LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASES AND CANCER

Organizers: Isaiah Fidler and Gabriel Lopez-Berestein
February 16 - 20, 1988

Liposomes in The Therapy of Infectious Diseases and Cancer

Keynote Address

W 001 TARGETING OF LIPOSOMES TO TUMOR CELLS *IN VIVO*, D. Papahadjopoulos and A. Gabizon*, Cancer Research Institute and Department of Pharmacology, University of California, San Francisco, CA 94143. *Liposome Technology Inc., 1050 Hamilton Ct., Menlo Park, CA 94025.

Liposomes have several important characterics that define their potential as drug carriers in three distinct areas: One, site avoidance: As liposomes are not taken up by certain sensitive tissues such as heart, kidneys and gut, encapsulation of certain drugs produces a lowering of their toxicity to these critical tissues. The reduction of cardiac toxicity of Adriamycin (1) and renal toxicity of Amphotericin B (2) are two examples of this, which have already given very promising results in currently conducted clinical trials. Two, clearance by the reticuloendothelial system (RES): Natural affinity of the RES for "foreign" particles results in a high uptake of liposomes and their encapsulated drugs by the liver and spleen. There, liposomes accumulate within the interior of resident macrophages, the same cells which engulf a variety of intracellular infectious microorganisms. Liposomes, in this case, have been proven to be useful as carriers of antiparasitic drugs (3) or immune stimulants (4), exhibiting a very high increase in the therapeutic index. Three, site-directed targeting: A relatively high uptake of liposomes by specific "target" cells in some tissues is theoretically possible as indicated by extensive *in vitro* studies, both in the laboratory and elsewhere. However, the applicability of this approach to clinically relevant situations is limited by the following two considerations: the short residence time of the liposomes in blood and the accessibility to the target cells, which is determined by the location of the target cells and the relative permeability of the endothelial barrier within each tissue.

Recent work in this area has revealed some new and important characteristics of liposomes: Inclusion of certain glycolipids within liposomes composed of phosphatidylcholine and cholesterol or sphingomyelin drastically prolongs the circulation half time and reduces their uptake by liver and spleen (5). Concomitantly, their accumulation in several implanted tumors is drastically increased. Furthermore, when liposomes are conjugated to monoclonal antibodies that recognize antigens on the tumor cell surface, there is a further increase in liposome uptake by the tumor, without a corresponding increase in liver and spleen (6). These studies suggest that controlling the circulation time of liposomes and limiting their non-specific uptake by the RES opens up new avenues for achieving specific targeting to tumors *in vivo*, with both diagnostic and therapeutic possibilities.

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Liposomes in Immunobiology

W 002 LIPOSOMES AS "CARRIERS" FOR ADJUVANTS AND VACCINES, Louis A. Chedid, M.D., Ph.D., Francoise Audibert, Ph.D., Department of Pharmacology & Therapeutics, College of Medicine, University of South Florida, Tampa, Florida, 33612.

Although in certain rare cases, virosomes have been built so as to offer a better presentation of viral components it is somewhat misleading for an immunologist to call a liposome "carrier" since this term is reserved for proteins to which haptens have been conjugated. More frequently, liposomes have been used as vehicles for protein or peptide antigens packaged alone or with an adjuvant. We will stress this latter approach (antigens encapsulated with adjuvants) and compare its merits to other modern vaccination procedures. New antigens obtained by synthetic or recombinant DNA technologies are weak immunogens and have created a surge of interest for safe and potent adjuvants. Yet amazingly, the only adjuvant in current clinical practice (aluminum compounds), have been discovered in 1926. In contrast a number of experimental adjuvants have been developed amongst which Freund's complete adjuvant (FCA) is clearly the most potent both for humoral (H.I) and for cell-mediated immunity (CMI). However FCA is too toxic for use outside of the laboratory because of the very strong side effects produced by its mycobacterial components. More recently, non toxic synthetic muramyl peptides (MDP) have been shown to replace mycobacteria in FCA for the induction of H.I. and CMI. With certain peptide antigens, MDP in Freund's incomplete adjuvant (FIA) has been shown to be more effective than FCA. Interestingly FIA has been administered with vaccines to very large numbers of patients but has been removed from clinical use because of concerns which were probably over emphasized. We are now entering an age which should bring a radical change of attitude since our immunization programs are no longer aimed at healthy children or adults but at a new category of population at high risk such as ARC patients, etc.... Therefore, need to identify the strongest adjuvant possible and to use it under the most favorable conditions for inducing prolonged HI and CMI. Since FIA alone increases only H.I., we are advocating the following strategy: comparison between FIA + MDP and liposomes + MDP in view of substituting later liposomes to FIA. A priori, limposomes have several advantages: they can decrease toxicity, target drugs, penetrate the macrophage. They can be helpful for oral use. Their effectiveness has already been demonstrated in animal models using various antigens with different MDP derivatives. There are many other new approaches of which the most fashionable in currently the use of the vaccinia vector. However the vaccinia virus offers some very serious limitations which will be discussed. It is also theoretically possible to use monokines or lymphokine adjuvants. Someday cytokines may be also packaged with adjuvants and antigens in liposomes.

W 003 pH-SENSITIVE AND TARGET-SENSITIVE IMMUNOLIPOSOMES FOR DRUG TARGETING, Leaf Huang, Dept. of Biochemistry, Univ. of Tennessee, Knoxville TN 37996-0840.

We have designed pH-sensitive and target-sensitive immunoliposomes, taking advantage of the polymorphic property of phosphatidylethanolamine (PE). Unsaturated PE by itself forms H_{II} phase at physiological conditions, but the bilayer phase of PE can be stabilized by the addition of a second lipid or an amphipathic protein (1). pH-sensitive liposomes were prepared by using a weakly acidic amphiphile such as fatty acid or fatty acyl amino acid as a bilayer stabilizer. These liposomes, bearing monoclonal antibody for target cell specificity, rapidly destabilize and become fusion competent when they encounter the acidic environment of the endosome, after endocytosis by the target cell. Enhanced cytoplasmic delivery by these liposomes was demonstrated for antitumor drugs (cytosine arabinoside and methotrexate) (2), toxin (diphtheria toxin fragment A) (3) and plasmid DNA (4). Furthermore, we have used an athymic nude mouse model to test the target-specific delivery of a foreign gene in vivo (4). These liposomes are very promising as an effective delivery system. The target-sensitive immunoliposome is a drug delivery vehicle which is independent of cellular endocytosis. The bilayer phase of PE is directly stabilized with fatty acylated antibody. These immunoliposomes rapidly lyse (few min.) at the cell surface when they bind to the cell surface antigen (5). Since drugs can be entrapped in these liposomes at high concentrations, significant drug uptake by the target cells, but not by the control cells, can be readily achieved. We have used mouse L-929 cells infected with Herpes Simplex Virus as a model system and demonstrated superior efficacy and greatly reduced toxicity with cytosine arabinoside and acyclovir (6). Work supported by NIH grant CA24553 and a contract from LipoGen, Inc.

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Novel Approaches in Liposomes Development

W 004 LIPOSOMES IN THE THERAPY OF FUNGAL DISEASES, Daniel P. Bonner and Junius M. Clark, Squibb Institute for Medical Research, Princeton NJ 08540

Amphotericin B has long been a mainstay in the chemotherapy of serious systemic fungal infections. While highly active against yeasts and fungi, the severity of toxic reactions elicited by amphotericin B, in particular nephrotoxicity, has restricted its aggressive use. In the past few years a number of investigators have incorporated amphotericin B into liposomes and have seen a reduction in toxicity with retention of efficacy in experimental animal infections. In limited human studies[1], these observations have been extended to man by Dr. G. Lopez-Berestein using liposomes constructed of amphotericin B with dimyristoyl phosphotidyl choline and dimyristoyl phosphotidyl glycerol. Using the same components as Dr. Lopez-Berestein but varying their relative amounts, an amphotericin B lipid complex has been generated and studied by us in a variety of animal model systems. This complex is significantly less toxic in acute studies in mice than Fungizone®, the current clinical formulation of amphotericin B containing desoxycholate. In experimental animal infections the complex has shown efficacy in systems involving *Candida albicans*, other *Candida sp.* and *Aspergillus*. Efficacy also has been observed in infected animals rendered leukopenic via cyclophosphamide treatment. This complex is targeted for further development and has the potential for clinical utility in man.

[1]Lopez-Berestein, G. (1986). Liposomal Amphotericin B in the Treatment of Fungal Infections. Ann. Int. Med. 105: 130-131.

W 005 INDUSTRIAL APPLICATION OF LIPOSOMES. Marc J. Ostro, Ph.D., The Liposome Company, Inc., 1 Research Way, Princeton Forrestal Center, Princeton, New Jersey 08540.

In the past 20 years, researchers have demonstrated that encapsulation of drugs into liposomes can lead to the enhancement of the drug's efficacy, the reduction of the drug's toxicity or the prolongation of the drug's therapeutic effect. In addition, work has been done on the application of liposome technology to the enhancement of subunit vaccines and to the development of novel diagnostic systems. Most of this work, with a few exceptions, has been done in small animals using liposomes produced in research laboratories in relatively small quantities. However, with the rapid progress of several liposome-encapsulated drugs into the clinic, the rigors of commercial scale pharmaceutical production must now be applied to liposomes. Those issues include the manufacture of liposomes in multi-hundred liter and multi-thousand liter batch sizes, the reproducibility of these preparations, their pyrogen content, integrity of the lipids, acute, subacute and chronic toxicity and quality control methods. All of these issues will be discussed using liposomal doxorubicin as an example.

