RATIONAL BASIS FOR CHEMOTHERAPY
Bruce Chabner, Organizer
April 18 – 23, 1982

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Immunologic Advances in Diagnosis, Staging, and Specific Targeting of Cancer Treatment

1016 MONOCLONAL ANTIBODY THERAPY OF MURINE LEUKEMIA. Irwin D. Bernstein, M.D., Pediatric Oncology Program, Fred Hutchinson Cancer Research Center and Department of Pediatrics, University of Washington, Seattle, WA 98104
The development of monoclonal antibody techniques has revitalized interest in the antibody therapy of cancer. With these techniques, virtually unlimited amounts of biologically relevant antibodies potentially useful for therapeutic purposes can be obtained. These homogenous antibodies have been exploited for the antibody therapy of mouse leukemias in vivo. Treatment of leukemia with monoclonal antibodies against a normal differentiation antigen has induced prolonged survival and cure in a significant proportion of treated animals. In these studies, monoclonal antibodies of different immunoglobulin isotypes with specificity against the normal T cell antigen, Thy 1.1, have been used to treat transplanted and spontaneous T cell leukemias. The results of studies examining the effectiveness of these antibodies, their mechanism of action, their biodistribution in the host, and factors which may limit antibody effectiveness will be described.

1017 IN VITRO GROWTH OF HUMAN CYTOTOXIC LYMPHOCYTES: IMPLICATIONS FOR THE IMMUNOTHERAPY OF CANCER, Steven A. Rosenberg, National Cancer Institute, Bethesda, MD 20205
Application of adoptive immunotherapy to the treatment of cancer in humans has been limited by the inability to obtain sufficient numbers of specifically sensitized lymphoid cells. The recent development of techniques for expansion of human lymphoid cells in vitro to large numbers using T cell growth factor (TCGF) has provided new possibilities for identifying, isolating, and expanding autologous human lymphoid cells cytotoxic for tumor. In this presentation we will summarize our efforts to develop lines of cytotoxic T lymphoid cells suitable for use in the adoptive immunotherapy of cancer in man. We have developed techniques for the production and partial purification of TCGF in both mouse and human systems. We have demonstrated in the human and in the mouse, that alloantigen reactive cytotoxic T cell lines and T cell clones can be maintained and expanded in TCGF for many months after sensitization with no loss of cytotoxic specificity. Further studies have demonstrated that these cytotoxic T cell lines are capable of mediating immunologic functions in vivo. Murine lymphoid lines with specificities to alloantigens are capable of accelerating the rejection of allogeneic skin grafts when lymphoid cells are injected intravenously one day after applying an allogeneic graft. Cytotoxic T cell lines and clones have been developed with specific cytotoxic reactivity to selected murine tumors. Studies using a murine lymphoma, FBL-3, have demonstrated that appropriately sensitized lymphocytes which are subsequently expanded in TCGF are capable of curing mice with disseminated lymphoma. These results have encouraged us to initiate studies of adoptive immunotherapy for human cancer. Cells from individuals with cancer when expanded in TCGF develop the capability to kill autologous fresh tumor but not normal cells. This tumor lysis appears to be a characteristic of activated human lymphocytes. Current studies are underway to analyze the mechanism of this tumor killing and to define both the cell population responsible for killing as well as to identify the characteristics of the tumor cell surface against which this cytotoxicity is directed. Preliminary experiments exploring the in vivo injection of cells expanded in TCGF into humans have been performed. Three patients have been infused with up to $5x10^8$ autologous T lymphoid cells expanded in human TCGF. The traffic of these injected autologous cells has been analyzed by labelling the cells with Indium-111. Radionuclide scanning and gamma camera imaging techniques were used to identify the organ localization of injected cells. These cells distribute initially to the lungs and then are slowly cleared over the course of one to two weeks to the liver and spleen. The safety of the injection of these autologous cells has been demonstrated in initial Phase I studies and no adverse effects have been seen in humans that have been infused with these cell preparations. We are currently attempting to isolate and expand autologous human lymphoid cells, specifically cytotoxic against human tumors. It is hoped that sufficiently sensitized and expanded cytotoxic T lymphoid cells may be of value in the future immunotherapy of cancer in man.

