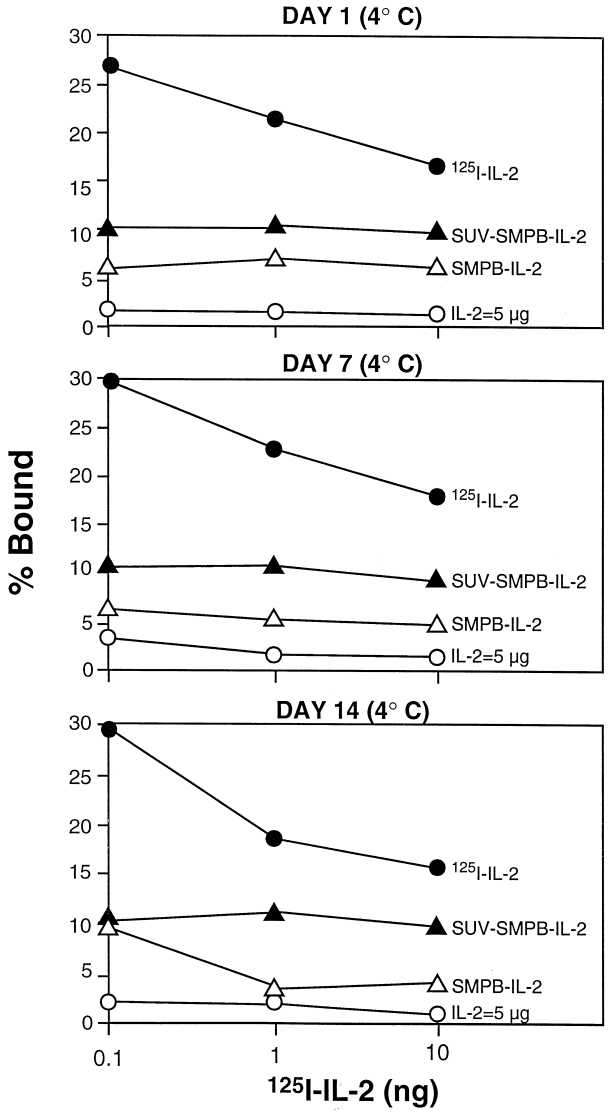

Fig. 2. Competitive receptor binding for the IL-2 receptor on murine HT-2 cells using SMPB-IL-2 (open triangles), liposomal IL-2 (closed triangles) and human recombinant IL-2 (open circles) to displace ^{125}I-IL-2 (closed circles) for occupancy on the receptor. Three concentrations of ^{125}I-IL-2 were used and competing forms of IL-2 were used at 5 μg per samples. Binding studies were carried out for 14 days during which time sterile samples of SMPB-IL-2 and SUV-SMPB-IL-2 were stored at 4°C. Data is expressed as '% bound'.

receptor, the ability of SUV-IL-2 or the derivatized but unconjugated SMPB-IL-2 to bind to all classes of the receptor remained constant during storage when followed over a fourteen day period.

SUV-IL-2 containing (^{111}In)EDTA was used to determine the receptor binding constants using mitogen-activated human peripheral blood lymphocytes and the data analyzed by Schatchard analysis. The binding constants obtained for (^{111}In)SUV-IL-2 were 3.2×10^{-11}M and 2.61×10^{-10} M. In the same system, (^{125}I)IL-2 had a binding constant of 2.08×10^{-12} M and 3.33×10^{-10} M. By comparison, Robb et al. [7] obtained receptor binding constants of 3.6×10^{-12} M and 2.83×10^{-11} M. Preliminary data indicated that SUV IL-2 competed with (^{125}I)IL-2 for binding to the β subunit of the IL-2 receptor visualized by autoradiograms.