W 006 A STANDARDIZED INDUSTRIAL LIPOSOME PREPARATION

Peter van Hoogevest, Ciba-Geigy Ltd, Research and Development, 4000 Basle, Switzerland

Although during the past twenty years many interesting studies have been published with liposomal drugs, up till now not a single liposome-drug formulation has been introduced on the market. One of the reasons for this apparent lack of interest of the pharmaceutical industry is the enormous technological hurdle to make stable, reproducible liposomes, suitable for large scale production.
With the Ciba-Geigy liposomal formulation developed for the synthetic immunomodulator MTP-PE the prerequisites for industrial development and for performing a full clinical program have been met.

This formulation comprises two preparation stages:

- first a stable, sterile, dry lyophilisate of liposomal components intended for storage and shipment is manufactured by industrial methods
- second a liposomal (MLV) suspension is prepared in the clinic shortly before use from the dry lyophilisate by means of a simple standardised preparation method.

It is characterised by:

- its increased therapeutic index as compared to unencapsulated drug
- efficient and complete incorporation of the drug in the liposomal structure
- reproducible chemical and physical characterisation of the dry lyophilisate as well as the constituted liposomes within and between batches
- its sufficient stability
- the sufficient safety margin of the in-situ preparation method
- its suitabilitty for large scale production

This list shows that at least for a special use with a special drug a major barrier for industrial development of a liposomal dosage form has been overcome.

Liposomes in The Therapy of Infectious Diseases and Cancer

Liposomes in Cancer

W 007 BIOLOGICAL HETEROGENEITY OF CANCER METASTASES AND IMPLICATIONS FOR THERAPY. Isaiah J. Fidler, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 1515 Holcombe Boulevard, Houston, Texas 77030.

Most deaths from malignant neoplasms are due to the uncontrolled proliferation of metastases which are resistant to conventional therapeutics. This resistance is due to a large extent to the constant evolution of cancer cells, resulting in the generation of neoplasms which are biologically heterogeneous. Recent data from our laboratory and others indicate that metastasis can arise from the nonrandom spread of specialized malignant cells that preexist within a primary neoplasm, that metastases can be clonal in their origin, that different metastases can originate from different progenitor cells, and that, in general, metastatic cells can exhibit an increased rate of spontaneous mutation as compared with benign nonmetastatic cells. These data provide an explanation for the clinical observation that multiple metastases often exhibit different sensitivities to therapeutic modalities and imply that the successful therapy of dissemintaed metastases will have to circumvent the problems of neoplastic heterogeneity and the development of resistance to therapy.

Macrophages can be activated to become tumoricidal by interaction with phospholipid vesicles (liposomes) containing various immunomodulators. Tumoricidal macrophages can recognize and destroy neoplastic cells in vitro and in vivo, while leaving non-neoplastic cells unharmed. Although the exact mechanism(s) by which macrophages discriminate between tumorigenic and normal cells is unknown, it is independent of tumor cell characteristics, such as immunogenicity, metastatic potential, and sensitivity to cytotoxic drugs. Moreover, macrophage destruction of tumor cells apparently is not associated with the development of tumor cell resistance. Intravenously administered liposomes are cleared from the circulation by phagocytic cells. The endocytosis of liposomes containing immunomodulators results in generating cytotoxic macrophages in situ, and the multiple administrations of such liposomes have been shown to bring about eradication of cancer metastases. Macrophage destruction of metastases in vivo is significant, provided that the total tumor burden at start of treatment is small. For this reason, we have been investigating various methods to reduce the tumor burden in metastases by modalities such as chemotherapy or radiotherapy. The ability of tumoricidal macrophages to distinguish neoplastic from bystander non-neoplastic cells presents an attractive possibility for treatment of these few tumor cells which escape destruction by conventional therapeutics.

W 008 *LIPOSOME THERAPY: A NOVEL APPROACH IN THE TREATMENT OF CHILDHOOD OSTEOSARCOMA*, Eugenie S. Kleinerman and Melissa M. Hudson, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Adjuvant chemotherapy following surgical resection of primary osteosarcoma has improved survival rates from 20% at 2 years to 60% at 5 years. Unfortunately 40% of patients will develop pulmonary metastases, most while receiving adjuvant chemotherapy. To improve these results, we propose employing liposome-encapsulated immunomodulators as an additional adjuvant therapy to eradicate the resistant and residual micrometastatic pulmonary disease. Human monocytes can be activated to the tumoricidal state following incubation with liposomal muramyl tripeptide (MTP-PE) and selectively lyse malignant but not normal cells. Multiple intravenous injections of liposomal MTP-PE into mice with microscopic pulmonary metastases has been shown to both activate pulmonary macrophages and eradicate micrometastatic disease. It is our hope that pulmonary macrophages activated by liposomal MTP-PE will eradicate the metastatic pulmonary lesions in patients with osteosarcoma. In order to salvage the significant patient population who develop metastases during therapy, we believe that liposomal therapy must be combined with other chemotherapy early in the adjuvant treatment. However, if chemotherapy interfers with monocyte function, the proposed therapy offers no advantage for increased tumor kill. Since Adriamycin (ADR) is a mainstay of many chemotherapeutic regimens, we determined the effect of ADR in vitro on the ability of liposomal MTP-PE to activate the tumoricidal function of normal blood monocytes. We also evaluated the in vitro activation of monocytes from patients with osteosarcoma pre and post ADR infusion. In vitro, ADR did not interfer with liposomal uptake or the ability of liposomal MTP-PE to activate normal monocytes to lyse tumor cells. Furthermore, monocyte tumoricidal activity could be stimulated in vitro by liposomal MTP-PE in 8/9 patients pretherapy and also 2, 3, and 4 weeks following ADR infusion (75 mg/m^2). The level of cytotoxic activity achieved was equal to or greater than pre therapy levels. Monocytes isolated from four additional patients one day after ADR infusion could also be activated in vitro by liposomal MTP-PE. These studies indicate that the monocyte function of osteosarcoma patients is intact and unaltered by ADR chemotherapy. We, therefore, propose that liposome therapy aimed at activating monocyte effector cells be combined with ADR in the adjuvant treatment of osteosarcoma.

W 009 DESIGN AND DEVELOPMENT OF LIPOSOME-DEPENDENT ANTITUMOR AGENTS. R. Perez-Soler, Immunobiology and Drug Carriers Section, Department of Clinical Immunology & Biological Therapy, The University of Texas M.D. Anderson Hospital & Tumor Institute, Houston, TX.

Liposomes have been extensively explored as carriers of antitumor agents and, have been shown to decrease doxorubicin cardiotoxicity and increase the antitumor activity against experimental liver metastases. However, formulation and stability problems have hampered their clinical application. Design of new antitumor agents for liposome entrapment may be a way to solve these limitations. More than 40 new lipophilic cisplatin analogues were synthesized. Efficient liposome entrapment (>90%) was achieved with most of them. Cis-bis-neodecanoato trans-R,R-1,2 diaminocyclohexane platinum (II) (NDDP) was selected for preclinical development. Characterization of NDDP and reproducibiliy of the synthetic method were assessed by elemental analysis, ^{195}Pt NMR, infrared spectroscopy, and HPLC. Liposomal-NDDP (L-NDDP) was formulated in a lyophilized powder containing dimyristoylphosphatidyl choline, dimyristoylphosphatidyl glycerol, and NDDP (weight ratio 10.5:4.5:1). Reconstitution of lyophilized L-NDDP with 0.9% NaCl in water results in a liposome suspension with >95% of the NDDP associated with the lipid vesicles and the following size profile: 15% <2 μm, 65% 3-5 μm, and 20% >5 μm. The liposome suspension was found to be stable for at least 6 hours as assessed by size profile, osmolality, PH, NDDP entrapment, optical microscopy, and NDDP integrity by thin layer chromatography. Antitumor activity studies were carried out in vitro against LOVO colon carcinoma cells sensitive and resistant to cisplatin. Against both types of cells, L-NDDP was 2-3 times more cytotoxic than cisplatin and non-entrapped NDDP. In vivo antitumor activity studies were carried out against ip L1210 leukemia, ip L1210/PDD leukemia, and liver metastases of M5076 reticulosarcoma. L-NDDP was significantly more active than cisplatin against all 3 tumor models. Non-entrapped NDDP had minimal antitumor activity against L1210/0 and L1210/PDD leukemias, and none against liver metastases of M5076 reticulosarcoma. In iv mouse toxicity studies, the LD50 of L-NDDP ranged from 45 to 60 mg/kg and that of cisplatin beween 20 and 25 mg/kg. At the LD50 dose, L-NDDP caused severe myelosuppression but no renal dysfunction while cisplatin was not myelosuppressive but resulted in a 7-fold increase of the BUN. In iv dog toxicity studies, the maximum tolerated dose (MTD) of L-NDDP was 150 mg/m^2. This dose caused moderate vomiting and myelosuppression but no renal dysfunction. Dogs that received several times the MTD at monthly intervals did not present weight loss, cumulative myelosuppression or signs of neurotoxicity. Renal dysfunction was minimal. Clinical studies with L-NDDP will be started shortly. Design of liposome-dependent analogues of other antitumor agents is being explored at this point.

W 010 ACTIVATION OF LIVER MACROPHAGES, Gerrit L. Scherphof and Toos Daemen, Laboratory of Physiological Chemistry, State University Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands.

Rat liver macrophages (Kupffer cells) were activated to a tumoricidal state by incubation of monolayers of isolated cells with free or liposome-encapsulated muramyldipeptide (MDP) or lipopolysaccharide (LPS). The cytolytic activity towards tumor cells was assessed by a ^{3}H-dThd release assay and cytostatic activity by a ^{3}H-dThd incorporation assay. Liposomal encapsulation resulted in a several hundred fold reduction in the amount of MDP required to reach maximal levels of cytotoxicity. The efficiency of the liposomal MDP, measured as extent and duration of the cytotoxic state, was influenced by the amount of encapsulating lipid, but, surprisingly, not by the lipid composition. The cytolytic activity of the Kupffer cells was shown to be specific towards tumorcells, in contrast to the cytostatic activity, which was also observed with respect to non-tumor cells.