Determinants of Steroid Hormone Response and Resistance

1018 GENETIC DETERMINANTS OF THE GLUCOCORTICOID RESPONSE IN LYMPHOID CELL LINES, Suzanne Bourgeois and Judith C. Gasson, The Salk Institute for Biological Studies, Regulatory Biology Laboratory, San Diego, California 92138.

The mouse thymoma line, WEHI 7.1 (W7), is lysed by glucocorticoids. All resistant variants induced by classical mutagens were shown to result from defects in the glucocorticoid receptor.[1] To increase the probability of inactivating another function involved in the response, we have turned to treatments by antitumor drugs known to induce large deletions and chromosome rearrangements or elimination. Mitomycin C, bleomycin, streptonigrin, colcemid and BD40 (an analog of ellipticine) were shown to be mutagenic in that they increased the frequency of glucocorticoid resistant variants.[2] Variants induced by mitomycin C, streptonigrin, colcemid and BD40 showed greatly reduced binding of dexamethasone to the glucocorticoid receptor. Bleomycin and mitomycin C plus dexamethasone induced variants with high glucocorticoid binding activity but reduced capacity to translocate the receptor-steroid complex into the nucleus. Thus, all these antitumor drugs also induce glucocorticoid receptor defects.

These results raise the possibility that, in combination therapies, antitumor drugs might induce glucocorticoid resistant lymphoid cell variants that could be selected by the presence of the hormone. Also, this study brings to a total of approximately 300 the glucocorticoid-resistant variants induced by a wide variety of mutagens in the W7 line. The fact that all these variants result from receptor defects is highly significant and raises the question of whether a function other than the receptor is involved in the cytolytic response.

We have obtained genetic evidence for the role of at least one other function by studying a different lymphoma line, SAK8. This line, derived from a spontaneous thymic lymphoma in an AKR mouse, is resistant to glucocorticoids, although containing normal receptor as determined by binding assays. This line was fused with a receptor-defective variant of W7. The hybrids obtained by fusion of these two glucocorticoid-resistant lines are lysed by the hormone. This phenomenon of complementation between two resistant lines further demonstrates that the receptor of the SAK8 line is functional and that this line is resistant to dexamethasone because of a defect in another locus (or loci) designated "l" for lysis. (Supported by NIH Grant #GM20868 and Fellowship #AM06179 and by a grant from the Whitehall Foundation.) 1. Bourgeois, S., Newby, R.F. and Huet, M. Cancer Research 38, 4279-4284, 1978.
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1019 RECEPTOR AND POST-RECEPTOR ABNORMALITIES IN PATIENTS WITH ANDROGEN RESISTANCE, *James E. Griffin, Department of Internal Medicine, University of Texas Health Science Center, Dallas, TX 75235*

Hereditary defects that impede androgen action frequently cause resistance to the hormone both during later life and during embryogenesis and hence frequently cause developmental defects of the male urogenital tract. Such defects in genetic men produce a phenotypic spectrum ranging from infertile but otherwise normal men to individuals with varying degrees of ambiguous genitalia to phenotypic women (1). In molecular terms, these disorders can be classified on the basis of the step in androgen action that is affected by the individual mutations. 5α-Reductase deficiency is an autosomal recessive enzyme defect that impairs the conversion of testosterone to dihydrotestosterone. As a consequence the internal male genital tract virilizes normally, but the external genitalia are predominantly female in character. A variety of disorders influence the androgen receptor that mediates the action of both testosterone and dihydrotestosterone. At least four phenotypic variants can be distinguished — complete testicular feminization, incomplete testicular feminization, the Reifenstein syndrome, and the infertile male syndrome, each of which is inherited as an X-linked trait. Absence of binding is found commonly in the phenotype of complete testicular feminization, but qualitative and/or quantitative defects in receptor function can be associated with all four variants. The quantitative defect is a partial decrease in the amount of binding of an apparently qualitatively normal receptor (2). Qualitative defects include thermolability (2-4), failure of stabilization by sodium molybdate (5), and altered affinity (3). A third type of disorder — termed receptor positive resistance — also causes a spectrum of defects in male development and is associated with normal 5α-reductase activity and normal levels of androgen receptor. The underlying defect is presumed to lie at the intranuclear site or sites of action of the hormone-receptor complex. Recent studies suggest that abnormal regulation of receptor number may be evidence for a post-receptor defect in some patients with receptor positive androgen resistance (6).