3.4. Leakage studies

The phospholipid formulation chosen was sufficiently stable since only a small fraction of encapsulated MTX had leaked out during a 72-h period. Leakage from SUV-MTX averaged 1% or less at 4°C and 4% at 37°C. Leakage from SUV-MTX-IL-2 averaged 2% or less at 4°C and 3% at 37°C. As expected, more of the drug leaked out when vesicles were incubated in serum-containing medium at 37°C. Covalently coupled IL-2 did not appear to alter the retention of this drug within the internal aqueous space of the liposome.

3.5. Growth stimulation by SUV-IL-2

Liposomal-IL-2 supported the growth of PHA-stimulated PBL in a concentration dependent manner

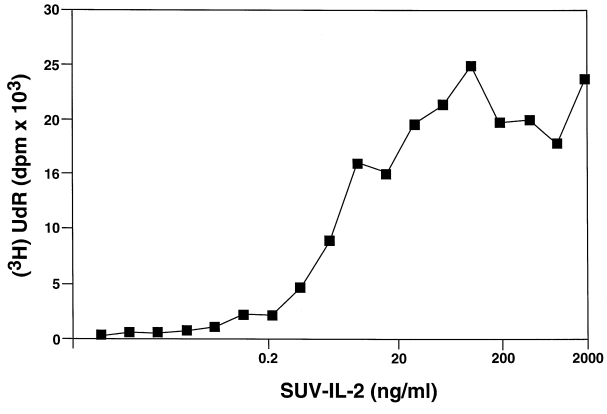

Fig. 3. The growth response of CTLL-2 cells to SUV-IL-2 as the IL-2 source was followed by incubation of the cells with SUV-IL-2 serially diluted in growth medium to generate the concentration range of coupled IL-2 indicated. Growth was measured by the incorporation of 0.5 μCi/ml uridine/well.

as shown in Fig. 3. Half maximal stimulation of growth occurred with 3.9 ng/ml IL-2 in the form of SUV-IL-2. This indicates that the IL-2 on the liposome was recognized by the receptor and internalized as is the unconjugated IL-2, supporting growth and proliferation. Further evidence demonstrating the SUV-IL-2 is internalized is provided by a study where CTLL-2 cells were incubated with vesicles containing 6-carboxyfluorecein (6-CF). Cells incubated for one hour at 37°C exhibit punctate fluorescence at the periphery of the cytoplasm as well as fluorescent material distributed within the cytoplasm as shown in Fig. 4. This would indicate that SUV-IL-2 had been internalized by the IL-2 receptor and had been sequestered in lysosomes.

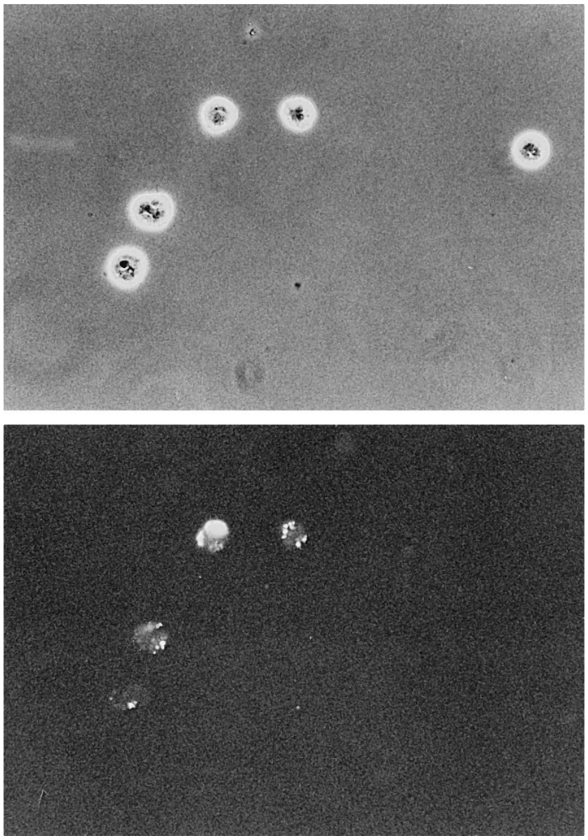

Fig. 4. The internalization of liposomal IL-2 was demonstrated by observing the presence of encapsulated 6-carboxyfluorescein after incubation of CTLL-2 cells with liposomes for 30 min at 37°C in growth medium. The cells were then cytospun onto glass slides. The brightfield image is presented along with the fluorescent micrograph at 100× magnification.