As regards the mechanism of activation of the macrophages by various immunostimulants, it is of interest to note that, in contrast to MDP, encapsulation of LPS in liposomes resulted in a decrease in the activating potency of the stimulant rather than in an enhancement. Concomitantly, the activating potency of LPS, but not that of MDP, was lost upon incubation of the two immunomodulators with a lysosomal fraction from liver.

Following intravenous injection of liposome-encapsulated MDP into rats, the Kupffer cells isolated from the livers of these animals are also cytotoxic to tumor cells. Subfractionation of the isolated liver macrophages, according to cell size, into a number of sub-populations revealed substantial differences in liposome uptake as well as in susceptibility to activation, the smaller cells being less active in liposome uptake but more susceptible to activation.

The activated state of the liver macrophages following systemic administration of liposomal MDP became also apparent by very substantial reductions in the development of liver metastatic nodules from intrasplenically injected colon adenocarcinoma cells. Intravenous injection with liposomal MDP elicits a transient two-fold increase in the size of the hepatic macrophage population, which is partly due to extrahepatic recruitment and partly to local proliferation of resident macrophages. In view of the observed refractoriness of Kupffer cells to a second activating stimulus following an initial activation response to liposomal MDP, it is believed that the increase in population size may be of crucial importance for successful immunotherapeutic treatment of liver metastases.

Liposomes in The Therapy of Infectious Diseases and Cancer

Liposomes in Infectious Disease - I

W 011 COMPARATIVE ACTIVITIES OF FREE AND LIPOSOME ENCAPSULATED AMIKACIN AGAINST MYCOBACTERIUM AVIUM COMPLEX (MAC), Pattisapu R.J. Gangadharam[1], Veluchamy K. Perumal[1], L. Kesavalu[1], Robert J. Debbs[2], Jayne Goldstein[2], and Nejat Duzgunes[2], [1]National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206 and [2]Cancer Research Institute, University of California at San Francisco, San Francisco, CA. Mycobacterium avium complex (MAC) causes serious disseminated disease in normal and immune deficient individuals, but more so in AIDS victims. In our multifaceted approach to identify new drugs active against MAC, we discovered in vitro activity of Amikacin against several strains of MAC. Dynamic studies using continuous, falling off, and pulsed exposures confirmed its bactericidal activity. More importantly, amikacin at 50 mg/kg dose given i.m. daily exhibited high activity against experimental MAC infections in the beige mouse model. When the drug was given once weekly i.v. in an encapsulated form in phosphatidyl-glycerol (PG)/phosphatidyl-choline (PC) liposomes, its activity against MAC infection was considerably greater than the free drug given i.v., as seen by the colony forming unit (CFU) counts in the spleen, liver, and kidney, but not in the lungs. PG/cholesterol liopsome encapsulated amikacin showed marked inhibition of intracellular multiplication of MAC inside resident and activated macrophages from beige, C57Bl/6 or S/W mice; in all these situations free amikacin was not active. Finally, both the free and liposome encapsulated forms were equally active against MAC growth inside J-774 cell lines. Our research is supported by NIH grant AI 21897, and NIH contract AI 42544, and a grant from the University-Wide Task Force on AIDS from the State of California.

W 012 COMPARISON OF FREE AND LIPOSOMAL MTP-PE: PHARMACOLOGICAL AND TOXICOLOGICAL ASPECTS, Gebhard Schumann, Research Department, Pharmaceuticals Division, CIBA-GEIGY Limited, Basle, Switzerland.
The lipophilic muramyl peptide MTP-PE (muramyl tripeptide-phosphatidyl ethanolamine) can be stably inserted into liposomes. Fidler and his associates could show that intravenously applied MTP-PE encapsulated into liposomes (multilamellar vesicles, MLV) consisting of egg phosphatidyl choline and beef brain phosphatidyl serine in a 7:3 weight ratio results in site specific targeting thus increasing the efficacy and the therapeutic window of the MTP-PE. In vitro and in vivo, MTP-PE/MLV appeared to be a more effective and longer lasting macrophage activator than MTP-PE alone. It showed increased therapeutic or prophylactic efficacy in tumor and infection models. Here we report about results obtained with a fully synthetic formulation consisting of MTP-PE and the endogenous lipids palmitoyl-oleoyl phosphatidyl choline (POPC) and dioleoyl phosphatidyl serine (OOPS). The data of Fidler obtained with liposomes consisting of natural products could be repeated and substantiated with the synthetic formulation by pharmacological experiments. For example, when compared with the free compound, MTP-PE/MLV given i.v. showed a 10 times higher efficacy in eradicating lung metastases in a melanoma model in mice (0.1 mg/kg MTP-PE/MLV versus 1 mg/kg MTP-PE in free form). On the other hand, in 2 weeks to 3 months toxicity tests in dogs and rabbits, the liposomal formulation was less toxic than the free form (toxic-no-effect level 0.1 mg/kg MTP-PE/MLV versus 0.01 mg/kg MTP-PE in free form). When MTP-PE was given intranasally, no superiority of the liposomal formulation could be demonstrated pharmacologically. In an ex vivo model where the induction of tumoricidal rat alveolar macrophages was measured the minimal effective dose of both MTP-PE/MLV and free MTP-PE turned out to be 0.001 mg/kg. The same dose showed also antiviral activity in a variety of virus models in mice. In a 3 months toxicity test in dogs, free MTP-PE applied intranasally did not show any toxicity up to the highest dose tested (1 mg/kg). Clinical Phase I trials are presently under way with the following formulations:
- MTP-PE/MLV, injected intravenously (tumor patients)
- MTP-PE, applied intranasally (virus challenge tests in volunteers).

Clinical Trials of Liposomes

W 013 EXPLORATORY CLINICAL PHASE OF LIPOSOME-ENTRAPPED DOXORUBICIN, A. Gabizon, A. Sulkes, N. Ben-Baruch, T. Peretz, S. Biran, S. Amselem, S. Druckman, and Y. Barenholz, Department of Oncology, Hadassah University Hospital, and Department of Membrane Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

A clinical study was designed to identify and evaluate the toxic effects of liposome-associated doxorubicin (L-DXR) after IV administration in cancer patients. The L-DXR formulation used was selected on the basis of preclinical work in rodents indicating: a. Reduced systemic toxicity and cardiotoxicity (1); b. Superior antitumor activity against liver metastases (2). The initial phase of this study consisted of twenty patients with primary or metastatic liver cancer refractory to conventional therapy who received a total of 35 courses of L-DXR. During this initial phase, no attempt was made at removing liposome-nonassociated (free) drug. The L-DXR and phospholipid doses were escalated from 20 mg/m² and 0.3 g/m² to 70 mg/m² and 1.2 g/m² respectively. A cumulative dose of 210 mg/m² with a three-week intermittent schedule was reached. Treatment was generally well tolerated and acute toxic effects such as nausea and vomiting were mild and infrequent. Bone marrow suppression was identified as the most likely single-dose limiting factor: leukopenia (grades 3 and 4) developed in 5 out of 20 evaluable courses at dose levels between 50 and 70 mg/m². As yet, the maximal tolerated dose (MTD) has not been reached. Quality control analysis pointed at the fraction of free drug (range 16-38%) as the most significant problem in batch to batch reproducibility. In the subsequent phase of this study, currently in progress, methodological improvements based on the affinity of free DXR for cation-exchange resins (3) have enabled us to produce L-DXR with minimal levels of free drug. This is a critical requirement for achieving a reliable MTD determination.

Pharmacokinetic analysis of L-DXR is complex because of the distribution of the drug in three plasma compartments (carrier-bound, protein-bound, and free diffusible fraction) and the difficulty in analyzing them separately. Overall plasma determinations of DXR suggests that liposome delivery substantially modifies various parameters of DXR pharmacokinetics resulting in reduced blood clearance and smaller volume of distribution. Supported by Liposome Technology, Inc. (Menlo Park, CA).

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W 014 PHASE I DEVELOPMENT OF CGP 19835A LIPID (MTP-PE ENCAPSULATED IN LIPOSOMES).
J.R. Hanagan, H. Frost, P. Trunet, D. LeSher, K. Andrejcio. CIBA-GEIGY Corp. Summit, N.J. and CIBA-GEIGY Ltd., Basle, Switzerland. CGP 19835A Lipid is a synthetic muramyl tripeptide encapsulated in liposomes that is believed to exert its effects by activating monocytes. Phase I trials have been initiated at four clinical centers involving patients with advanced malignancy and at one center involving patients with AIDS-related Kaposi's Sarcoma. Drug is administered either once or twice weekly intravenously on either an intra or inter patient dose escalating schedule. Eighty-two patients have been enrolled to date exploring doses from 0.01-4.0 mg/m². No serious adverse reactions have been reported to date. Acute reactions include fever (79%) rigors (59%) and nausea(44%) (percent of patients in parentheses). Macrophage activation by cytotoxicity assay is measured at various time points pre-and post-dosing. To date no clear dose-response or dose-duration trends are apparent for either clinical tolerability or macrophage activation. The phase I trials are continuing.