Since normal androgen action is essential for reproduction but not for the life of individuals and because even slight abnormalities in androgen actions are usually manifested by anatomic and/or functional abnormalities (and hence come to the attention of physicians frequently) the syndromes of androgen resistance provide a remarkable opportunity to utilize single gene mutations for the simultaneous elucidation of the normal pathway of action of a hormone and of the various types of receptor and post-receptor defects which might be present in other steroid receptor systems.

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1020 CEM CELLS: A PARADIGM FOR GLUCOCORTICOID ACTION IN HUMAN ACUTE LYMPHOBLASTIC LEUKEMIA, E. Brad Thompson, Jeffrey M. Harmon, Michael R. Norman, Thomas J. Schmidt, and Robert Zawydiwsky, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, Maryland 20205

Examination of abnormal cells from patients with various leukemias has shown that frequently the simple presence or absence of glucocorticoid receptor sites does not predict steroid sensitivity. Most patients have receptors regardless, a result not predicted by existing rodent cultured cell systems. For this reason we have sought a human model system in which to study glucocorticoid effects on sensitive cells, as well as the mechanism(s) by which resistance to steroids develops. The CEM cell line of childhood ALL cells, bearing T cell markers has proved useful for these purposes. From this line we have isolated both glucocorticoid-sensitive and -resistant clones.

We have shown that spontaneous resistance from a sensitive, near-diploid clone of CEM cells occurs as separate, independent (mutational?) events at a rate of about 10^{-5} per cell per generation. The resistant clones are stable. All possess glucocorticoid receptors in amounts reduced from those in the wild-type, sensitive parent, with normal or near-normal steroid affinity. These receptors fail to "translocate" to cell nuclei as well as do wild-type. In vitro, the receptors from resistant cells show abnormal lability, and at least some display a unique phenotype we have termed "activation labile" (r^{actl}).

Sensitive clones are killed by glucocorticoids, and continuous exposure to receptor-saturating concentrations of steroid for one to several days is required. CEM-C7, the most thoroughly studied sensitive clone, requires exposure to steroid for 24 $\pm$ 6 hr before cell killing commences. Cells then increasingly accumulate in G_1 phase, in which they die. Treatment with drugs which reversibly block cells in S phase protects against steroid.

Exogenous mutagenesis of CEM-C7 cells increases the rate at which resistant clones appear. The predominant phenotype of mutagen-treated, steroid-resistant cells is that of absence of glucocorticoid receptors (r°). This is similar to the phenotype most frequently seen in rodent model systems and is in sharp contrast to that of the spontaneously resistant CEM cells (see above).

CEM cells have revealed a potential marker for functional glucocorticoid receptors in human leukemias. Glutamine synthetase (GS) is induced in these cells prior to cell death. With one exception, it is not inducible in steroid-resistant clones. Assay of GS may thus allow assessment of potential response to steroid therapy by use of a simple enzyme assay requiring relatively few cells. It has been shown to be inducible in Sézary syndrome cells.