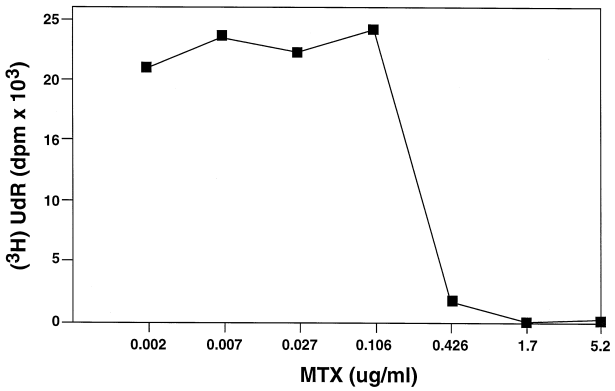

Fig. 5. The inhibition of CTLL-2 proliferation by SUV-MTX-IL-2 at a constant concentration of IL-2 was monitored by incubation of cells for 72 h. SUV-MTX-IL-2 was diluted with SUV-IL-2 in growth medium to a final IL-2 concentration of 25 ng/ml. CTLL-2 at 2×10^5 cells/ml were incubated with liposomes and then pulsed for 5 h with 0.5 μCi/well of ^{3}H uridine.

3.6. Growth inhibition of mitogen-stimulated PBLs by SUV-MTX-IL-2

In order to directly follow the dose-related inhibition of proliferation by SUV-MTX-IL-2, IL-2 receptor positive cells were incubated with a constant concentration of IL-2 while varying the concentration of the drug. This was achieved by using a mixture of SUV-MTX-IL-2 and SUV-IL-2 where the variable was MTX. The concentration of liposomal IL-2 generated in this way was maintained at 25 ng/ml. The data in Fig. 5 present a clear dose-response inhibition of proliferation due to the presence of MTX, where the source of the drug was SUV-MTX-IL-2. In addition, inhibition would not have occurred if the drug had not been delivered into the cytoplasm by receptor-mediated endocytosis. No inhibition of proliferation was due to the presence of SUV MTX under the same conditions. cells if the cells were incubated with SUV-IL-2 in combination with the free drug. Other immunoliposomes have been shown to deliver daunorubricin to the cytoplasm leading to cell death [14,15].

4. Discussion

Liposomes have long been used as a research tool to study the physical and chemical properties of biological membranes. Phospholipid vesicles both as

multilamellar or small unilamellar vesicles are now being used as drug delivery systems as well as probes of cell structure and function. Liposomes are biodegradable, non-toxic, and enable encapsulated pharmacological agents to exhibit longer circulation lifetimes in vivo as compared to the non-encapsulated drug [13]. The limitations of current therapies to treat graft rejection has led to the development of a liposome-based drug delivery system that would restrict the uptake of immunosuppressive drugs to only those cells of the immune system that were actively engaged in graft rejection rather than producing systemic delivery of the drug as is usually the case. Another aspect of the study is that this form of targeted liposome can be used to modulate intracellular activity such as DNA synthesis in the absence of the drug.

Targeting a vesicle to the IL-2 receptor mandates that a critical concentration of the β subunit is present to elicit delivery of the drug. This situation would be found on activated $CD8^+$ cells. In addition, there is a need to develop other modes of drug therapy since the toxicity of many immunosuppressive agents often compromises their efficacy.