W 015 LIPOSOMAL ENCAPSULATED DOXORUBICIN (LED): PHASE II STUDY IN BREAST CANCER, Joseph Treat and Aquilur Rahman, Division of Medical Oncology, Lombardi Cancer Center, Georgetown University, Washington, D.C. 20007. Our Phase I study with LED defined a maximum tolerated dose of less than 90 mg/m^2. At 90 mg/m^2 grade 4 neutropenia was seen in some of the patients. However, at a dose of 60 mg/m^2, the three patients treated had experienced no myelosuppression, one had mild nausea and vomiting and one exhibited alopecia. The clinical pharmacology studies at a dose of 60 mg/m^2 demonstrate that drug pharmcokinetics administered in liposomes is markedly altered. The peak plasma concentration of doxorubicin was 6.79 + 1.04 ug/ml at 5 minutes, the AUC was 26.72 ug.hr.ml^{-1} and t 1/2 was 22.0 hours, and volume of distribution of drug being 107.83 liters. Based on the toxicity profile of LED in Phase I trials, we have begun a Phase II trial in recurrent measurable breast cancer patients at a dose of 75 mg/m^2 to be infused in 45 minutes every 21 days. Response rate and toxicities, particularly cardiac, will be assessed in this trial. Radionucleotide resting ventriculograms are performed to assess cardiac function prior to entry in the study. All patients who have entered in this Phase II study have failed first line chemotherapy with recurrent disease. To date 6 patients have been entered and 3 patients have received 3 cycles and are evaluable for response rate and toxicities. Patient one had complete resolution of biopsy proven malignant pleural effusion. Patient two had complete resolution of skin metastasis. Patient three had complete regression of a supraclavicular lymph node. In one of the patients there has been grade 4 granulocytopenia requiring dose reduction. In all of the patients there has been no nausea or vomiting (antiemetic at low doses were given before chemotherapy). Alopecia has been seen in all patients. Patient accrual is ongoing in this Phase II trial and updated results as well as pharmacokinetics data at this dose level will be presented.

Supported by LyphoMed, Inc., Rosemont, Illinois

Liposomes in Infectious Disease - II

W 016 **ROLE OF MACROPHAGES (MØ) IN VIRAL INFECTIONS,** Page S. Morahan, Deneen Stewart and Angelo J. Pinto. Department of Microbiology and Immunology, The Medical College of Pennsylvania, Philadelphia, PA 19129

The importance of MØ in viral infections has been established by several experimental approaches. These have involved: (i) kinetic correlations of MØ with viral pathogenesis, (ii) selective depletion of MØ, (iii) selective augmentation of MØ functions, and (iv) genetic and age-related correlations. Our laboratory has focused on herpes simplex (HSV) and encephalomyocarditis (EMC) virus infections in mice. We have established that peritoneal and liver MØ have potent intrinsic antiviral activity (ability to restrict virus replication within MØ), MØ with extrinsic antiviral activity (ability to restrict virus replication in permissive cells) appear very early after HSV-2 infection, transfer of antivirally active MØ can transfer antiviral resistance to HSV-2, activation of MØ with various immunomodulators can be correlated with enhanced antiviral resistance, and that selective depletion of certain tissue MØ can markedly decrease antiviral resistance (1-4). Moreover, if circulating monocytes and NK cells are depressed, but not tissue MØ, antiviral resistance remains relatively normal (5-6). We have recently begun investigation of selective depletion of various tissue MØ with toxins encapsulated in liposomes. Preliminary data indicate that depletion of splenic and liver MØ markedly decreases resistance to i.v. infection with HSV-2 and Listeria. Taken together, these data support the concept that tissue MØ are very important in providing early nonspecific resistance to viral infections. MØ also appear to be important in amplification of specific immune responses to viruses, such as enhancement of virus specific antibody activity through the antibody dependent cell mediated cytotoxic reaction and enhancement of virus specific T cell activity through lymphokine activation of MØ for enhanced extrinsic antiviral activity. (Supported by AI25751, DAMD17-86-C-117, N00014-82-K-0669, ACS IM462)

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W 017 LIPOSOMES AS CARRIERS OF LIPOPHILIC DRUGS FOR USE IN AEROSOL THERAPY OF RESPIRATORY VIRUS INFECTIONS, Howard R. Six, Brian E. Gilbert, Philip R. Wyde, Sam Z. Wilson and Vernon Knight, Department of Microbiology, Baylor College of Medicine, Houston, Texas.

Enviroxime inhibits the replication of all rhinoviruses tested in vitro at very low concentrations (10-1000 ng/mL), but evaluations in humans have not consistently shown efficacy. Lack of an appropriate method for administering this water insoluble drug may have contributed to the latter result. Present studies demonstrate that enviroxime:phoshatidylcholine mixtures form liposomes. Examination of negatively stained prepartions by electron microscopy revealed the presence of multilamellar structures. Formation of a permeability barrier was confirmed by entrapment of umbelliferone phosphate, a marker known to reside in the aqueous compartments of liposomes. Quantitative asssociation with the lipid bilayers was confirmed using ^{14}C-enviroxime. Enviroxime-containing liposomes (LE) retained their antiviral activity when tested against rhinoviruses strains 1A and 13; however, these same preparations had significantly less toxicity to KB, HeLa, and MDCK cells. Thus, the therapeutic index was increased by 10 to 50-fold.

Enviroxime content of liposomes and biological fluids was quantified by high performance liquid chromatography using C18 resin, mobile phase of 60:40 acetonitrile:water, and monitoring at 215 nm. Small particle aerosols of LE generated by Puritan-Bennett nebulizers had mass median diameters of 2.4-3.1 µm. The concentration of enviroxime in the aerosol particle was proportional to the reservoir concentration; mean concentration was 20 µg of enviroxime/L of aerosol/hr. Liposome particles in the reservoir, initially heterogeneous in size (0.2 to >1 µm), were processed by passage through the nebulizer to smaller, more homogeneous particles; the majority were less than 0.2 µm. There was no loss of antirhinovirus activity due to aerosolization and the multilamellar structure of the liposome was maintained.

A preliminary evaluation of the LE aerosol was conducted on 5 volunteers exposed for 1 hr. At 2 hr posttreatment, large amounts of enviroxime were still present in the nasal wash as determined both by HPLC and biological assay. Enviroxime was not detected in any urine sample and was detected in only 1 of 5 serum samples. No side effects were noted. This data suggests that liposome aerosols offer a method for the delivery of hydrophobic, as well as hydrophilic, compounds for the treatment of respiratory diseases.

Late Additions

W 018 LIPOSOMES IN THE THERAPY OF RIFT VALLEY FEVER VIRUS (RVFV) INFECTION, Meir Kende[1], Peter G. Canonico[1], Michael Contos[1], and Alberto Gabizon[2], [1]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011, and [2]Liposome Technology, Inc., Menlo Park, California 94025.

By using liposomes as drug carriers, a nontoxic, low-dose regimen of ribavirin administered to CD-1 mice simultaneously with Rift Valley fever virus (RVFV) challenge had a therapeutic effect that was comparable to that achieved with higher but potentially more toxic doses of ribavirin (1). However, the increased efficacy of liposome-encapsulated ribavirin on therapeutic schedules compared to "free" ribavirin varied with each batch. Probable reasons for the variation are wide range in the size distribution of the liposome particles and the rapid dissemination of RVFV from macrophages to liver hepatocytes and other cell types in different organs. To help solve this problem, liposome-encapsulated ribavirin was subjected to: (1) dialysis with continuous a flow-plate unit, (2) sonication of vesicles prepared by rotary evaporator, and (3) filtration by membrane extrusion to produce liposomes of homogeneous size. Due to their lipid composition, liposomes prepared with membrane filtration remain in the circulation for an extended time and can confer antiviral state to cells other than macrophages, either via endocytic pinocytosis or by active or passive transport. With continuous flow equipment using egg yolk lecithin and n-octylglucoside detergent, 80% of the liposome-encapsulated ribavirin vesicles were 0.2µm in diameter, as revealed by laser submicron particle analyzer; however, the encapsulation yield was 4-5 times lower than methods 2 and 3. The large majority of liposomes prepared by 5-10 min sonication were 0.2-0.3 µm. Such liposome-encapsulated ribavirin preparations gave significantly better efficacy compared to "free" ribavirin when administered to CD-1 mice 24 hr postinfection with a rapidly lethal challenge of RVFV. The majority of liposomes made with the membrane extrusion method were 0.2 µm in diameter. Treatment of RVFV infections with such preparations was more efficacious than with "free" ribavirin. These results suggest that the size of the liposomal vesicles is a contributing factor to macrophage targeting, and macrophage targeting should be coupled with longer liposomal half-life to allow drug uptake by cells without phagocytic activity.

Kende, M., Alving, C.R., Rill, W.L., Swartz, G.M., Jr., and Canonico, P.G. Antimicrob. Agents Chemother. 19:1042-1049, 1985.

W 019 *TARGETED DRUG DELIVERY VIA PROTEIN-MEDIATED MEMBRANE FUSION. SPECIFIC KILLING OF HIV-IFECTED HUMAN LYMPHOCYTES,* *Claude Nicolau[1,2], Garret M. Ihler[2], Joseph L. Melnick[3], Christine Noonan[3], S.K. George[2], Francois Tosi[1,2], Tudor Arvinte[2], and Amelia Cudd[1], [1]Biophor Corporation College Station, TX 77843, [2]TAMU College of Medicine, College Station, TX 77843, [3]Baylor College of Medicine, Houston, TX 77010.*

We reconstitute in the bilayer of giant SUV liposomes the CD4 molecule extracted from a line of human GEM lymphocytes. A plant toxin, naturally lacking chain A, gelonin is encapsulated in the liposomes. Upon incubation of these liposomes with HIV infected H-9 cells and normal H-9 cells fusion, inhibition of protein symthesis leading to cell death is observed with the infected cells while no effects appear with the normal cells. This difference is ascribed to the interaction of the liposomal CD4 molecule with the viral protein gp120 present on the HIV infected H-9 cells.

Insertion of CD4 antigen in the membranes of intact red blood cells leads to interaction of these RBC-CD4+ and HIV-infected H9 cells. This provides for a potentially long-lived CD4 carrier in the circulation.

Potential therapeutic implications are discussed.