Determinants of Drug Action

1021 CELLULAR GENETICS OF DRUG RESISTANCE, June L. Biedler, Robert H. F. Peterson, Peter W. Melera*, Marian B. Meyers, and Barbara A. Spengler, Laboratories of Cellular and Biochemical Genetics and *RNA Synthesis and Regulation, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Acquisition of resistance to a wide variety of antibiotic and chemotherapeutic agents by prokaryotic and eukaryotic cells is a well-known biologic phenomenon. However, only recently has the genetic basis of drug resistance development in diploid mammalian cells yielded to molecular analysis. Studies of antifolate-resistance Chinese hamster lung cells have shown that sublines with high target enzyme dihydrofolate reductase (DHFR) levels have specifically altered, homogeneously staining regions of metaphase chromosomes known as HSRs, whereas low enzyme lines have distinctive, abnormally banded chromosome segments (1). Resistant sublines contain selectively amplified DHFR genes (2) clustered in both HSRs and the abnormally banded regions of sublines with low DHFR gene copy numbers (3). Studies of highly resistant Chinese hamster lung cells selected with actinomycin D, daunorubicin, or vincristine revealed that the vincristine-resistant cells likewise contain an HSR (4). We have also found HSRs or double minute chromosomes (DMs), alternative aberrant chromosome structures indicative of gene amplification, in vincristine-resistant mouse and human cell lines (3) and have demonstrated that a 19 KD acidic protein (V19) or analogous peptides are oversynthesized in resistant cells and are barely detectable in control and actinomycin D-resistant HSR-lacking cells (5). The Chinese hamster sublines with acquired resistance to actinomycin D, daunorubicin and vincristine display decreased permeability to drug and mutual cross-resistance (4). These variously resistant cells have qualitatively and quantitatively similar alterations in plasma membrane composition, notably a relative increase in a 150 KD glycoprotein species and a decrease in a 100 KD species coinciding with increase in resistance and reversion from a malignant to a more normal cell phenotype (4). The apparently nonspecific plasma membrane alterations, the size of the vincristine-specific HSR, and synthesis of V19 all decrease with time in drug-free medium. Understanding of the potential role of gene amplification in these cellular events will aid in identifying determinants of these drug resistant phenotypes.

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1022 IMPROVED MARROW TOLERANCE OF CHEMOTHERAPY BY GENE INSERTION TECHNIQUES, Martin J. Cline, Menashe Bar-Eli, Howard D. Stang, and Karen E. Mercola, Department of Medicine, University of California, Los Angeles, CA 90024

Gene sequences coding for a mutant mouse dihydrofolate reductase (DHFR) have been introduced into mouse hematopoietic cells by DNA-mediated gene transfer. The mutant DHFR was obtained from a methotrexate(Mtx)-resistant mouse cell line (3T6R1) and was characterized by altered Mtx affinity and electrophoretic mobility. High-molecular-weight DNA was extracted from 3T6R1 cells, precipitated with calcium phosphate, and incubated with bone marrow cells from CBA mice. These cells were then injected into irradiated syngeneic recipient animals. After 30 days, mice receiving marrow transformed with DNA from Mtx-resistant 3T6R1 cells demonstrated reduced hematopoietic suppression from Mtx and had Mtx-resistant DHFR in hematopoietic cells but not in other tissues. Mutant DHFR was identified by its Mtx resistance and mobility on two-dimensional gels.

Insertion of drug resistance genes into hematopoietic stem cells offers the possibility of enhancing the therapeutic-to-toxic ratio of certain anticancer drugs.

Physical and Biochemical Targeting of Antineoplastic Therapy

1023 BIOCHEMICAL MECHANISMS RESPONSIBLE FOR THE SENSITIVITY OF T-LYMPHOCYTES TO PURINE NUCLEOSIDES, Michael S. Hershfield, Departments of Medicine and Biochemistry, Division of Rheumatic and Genetic Diseases, Duke University, Durham, N.C. 27710