Decreasing drug toxicity is a hallmark of liposome encapsulated drugs. Liposomal FK506 tested in a canine liver transplantation model was found in higher concentrations in the liver and spleen and had decreased levels in the kidney [16,17]. Decreased nephrotoxicity was observed using liposomal cyclosporine [18]. Liposome-encapsulated methylprednisolone prolonged cardiac allograft survival in rats [19]. However, none of these liposomal systems could be targeted to a specific T-cell subset or any other group of cells actively involved in the disease process and can potentially interact with non-involved cells. Anti-transferrin and anti-TAC liposomes have been developed as a targeted immunoliposome to treat graft rejection [20,21]. IL-2 has been incorporated into multilamellar liposomes as another means of delivering it with greater circulating life time. This type of complex has also exhibited bioavailability to some extent [5]. Our method of covalently coupling IL-2 to the external surface of liposomes implies that bound molecules demonstrate bioavailability as seen in Fig. 3.

The use of IL-2 as a targeting ligand is also being exploited by the development of immunotoxins.These are constructed from IL-2 or monoclonal antibodies linked chemically to toxins or as fusion proteins of IL-2 and bacterial toxins [6,2–4,9–11]. These hybrid molecules have also been prepared as another means of delivering antiproliferative drugs to cells involved in graft rejection in a cell-specific manner [9,14,22]. However, these immunotoxins may themselves be immunogenic. Moreover, the concentration of drug delivered by immunotoxins is limited by the total number of toxin-conjugated molecules of IL-2 present even though the toxins themselves are quite potent. A far greater concentration of drug is delivered using a liposome as a drug carrier rather than a single toxin molecule.

Confocal microscopy studies demonstrate that the entry of liposomes is into intracytoplasmic vesicles [14]. Liposomes with anti-c-erb-2 conjugated to the outside and doxorubicin encapsulated internally have been found to suppress the growth of human breast tumor cells in culture [23]. Efficient internalization of anti-p185 Her 2 liposomes was observed by light and electron microscopy and had occurred by receptor-mediated endocytosis [24].

These data do reflect an in vitro mode of targeted drug delivery to IL-positive receptor cells. Such cells in vitro would also be mainly those of T-cell lineage. Macrophage do phagocytose particulate antigens and process them for antigen presentation. It remains to be seen how SUV-MTX-Il-2 would be partitioned, if at all, between various groups of lymphocytes during therapy.

Methotrexate was chosen only as a candidate immunosuppressive drug because of its chemical properties. It is often used as an immunosuppressive agent in the treatment of rheumatoid arthritis, in addition to being an antineoplastic agent [15,13,25]. It is water soluble and would be sequestered within the internal aqueous space, is easily monitored in radioactive form, and is largely retained during storage.

SMPB-modified IL-2 is recognized by anti-IL-2 and quantitated by an ELISA is biologically active (24). An interesting feature of this work is that modified IL-2 is internalized by the receptor and therefore initiates proliferative events as seen in Fig. 2. This in turn makes them more sensitive to inhibition by MTX.

In addition, modified IL-2 is stable in solution at 4°C and was able to compete for binding to the IL-2

receptors on HT-2 cells, a murine T-cell line, over a fourteen day period (Fig. 2). Both SATA-PE and IL-2 are stable and can be stored at 4°C prior to coupling [15,13,25]. The stability of SUV-IL-2 also makes it a useful reagent for examining receptor-mediated endocytosis in culture.

The methods described feature IL-2 as a targeting agent to create cell specificity in a liposome-based drug delivery system that otherwise lacks any cell or tissue specificity. Clearly, any cell specific ligand that has a unique high affinity receptor that is unique to a particular tissue or appears to be developmentally regulated could be used in this scheme to achieve the same function as IL-2 does with lymphocytes in the immune system thereby achieving specific drug delivery.

These data do reflect an in vitro mode of targeted drug delivery to IL-positive receptor cells. Such cells in vitro would also be mainly those of T-cell lineage. Macrophage do phagocytose particulate antigens and process them for antigen presentation. It remains to be seen how SUV-MTX-IL-2 would be partitioned, if at all, between various groups of lymphocytes during therapy in vivo.

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