Liposomes in the Therapy of Infectious Diseases and Cancer

Liposomes in Cancer

W 100 Liposomal Interleukin 2 Synthesis and Biologic Activity. Peter M. Anderson, Douglas Schow, and Arnold Leonard, University of Minnesota, Minneapolis, MN
Interleukin 2 (IL2) is a potent lymphokine produced by T lymphocytes which facilitates T lymphocyte proliferation and the development of non-MHC restricted killing of tumor by activated NK and/or T cells. IL2 has a short T1/2 (3 min) and is metabolized by the kidney. Systemic toxicity, especially a capillary leak syndrome has been associated with IL2 infusions thus severely limiting the therapeutic effectiveness of this biologic response modifier. Thus we have investigated methods to entrap IL2 in liposomes and have studied the pharmacokinetics and pharmacodynamics of liposomal IL2. Detergent dialysis using sodium cholate, DMPC, and DMPG in a 25:4:1 molar ratio resulted in 42 percent entrapment. The best entrapment, however, was obtained with freeze and thaw multilamillar vesicles (FATMLV) which were produced by addition of aqueous IL2 (1 mg/ml H_2O) to lipids (DMPC 7:DMPG 3). FATMLV's had 80 percent IL2 entrapped and little or/no leakage of contents after 3 weeks at 4°C. The addition of trehalose (125 mM) to lipids permitted freezing and storage of FATMLV in liquid nitrogen with no loss of IL2 from FATMLV's when thawed. IL2 liposomes were active in the CTLL-2 IL2 bioassay. Pharmacokinetic analysis of serum IL2 levels after administration of 250,000u liposomal IL2 sq to mice indicated prolonged levels (> 48 hr) could be obtained with this preparation; the calculated biphasic T1/2 of FATMLV IL2 was 5 min hours 2-12 and 68 min hours 12-48. Liposomal IL2 may have potential as a potent immunoadjuvant and potential immunologic anti-cancer agent.

W 101 MONOCYTE TUMORICIDAL ACTIVITY IN BRAIN TUMORS: IN VITRO ACTIVATION BY LIPOSOMAL C-REACTIVE PROTEIN (CRP), B. Barna, J. Pettay, S. Malcolm-Kohn, J. Bay, M.J. Thomassen and S. Deodhar, Cleveland Clinic Foundation, Cleveland, OH 44106.
Because malignant brain tumors respond poorly to conventional therapy, biological response modifiers (BRM) may be of use after surgical reduction of tumor mass. The purpose of this study was to determine: (1) if monocytes (Mos) from steroid-treated brain tumor patients develop enhanced in vitro cytotoxic activity (CTX) against astrocytoma cells after exposure to soluble human CRP, multilamellar vesicle (MLV) associated CRP, or other BRM; and (2) whether Mo CTX after craniotomy (when BRM therapy might be most effective) differs from that before surgery. Results from 14 patients (7 astrocytoma, 2 meningioma, 2 metastatic carcinoma, 2 craniopharyngioma and 1 lymphoma) indicated that frequency of Mo CTX (defined as CTX > 20% against NK-insensitive CRL-1718 astrocytoma cells) induced by soluble CRP (50 µg/ml) was not different from normal: 9/14 (64%) patients responded vs 6/9 (67%) normals. Spontaneous Mo CTX, however, was significantly more frequent among brain tumor patients (11/14, 79%, $p < .05$) than among normals (1/9, 11%). In post-surgical patients, mean percentage Mo CTX was significantly ($p < .025$) greater with 300 nmoles/ml CRP-MLV (1.0 µg CRP/nm phospholipid) (18.9 ± 5.4% SE, n = 12), or MTP-PE-MLV (5.0 µg/µm) (23.7 ± 6.4%, n = 10) than with control (buffer) MLV (4.1 ± 2.2%), and was also higher than pre-surgical CTX with CRP-MLV (12.8 ± 5.2) or MTP-PE-MLV (10.1 ± 3.9) ($p < .05$). Normal Mos did not respond to MLV reagents. Data indicate that soluble and liposomal CRP, like other BRM, enhance Mo CTX in steroid-treated brain tumor patients and that CTX is elevated after craniotomy. Results support BRM use in malignant brain tumors.

W 102 ANTIVIRAL PROPERTIES OF FREE AND LIPOSOME-ENCAPSULATED MURAMYL TRIPEPTIDE, J. David Gangemi, Eugene Mayer, and Abdul Ghaffar, Microbiology and Immunology, University of South Carolina, School of Medicine, Columbia, SC.
We have examined the ability of both free and liposome-encapsulated MTP-PE (muramyl tripeptide-phosphatidyl ethanolamine) to activate murine macrophages and to enhance resistance to viral pneumonitis, hepatitis and encephalitis. Macrophages from several anatomic compartments (i.e. lung, liver and peritoneum) were obtained from mice receiving intravenous inoculations of either free or liposome-encapsulated MTP-PE and their biological activity assessed. The in vitro tests used to measure biological activity included phagocytic, antimicrobial and antitumor assays. Regardless of their anatomic location, macrophages obtained from mice receiving liposome-encapsulated MTP-PE were more highly activated than those obtained from mice receiving free MTP-PE. The enhancement of alveolar and liver macrophage functions following administration of liposome-encapsulated MTP-PE appeared to result from both increased drug deposition and sustained release in the lung and liver. In addition to the augmentation of macrophage activity as determined by in vitro assays, liposomal MTP-PE also enhanced resistance to herpesvirus-induced hepatitis and pneumonitis. Protection (> 80 % survival) was best observed when liposomal MTP-PE was administered several days prior to virus infection, and was correlated with a reduction in virus replication in target organs. Free MTP-PE had little or no effect in these animal models of disease. In contrast, free MTP-PE was more effective than liposome-encapsulated MTP-PE in enhancing resistance to herpesvirus-induced encephalitis. This observation may be due to the ability of free MTP-PE to pass the blood-brain barrier and activate immune elements within the central nervous system.

W 103 Reversal of Drug-Sensitivity Profiles in Wild Type and Multidrug-Resistant MCF-7 Breast Cancer Cells by Liposomes Comprised of Cytotoxic Phospholipids. Marti Jett, Robert L. Fine, K.C. Cowan and B.A. Chabenr, Div. Medicine, Walter Reed Army Inst. Res. Washington, D.C. 20307-5100 and Clinical Pharmacology Branch, NCI, Bethesda, Maryland 20875 Multidrug-resistance is characterized by a broad spectrum of resistance to drugs of the natural product type. Cells exposed to one of these drugs (e.g. adriamycin) develop resistance, as a stable genetic trait, not only to that drug but to many other structurally unrelated drugs (e.g. colchicine, vinca alkaloids). The MCF-7 breast cancer cells were exposed to adriamycin in vitro and a stable cell line emerged which was 100-fold more resistant to adriamycin than were the wild type cells. We have found that liposomes comprised of phospholipids characterized as having antitumor properties reversed the order of sensitivity; the multidrug-resistant cells required a 10-fold lower concentration of drug to achieve IC_{50} than the wild type cells. This is the first drug which is more cytotoxic to multidrug-resistant than wild type cells, and may indicate that the cytotoxic phospholipids affect a pathway crucial to survival of multidrug-resistant cells. The two likely mechanisms were the signal transduction pathway and phospholipase A_2 activity, both known to be affected by presentation of cytotoxic phospholipids. The two cell lines were compared and striking differences were observed in the signal transduction cascade. Incubation with sublethal concentrations of the cytotoxic phospholipids casused the multidrug-resistant cells to resemble the wild type cells in their responsiveness to signal transduction. These data suggest that cytotoxic phospholipids may have therapeutic usefulness in the treatment of multidrug-resistant tumors.

W 104 ANTIBODY-TARGETED LIPOSOMES CONTAINING PHOSPHOLIPID DERIVATIVES OF METHOTREXATE : CYTOTOXICITY IN VITRO, Christine Noe (1), Jordi Hernandez-Borrell (1), Eiji Matsuura (2), Stephen C. Kinsky, (2) and Lee Leserman (1). (1) Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France. (2) National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

Antibody-bearing liposomes containing methotrexate have shown their capacity to specifically inhibit cell proliferation in vitro. The lability of these liposomes, as manifest by non-specific effects due to leakage, limit their in vitro and potential in vivo applications.

To reduce the loss of liposome contents, a phosphatidylethanolamine conjugate of methotrexate, MTX-γ DMPE, was incorporated into small liposomes. In an in vitro model system, these liposomes specifically inhibited the proliferation of murine tumor cells when targeted by moncolonal antibodies recognizing major histocompatibility-complex-encoded molecules. Evidence suggests that these liposomes are taken up by an endocytic process and that methotrexate is liberated intracellularly by phospholipases. These liposomes remain stable and retain their specificity under conditions, such as exposure to acids and repeated freezing and thawing, that induce the leakage of the water-soluble drug.

W 105 TREATMENT OF RETROVIRUS-INDUCED LYMPHOMATOUS MICE WITH DAUNORUBICIN AND AMPHOTERICIN B,FREE AND LIPOSOME-ENCAPSULATED FORMS, *Bijay K. Pal, Jill P. Adler,Daniel A. Guerra, and Jim Pieratos, Bio. Sci. Dept., Cal Poly Univ., Pomona , CA 91768.*

Wild mouse retroviruses produce splenic lymphoma and cause splenomegaly in wild as well as susceptible inbred mice. We standardized a wild mouse retrovirus (10A1)-induced splenic lymphoma model in NIH-Swiss mice and studied therapeutic efficacy of the anticancer drug, Daunorubicin (DN) in this system. The lymphomatous mice (5-7 mo.old) were treated for 3 weeks by i.v.injections of DN every 3 days at 5 and 15mg/kg dose levels. Our results showed that DN was effective in reducing lymphomatous spleen weight and viral antigen titer in spleen indicating successful lymphoma therapy. However, DN appeared to show severe cardiac damage. To alleviate inherent cardiotoxicity of free DN, it was encapsulated in multilamellar liposome composed of phosphatidyl serine, phosphatidyl choline and cholesterol (at a 3:7:10 molar ratio). Our results showed that ,at both therapeutic dose levels, the liposome-encapsulated DN was more effective , compared to the free drug, in reducing cardiac damage and treating the splenic lymphoma.

Since a frequent complication of patients with lymphoma is susceptibility to the opportunistic fungal infection, Candidiasis, we established the conditions for producing a lethal systemic Candidiasis in retrovirus-induced lymphomatous mice. One day post infection mice were treated (1x/day for 3 days) with 0.4mg/kg Amphotericin B (AmB) or 1.25 to 5mg/kg AmB multilamellar liposomes (phosphatidyl glycerol : phosphatidyl choline : AmB in a 7:3:1 molar ratio). Two weeks post infection , surviving mice were sacrificed and their kidneys assayed for colony forming units (CFU)/mg kidney. The results showed that the non-toxic dose 1.25mg/kg of AmB liposomes reduced CFU significantly more than the non-toxic dose (0.4mg/kg) of AmB ; 5mg/kg dose reduced the CFU to <1.0 and was also non-toxic. Additional treatment with immunostimulating mycoviral dsRNA liposomes did not improve treatment efficacy in these mice. In conclusion, the data showed that non-toxic dose of AmB liposomes alone could be used to very effectively treat established systemic Candidiasis in these mice.

W 106 LIPOSOMAL TUMOR ANTIGENS AS IMMUNOTHERAPY AGENTS, Nigel C. Phillips, Montreal General Hospital Research Institute, Montreal H3G 1A4, Canada. **A number of studies have demonstrated that liposomes can act as immunological adjuvants when proteins are incorporated within or attached to the external membrane. The ability of liposomes to act as immune adjuvants for a human tumor-associated antigen, CEA, has been evaluated in a xenogeneic assay system. Immunization of mice with CEA alone induced a small increase in the stimulation index (SI) of spleenocytes (2.0). Immunization with CEA in FCA or in liposomes resulted in increased SI's (2.6 and 4.3 respectively). Immunization with CEA in liposomes containing a lipophilic muramyl dipeptide - N-acetylmuramyl-L-alanyl-D-isoglutamyl-glyceroyl dipalmitate (MDP-GDP) - resulted in the highest SI (8.7). Mice immunized with CEA/liposomes/MDP-GDP had antibodies against CEA (<1/250 dilution): all other groups had antibody titers >1/32. The effect of administering liposomes containing autologous melanoma antigens to patients with stage III melanoma on peripheral blood lymphocyte mitogenesis has been evaluated. 15 patients received between 3 and 9 SC injections of liposomal tumor antigen preparations. No local or systemic toxicity was observed in any patient, and 4/15 patients demonstrated increased lymphocyte mitogenic responses to tumor antigens *in vitro* with a concomitant stabilization of disease progression. Liposomal therapy may therefore provide a means of optimizing the impact of tumor antigens on the immune system of patients with cancer.**

W 107 LIPOSOMAL ENCAPSULATED DOXORUBICIN - ITS ROLE IN CANCER CHEMOTHERAPY. A. Rahman, J. Treat, P. Woolley. Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington, DC 20007

Doxorubicin (Dx), an important antineoplastic agent, has treatment-limiting cardiotoxicity. To protect from cardiotoxicity and enhance therapeutic activity, Dx was encapsulated in cardiolipin liposomes. These Dx-liposomes substantially reduced the uptake of drug in mouse cardiac tissue compared to free drug. The drug concentration in plasma, liver and spleen was higher when administered in liposomes. Chronic cardiotoxicity studies in dogs at a cumulative dose of 245 mg/m^2 Dx were performed for 21 weeks. Dogs treated with Dx-liposomes exhibited complete protection whereas those treated with free drug suffered extensive myocardial damage. Antitumor evaluation of Dx-liposomes in P_{388} leukemia, Gross leukemia and advanced mammary carcinoma in mice demonstrated enhanced therapeutic effect with liposomal drug. Based on these preclinical studies, we performed a Phase I clinical trial of Dx-liposomes in patients with advanced cancer. Doses of Dx-liposomes were escalated from 30 - 90 mg/m^2. At 60 mg/m^2 of Dx-liposomes, 3 of 3 patients experienced no myelosuppression. At 90 mg/m^2, 2 of 5 patients experienced grade 4 neutropenia. Hence, MTD of Dx-liposomes was defined as 75 mg/m^2. Clinical pharmacology of Dx-liposomes at 60 mg/m^2 exhibited substantially altered pharmacokinetics. The peak plasma concentration of drug was 6.79 ug/ml with AUC being 26.72 ug.hr.ml^{-1}, with t 1/2 of 22.0 hrs, volume of distribution of 107.8 liters and clearance of 3.65 liters/hr. These studies in patients demonstrate that Dx-liposomes are very well tolerated and toxic manifestations are substantially reduced. Supported by LyphoMed Inc., Rosemont, IL.

W 108 EFFECTS OF LIFE LONG REPEATED ADMINISTRATION OF LIPOSOMES, LIPID A AND LIPOSOMAL LIPID A IN BALB/c MICE. Earl C. Richardson, Glenn M. Swartz Jr., James B. Moe, and Carl R. Alving, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Liposomes, lipid A, liposomes containing lipid A or saline were administered intravenously at six week intervals over the life-time of BALB/c mice. The liposomes contained dipalmitoyl phosphatidylcholine and cholesterol (1/0.75). There were no statistical differences between the treatment groups when longevity and body weights were compared. Animals that were injected with liposomes or liposomal lipid A progressively developed a visual appearance of "rough" fur when compared to the mice that received lipid A or saline. No other differences in appearance or vitality were seen. All mice that were tested from each group eventually produce antibodies to one or more of nine phospholipid antigens after 25.5 months. When half of the highly aged mice in each group had died from natural causes, the survivors were euthanized and detailed gross and histopathological examinations were made. Individuals in each of the four groups exhibited pathological changes in the liver, spleen or lung but none of the changes was identifiable primarily to a treatment group. Approximately half of the animals of each group had lymphoproliferative disorders (hyperplasia and/or lymphoma). No bone marrow adnormalities or splenic granulomatous reactions were found. We conclude that the chronic administration of liposomes, lipid A and liposomal lipid A does not produce changes in longevity or appearance of distinctive pathological effects in mice. Antibodies to lipid A and various phospholipids may occur naturally in mice injected with saline, but the long-term appearance of such antibodies was not enhanced by the use of liposomes.

W 109 BENEFICIAL EFFECTS OF DOXORUBICIN-CONTAINING LIPOSOMES IN CANCER CHEMOTHERAPY: MODE OF ACTION, G. Storm*, U.K. Nässander*, P.A. Steerenberg**, F.H. Roerdink***, J.H. Beijnen*, W.H. de Jong** and D.J.A. Crommelin*, * Dept. of Pharmaceutics, University of Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands, ** National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands, *** Lab Physiological Chemistry, University of Groningen, The Netherlands.

The application of liposomes as carrier system for chemotherapeutic agents has been proposed as a means to enhance the therapeutic value of the presently available cytostatics. One of the most promising examples is the encapsulation of doxorubicin (DXR) into liposomes. Several groups of investigators, including our group, have reported that the systemic toxicity and, specifically, the cardiotoxicity of DXR is significantly reduced while the antitumor activity is preserved after encapsulation of the drug into liposomes.
The precise mechanism responsible for the increased therapeutic index was unclear. However, insight into the mechanism was needed to improve liposomal chemotherapy by rationale and of great value when clinical applications are considered. Therefore, experiments were designed to elucidate the mode of action of liposomal DXR. Special attention was paid to the role of macrophages of liver and spleen which are known to take up the majority of i.v. administered liposomes. The results indicate that the beneficial effects of liposomal encapsulation of DXR are due to slow release of DXR from the liposomes, which occurs partly within the blood compartment and partly after uptake of the liposomes by macrophages. We concluded that a successful in vivo performance of liposome-encapsulated cytostatics heavily depends on the ability of drug molecules to 'escape' in active form from macrophages.

W 110 SITE-SPECIFIC ANTI-LEISHMANIAL EFFECT OF SODIUM STIBOGLUCONATE IN L.donovani INFECTED MICE, Carter, K.C.[1,2], Dolan, T.F.[1], Alexander, J.[2] and Baillie, A.J.[1], Pharmacy Department[1] and Immunology Division[2], University of Strathclyde, Glasgow, UK
The problems of multiple dose regimes and associated cumulative drug toxicity which compromise the treatment of visceral leishmaniasis (VL) in man are a direct consequence of the intracellular location of the parasite in the RES and the unfavourable pharmacokinetics of the organic pentavalent antimonials which are the drugs of choice. Carrier mediated therapy provides a novel means of manipulating drug distribution in vivo. The BALB/c mouse is genetically susceptible (non-cure) to VL and the diffuse location of the parasite in liver, spleen and bone marrow of the infected animal provide a stringent environment for the evaluation of the efficacy of drug delivery strategies. Using this mouse model infected with L.donovani we have studied the effect of treatment with free or vesicular (niosomal and liposomal) forms of stibogluconate on parasite numbers and their in vivo distribution. Although liver parasite burdens were readily suppressed with the equivalent of 40-50 $mgKg^{-1}$ Sb given i.v. as free drug and with a tenth of this dose in vesicular form, spleen and bone marrow burdens were apparently resistant to either form of the drug. Multiple dosing with free or vesicular drug had a minimal effect at these deep sites. Reducing the size of the drug loaded vesicles by sonication facilitated suppression of spleen but not bone marrow parasites. Livers of infected, drug treated mice were recolonised apparently by parasites from other foci of infection. Although bone marrow represents only 5% of the RES, a successful carrier mediated therapy of VL must address the problem of efficient drug delivery to this inaccessible reservoir of infection.

Liposomes in Infectious Diseases

W 200 EFFECT OF LIPOSOME-ENCAPSULATED MDP ON THE UPTAKE AND INTRACELLULAR SURVIVAL OF *LISTERIA MONOCYTOGENES* BY PERITONEAL MOUSE MACROPHAGES

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Macrophages in monolayer culture were exposed to free or liposome-encapsulated muramyl dipeptide (MDP) at various concentrations during different time periods before a 30-min incubation with *L. monocytogenes*; after uptake of bacteria the macrophages were reincubated during 6 hours. In macrophages not preincubated with MDP an average number of 5 bacteria per macrophage were taken up; the intracellular bacteria multiplied, and increased about 7-fold in number within 6 hours. An increase in bacterial uptake depending on the concentration of MDP and the period of exposure to MDP was observed. Exposure of macrophages to 200 µg of free MDP per ml during 15 hours led to a 3-fold increase in bacterial uptake; in addition, 50% of the intracellular bacteria were killed. Encapsulation of the MDP within liposomes (multilamellar vesicles, 0.4 µm in diameter, consisting of egg phosphatidylcholine/cholesterol/phosphatidylserine, molar ratio 4/5/1) led to a 1000-fold reduction in the amount of MDP required to obtain these effects: exposure of the macrophages during 15 hours to 0.2 µg of liposome-encapsulated MDP per ml led to a 4-fold increase in bacterial uptake, followed by killing of 80% of the intracellular bacteria. Empty liposomes had no effect on the uptake or killing of the bacteria. MDP or liposome-encapsulated MDP in itself was not bactericidal for *L. monocytogenes*.

W 201 LIPOSOME-ENCAPSULATED AMPICILLIN AGAINST *LISTERIA MONOCYTOGENES* IN VIVO AND IN VITRO

Irma A.J.M. Bakker-Woudenberg[1], August F. Lokerse[1], Joke C. Vink[1] and Frits H. Roerdink[2]
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Encapsulation of ampicillin within liposomes (multilamellar vesicles, 0.4 μm in diameter, consisting of egg phosphatidylcholine/cholesterol/phosphatidylserine, molar ratio 4/5/1) led to a 90-fold reduction in the amounts of ampicillin required to obtain a therapeutic effect in experimental infection caused by *Listeria monocytogenes* in mice. The mechanism by which liposomes improved the therapeutic activity of ampicillin appeared to be an increased delivery of the antibiotic to the site of infection, 56% in the liver and 23% in the spleen. Substantial amounts of liposomal ampicillin could be recovered from isolated Kupffer cells, the target cell of *L.monocytogenes* after intravenous inoculation. In studies on the survival of *L.monocytogenes* within mouse peritoneal macrophages in vitro it was found that liposome-encapsulated ampicillin killed almost all of the intracellular bacteria, whereas the same concentration of free ampicillin plus empty liposomes had no effect upon the intracellular bacteria. Changing the lipid composition of the liposomes into distearoylphosphatidylcholine/cholesterol/dipalmitoylphosphatidylglycerol, molar ratio 10/10/1, did not change the cellular uptake of the liposomes but resulted in a delayed degradation of the liposomes, and influenced the release of the encapsulated ampicillin intracellularly as reflected in absent or delayed intracellular killing of *L.monocytogenes*.

W 202 LIPOSOME-ENCAPSULATED GENTAMICIN (L-GENTAMICIN) TREATMENT OF MICE INFECTED ORALLY WITH SALMONELLA DUBLIN, Joshua Fierer, Paul Mihalko, and Annie Yau-Young, U.C. San Diego School of Medicine, V.A. Medical Center, San Diego, CA 92161, and Liposome Technology, Inc., Menlo Park, CA. Systemic salmonella infections are difficult to treat because the pathogens are intracellular and so protected from many antibiotics. We used experimental S. dublin infection to test the therapeutic efficacy of l-gentamicin (> 90% encapsulated in MLV). The in vitro sensitivity of the S. dublin to gentamicin (MIC) was 1.0 mg/L. Female Balb/c mice were infected by gavage with 2×10^7 colony forming units (CFU) in 0.1M $NaHCO_3$. Three days after infection mice were given one injection, either i.v. or i.p., of 2, 10, or 20 mg/kg of gentamicin or l-gentamicin. Controls received liposomes alone, or buffer, and they all died 6 to 10 days after infection. At 28 days after infection the mortality of l-gentamicin treated mice was 3/10, 2/10 and 0/10 in the three i.v. groups, and 8/10, 2/10 and 0/10 in the three i.p. groups. We autopsied three surviving mice from each i.v. treatment group four weeks after infection. The low dose treated mice had abscesses in mesenteric node which had $>10^7$ CFU/node. The other two groups had 3×10^2 CFU/node. Spleens from the three groups had a mean of 1.5×10^3, 8×10^2, and $<10^1$ CFU. Therefore, l-gentamicin treatment is more effective than free gentamicin, and i.v. route is more effective than i.p. against S. dublin. L-gentamicin effectively treats mesenteric adenitis, an important site of Salmonella infection acquired by the enteral route. (Supported by the VA, M.R.S., and NIH SBIR Grant No. N44-AI-62600 to Liposome Technology, Inc., Menlo Park, CA.)

W 203 PARASITE LIPIDS ALTER MEMBRANE PROPERTIES OF HOST CELLS, Stephen T. Furlong, David E. Golan, and John P. Caulfield. Harvard Medical School, Boston,, MA. 02115. The development of a vaccine against the human parasite Schistosoma mansoni has focused on surface antigen (Ag). However, the surface is comprised of two lipid bilayers and the Ag are shed by the parasite in vitro. Lipids associated with the shed Ag may influence the immune system. After biosynthetic labeling, only two phospholipids were released, phosphatidylcholine and lysophosphatidylcholine (LPC). Since the parasite fuses with neutrophils and lyses RBC's and since LPC alters membrane function, we compared properties of RBC's adherent to the parasite surface (a-RBC) with those treated with LPC. By fluorescence photobleaching recovery both lipid and protein probes were immobilized in a-RBC ghosts and in ghosts treated with 8.4 μg/ml LPC but not with other agents tested. Monopalmitoyl-PC (MPPC) was the predominant LPC produced by schistosomula and was the most effective of a series of synthetic LPC's of different chain lengths in causing lipid and protein immobilization in RBC's. The LPC mole fraction increased from 0.2 to 25% of the total RBC ghost lipid when treated with 0-16 μg/ml MPPC. The molar sterol/phospholipid ratio (exclusive of LPC) was inversely dependent on MPPC concentration, decreasing from 1.0 at 0 μg/ml to 0.7 at 8 μg/ml but not decreasing further at higher concentrations. These data suggest that parasite MPPC is transferred to a-RBC, causing their lysis. Immobilization of protein and lipid probes is due to a high concentration of MPPC in the RBC membranes but may also be influenced by decreased cholesterol content. MPPC may cause Ag shedding and influence the presentation of Ag to the immune system. These studies could help in designing liposomes to carry schistosome Ag for vaccines. Supported by NIH AI15311, HL32854, AI19581, AI23083

W 204 PHYSICAL CHARACTERIZATION OF LIPOSOMES BEARING AMPHOTERICIN B, Chris W.M. Grant and Kathryn R. Barber, Univ. of Western Ontario, London, Ont., Canada N6A 5C1.
Impetus to clarify structural details of liposomal amphotericin B at the molecular level comes from a variety of sources. We have approached this problem using two major physical techniques: EPR spectroscopy to monitor the effect of temperature variation on drug/liposome molecular organization, and freeze-etch electron microscopy to visualize drug/liposome structure to a resolution of 2.5 nm. We chose an unsaturated phospholipid, dielaidoyl phosphatidylcholine, as well as several saturated phospholipids including the 7:3 (mol ratio) mixture of dimyristoyl phosphatidylcholine/dimyristoyl phosphatidylglycerol for study. Phase diagrams were derived for the different lipids with amphotericin B from 0 to 25%. Phase diagrams for dielaidoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine were very similar to one another in spite of their different protective properties regarding amphotericin B toxicity. Phase diagrams of amphotericin B with dimyristoyl phosphatidylcholine and its mixture with phosphatidylglycerol showed evidence of greater dispersion of drug into the bilayer membrane. The latter may reflect a difference in behaviour of shorter chain phospholipids. Fluidity of (unprotective) dielaidoyl phosphatidylcholine membranes containing drug was found to be the same as that of some (protective) saturated bilayers. These data correlated with liposome appearance in the electron microscope.

W 205 SELECTIVE KILLING OF HUMAN IMMUNODEFICIENCY VIRUS-PRODUCING CELLS BY LIPOSOMES CONTAINING DIPHTHERIA TOXIN FRAGMENT A, Kazuyoshi Ikuta, Shigeharu Ueda, Tsuyoshi Uchida, Yoshio Okada and Shiro Kato, Osaka University, Suita, Osaka 565, Japan
Acute lymphocytic leukemia-derived cell lines, such as MOLT-4 or TALL-1, are susceptible to human immunodeficiency virus (HIV), but can be passaged as continuous producers of HIV. Liposomes containing fragment A of diphtheria toxin [Lip(Fr.A)] were used to selectively kill HIV-producing cells. Diphtheria toxin consists of two functional domains, fragment A and fragment B. Fragment B is responsible for binding and entry of the toxin fragment A into susceptible cells, and so fragment A alone is not toxic to the cells. In this work, fragment A was obtained from the C7(ß22) strain of Cornebacterium diphtheriae, which produces only fragment A. Lip(Fr.A) did not kill HIV-uninfected MOLT-4, and had only a slight lethal effect on TALL-1, but had definite lethal effects on all MOLT-4 and TALL-1 cells producing HIV continuously. The sensitivities of the various HIV strains to lip(Fr.A) differed. On treatment with lip(Fr.A) the cell numbers decreased to 2-3 % of those of cells treated with the four controls, 1) empty liposomes, 2) fragment A, 3) a mixture of 1) and 2), and 4) PBS. This killing was not affected by the presence of HIV antibodies. These results showed that lip(Fr.A) selectively killed cells expressing HIV antigens or producing the virus in vitro. A possible explanation for the selective killing is that lip(Fr.A) could not reach the lipid bilayer of the plasma membrane of uninfected cells, but could selectively reach the lipid bilayer on the surface of HIV-producing cells as a result of a modulation of the cell surface by infection with HIV and/or production of HIV.

W 206 INFLUENCE OF PROTEIN CONTENT AND LIPOSOMAL BILAYER COMPOSITION ON THE IMMUNOGENICITY OF LIPOSOMES AND ISCOMS CONTAINING THE MAJOR OUTER MEMBRANE PROTEIN PI OF NEISSERIA GONORRHOEAE. Gideon F.A. Kersten, Anne-Mary v.d. Put, Tom Teerlink, E. Coën Beuvery and Daan J.A. Crommelin. National Inst. for Public Health and Environmental Hygiene, Bilthoven and University of Utrecht, the Netherlands.
The influence of epitope density, cholesterol content and the phase transition temperature Tc of the phospholipids on the IgG response against PI containing liposomes was studied and compared with iscom preparations differing in their protein: saponin ratio. Cholesterol content, Tc of the phospholipids as well as the overall membrane fluidity influenced the primary response. After a booster injection these effects disappeared, indicating that the immunological memory was not affected by these variables.

W 207 PROTECTIVE ACTIVITY OF LIPOSOMAL MURAMYL DIPEPTIDE IN MICE WITH GENETIC DEFECTS IN ANTI-INFECTIOUS ACTIVITY. **Nigel C. Phillips, Montreal General Hospital Research Institute, Montreal H3G 1A4, Canada.** **A/J mice possess a genetic defect in their ability to mount an inflammatory response against Listeria monocytogenes (Lm), and CBA/N mice possess a sex-linked defect in their ability to raise antibodies against gram-negative bacteria. The effect of therapy with liposomes containing a lipophilic muramyl dipeptide-N-acetylmuramyl-L-alanyl-D-isoglutamyl-glyceroyl dipalmitate -(MDP-GDP) on the ability of these mouse strains to recover from lethal challenges of Listeria monocytogenes or Salmonella or Streptococci has been determined. Liposomal MDP-GDP was significantly more effective than free MDP in protecting A/J mice from Lm infection (15-fold increase in potency). Liposomal MDP-GDP was also more active than free MDP in reducing bacteria counts in the liver and spleen of infected mice. *In vitro* studies showed that macrophages from A/J mice had increased phagocytic and Listericidal activity after exposure to liposomal MDP-GDP. Treatment of CBA/N mice with liposomal MDP-GDP also resulted in increased resistance to infection with gram-negative bacteria. Liposomal MDP-GDP was 10 fold more potent than free MDP. Treatment of CBA/N mice with liposomal MDP-GDP resulted in an enhanced clearance of bacteria from the blood compared to free MDP. The ability of liposomal MDP-GDP to induce anti-infectious activity in genetically susceptible mice would appear to depend on a stimulation of tissue-fixed macrophage defense activities.**

W 208 MARKED REDUCTION IN THE IN VIVO ACTIVITY OF BACTERIAL ENDOTOXIN BY LIPOSOME INCORPORATION, John W. Mellors, Jan Dijkstra, John L. Ryan,
Yale Univ. School of Medicine, Veterans Administration Medical Center, West Haven, CT 06516.

Bacterial endotoxin, a major component of the outer membrane of gram-negative bacteria, elicits many of the pathological sequelae of gram-negative sepsis. The precise mechanisms by which endotoxin triggers inflammatory and immune responses are unknown, but macrophages appear to be the primary target cells. Previously we have shown that incorporation of endotoxin into liposomes results in a 100 to 1000-fold reduction in its ability to stimulate murine macrophage interleukin-1 secretion and Fc-receptor mediated phagocytosis in vitro (J Immunol 1987; 138: 2663). To determine whether liposomal delivery alters endotoxin activity in vivo, we incorporated lipid A, the active component of endotoxin, into multilamellar vesicles composed of egg-phosphatidylcholine /bovine-brain phosphatidylserine /cholesterol in a molar ratio of 4:1:4, and compared the activity of lipsomal lipid A with that of free lipid A by assessing peritoneal macrophage activation 4 days after intrapertioneal injection of the preparations into Balb/c mice. Macrophage activation was measured by determining phorbol myristate acetate stimulated H_2O_2-release from freshly harvested cells. Liposomal lipid A (10-0.01 ug in 2.5 umole total liposomal lipid/mouse) was 100-fold less active than free lipid A (10-0.001 ug/mouse). The dose per mouse of lipsomal lipid A required to activate peritoneal macrophages was 10 ug compared with 0.1 ug for free Lipid A. These data indicate that liposome incorporation profoundly affects the interaction of lipid A with macrophages in vivo. Further study of the effects of liposome incorporation on the activity of lipid A may provide insight into the mechanisms of endotoxin action in vivo.

W 209 Lipofection: An Efficient Lipid Mediated Transfection Procedure.
Philip L. Felgner and Marilyn Holm. Institutes of BioOrganic Chemistry and Cancer and Developmental Biology, Syntex Research, Palo Alto, Calif. 94304
A novel transfection protocol has been developed which makes use of a newly synthesized cationic lipid, DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N,-trimethylammonium chloride). Small unilamellar liposomes, containing a mixture of DOTMA and dioleoylphosphatidyl ethanolamine (PE; 50:50), interact spontaneously with DNA to form complexes with 100% entrapment of the DNA. DOTMA facilitates fusion of the complex with the plasma membrane of tissue culture cells resulting in both uptake and expression of the DNA. Replacing the PE with phosphatidylcholine in the liposomes inhibits the transfection; this observation supports the fusion mechanism. The technique, termed lipofection, is simple highly reproducible and effective for both transient and stable expression of transfected DNA. Depending upon the cell line, lipofection can be greater than 100 fold more effective than either the calcium phosphate or the DEAE-dextran transfection techniques. Experiments using fluorescent DNA and fluorescent lipid show that lipofection delivers both DNA and lipid to 100% of the cells exposed to the reagent. Radiolabeled DNA indicates that about 10% of the input DNA, or 100,000 copies of plasmid, are delivered per cell; probing the nuclear extract reveals 300 copies per nucleus. Liposomes comprised of either stearylamine or dimethyl-dioctadecylammonium, instead of DOTMA, are inactive in the transfection assay. Stearylamine liposome are significantly more toxic to tissue culture cells than DOTMA liposomes. Lipofection is inhibited by negatively charged liposomes and fetal calf serum.

Late Additions

W 210 PHASE I STUDY OF MTP-PE ENCAPSULATED IN LIPOSOMES. P.J. Creaven, D.E. Brenner, J.W. Cowens, B. Dadey, R. Huben, C. Karakousis, S. Arbuck, M.K. Cushman, T. Han, K. Andrejcio. Roswell Park Memorial Institute, Buffalo, NY and Ciba-Geigy, Summit, N.J. Twenty five patients (pts) with advanced malignancy have received 34 evaluable courses of MTP-PE encapsulated in liposomes. Each course consisted of 8 twice weekly doses. There was no intrapatient escalation. Doses of 0.01-1.2 mg/m² have been explored. Acute toxic manifestations included fever (68%), rigors (41%), tachycardia (32%), tachypnea (29%), nausea and vomiting, hypertension, hypotension (26% each), headache, fatigue, vertigo, anorexia (<20%) (percent of evaluable courses in parentheses). Skin rash, possibly drug related, was seen in 2 cases. In 1, at a dose of 0.05 mg/m², a biopsy revealed vasculitis Fever was most marked after the first dose (median maximum 38.2°, range 37.3°-40.4°). After doses 2-8 median maximum was 37.3°-37.5° (range 36.0°-39.5°). None of the toxicities appear to be clearly dose related. There were no consistent changes in hematologic or blood chemistry parameters, acute phase reactants or immunoglobulins. Macrophage activation by a cytotoxicity assay was measured before and 24h after drug administration (generally doses 1,3,5 and 7) and was highly variable. No significant trends could be detected following treatment. For dose 1, cytotoxicity was a median of 16.1% pretreatment (range 0-58.2%) and 19.1% at 24h (range 0-58.9%, n=24). For the last dose measured (5-8) it was a median of 15.9% pretreatment (range 0-70.2%) and a median of 22.2% at 24h (range 0-50.2%, n=20). The study is continuing. The current dose being evaluated is 1.8 mg/m².

W 211 ACTIVATION OF HUMAN MONOCYTE-MACROPHAGES TO THE TUMORICIDAL STATE BY LIPOSOMAL MACROPHAGE ACTIVATORS AND ITS CLINICAL IMPLICATION, Saburo Sone, 3rd Dept. of Internal Medicine, University of Tokushima School of Medicine, Tokushima, Japan.
The significance of activation of the antitumor properties of human monocyte-macrophages by macrophage activators encapsulated in liposomes and its effector mechanism is discussed. Human alveolar macrophages and blood monocytes can be activated to the tumoricidal state by incubation with liposomes containing muramyl dipeptide (MDP) or its lipophilic analog (MTP-PE) in vitro. Macrophages activated by this procedure destroy allogeneic tumor cells, but leave nontumorigenic cells unharmed. MDP or its lipophilic otherwise MTP-PE encapsulated in multilamellar vesicle (MLV) liposomes activated human monocyte- macrophages at lower concentrations than free MDP and also maintained the activated state for a longer period than free MDP. Tumoricidal monocytes induced by liposomal MTP-PE did not release monokines such as IL-1 and tumor cytotoxic factor (TCF) into their culture supernatant.
Recombinant human interferon (IFN) alpha, beta and tau also rendered human monocytes tumoricidal, but IFN tau had a much higher synergistic effect than IFN alpha or IFN beta with soluble MDP analog for monocyte activation. Similarly, a combination of IFN tau and liposome-entrapped MTP-PE at suboptimal concentrations also induced synergistic activation of monocytes to the tumoricidal state. Thus, encapsulation of macrophage activators in liposomes may be a valuable approach in in situ activation of monocyte-macrophages for treatment of cancer metastases in humans.