Genetic deficiency of adenosine deaminase (ADA) causes a form of severe combined immunodeficiency disease in which selective, profound lymphopenia is thought to result from lymphocytotoxic effects of the naturally occurring ADA substrates, adenosine (Ado) and 2'-deoxyadenosine (dAdo). This experiment of nature and the information gained in studies of its pathogenesis, have provided a compelling rationale for attempts during the past 3-4 years to use a potent ADA inhibitor, 2'-deoxycoformycin (dCF, Pentostatin) in the treatment of lymphoid malignancies. More recently clinical trials have begun of the combination of dCF and adenine arabinoside (Ara-A), a substrate analog for ADA. Responses and occasional remissions have been obtained, primarily in patients with refractory T-cell acute lymphoblastic leukemia. Selective depletion of non-malignant circulating lymphocytes has also been observed in patients with non-lymphoid malignancies who have received the drug. However, non-lymphoid toxicity (CNS, hepatic, renal, pulmonary) has been encounted in some patients, and this experience may limit the use of dCF unless the factors responsible for toxicity can be identified and avoided. I shall review aspects of the biochemical actions and metabolism of Ado, dAdo, and Ara-A that may determine the nature of the response to treatment with dCF. The following biochemical effects will be discussed: 1) The much greater efficiency of T-like than B-like lymphoblasts in accumulating dATP (or Ara-ATP), which is responsible for the greater sensitivity of T-lymphoblasts to the cytotoxic effects of dAdo (or Ara-A). 2) The ability of Ado to inhibit, and of dAdo and Ara-A to act as suicide-like inactivators of S-adenosylhomocysteine (AdoHcy) hydrolase. Accumulation of AdoHcy, a potent inhibitor of many transmethylation reactions, may contribute to the hepatic and CNS toxicity observed with dCF alone, or with the combination of dCF and Ara-A. 3) Recent studies from our laboratory of the mechanism by which accumulation of dATP causes ATP depletion in the lymphoblasts and erythrocytes of patients undergoing treatment with dCF. Among the metabolic factors to be discussed are 1) The ability of dCF to cause long lasting, virtually stoichiometric inhibition of ADA 2) The importance of inhibition of ADA in non-lymphoid tissues in permitting plasma concentrations of ADA substrates to be maintained at sufficient levels and for sufficient time to acheive their effects in malignant lymphoblasts 3) The extraordinarily efficient renal clearance of ADA substrates which limits the plasma levels of these nucleosides, a corollary of which is that any limitation in renal function can cause plasma concentrations of ADA substrates to rise to levels which are capable of causing toxicity to non-lymphoid organs 4) The effect of cell lysis in contributing to sudden rises in plasma dAdo to toxic levels.

1024 PHYSICAL TARGETING OF ANTI-TUMOR THERAPY: THE INTERACTIONS OF HEAT AND DRUG, George M. Hahn, Dept. of Radiology, Stanford University School of Medicine, Stanford, CA94305

Elevated temperatures, induced either locally in tumor volumes, or systemically over regions or over the whole body, can affect in a variety of ways the efficacy of anti-cancer drugs. On a physiological level, blood flow rates in tumors and in normal tissues can either be decreased or increased, depending upon temperature and duration of heating. In general, at temperatures below about 43°C, blood flow rates in both types of tissues are increased significantly over 37°C controls; at higher temperatures a profound inhibition of blood flow occurs in many tumors but is not seen in normal tissues. Such modulations of blood flow can greatly modify drug pharmacokinetics.

At the cellular level, heat can not only influence drug delivery, it can also affect cytotoxicity (1). Elevated temperatures change the permeability of plasma membranes, allowing at least initially more of some drugs to enter the cell. For example, cells treated with adriamycin for periods of 30 min or less are appreciably more sensitive to the drug at 43°C than at 37°C (2). With increasing time, however, the cells react to heat in a manner not currently understood, and as a consequence their membranes become very impermeable to adriamycin. Studies with misonidazole indicate that at or near 43°C a thermotropic transition modifies the cells' membranes so that above that temperature much more of this agent enters the cells than below (3).

Some cytotoxic reactions, particularly alkylations, are speeded up at elevated temperatures, resulting in increased cell killing. This is true, for example, for the nitrosoureas, and dose-response curves obtained with these drugs over the range of 37°C-45°C show progressively increasing slopes (4). Dose modifying factors of 5 and even larger are seen at the higher temperatures. Heat can also interfere with cellular recovery processes; this can be demonstrated with bleomycin (5). Finally, some drugs that are relatively benign at 37°C become potent cell killers at elevated temperatures. An example here is amphotericin B (6). Interestingly, dose-response curves for bleomycin and amphotericin B do not change much between 37-41°C, but differ appreciably at 43°C. Above that threshold temperature, dose modifying factors of 10 or larger are routinely observed.

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1025 TWO STRATEGIES FOR TARGETED DELIVERY OF DRUGS IN LIPOSOMES, John N. Weinstein (1), Richard L. Magin (2), Lee D. Leserman (3). 1. LTB, NCI, NIH, Bldg 10, Rm 4B-56, Bethesda, Md 20205; 2. Dept Elect. Eng., U. Ill. at Urbana-Champaign, Urbana, Ill. 61801; 3. Centre d'Immunologie de Marseille-Luminy, Case 906, 13288 Marseille, France

Liposomes can be thought of as microscopic "capsules" composed of lipid bilayers, with enclosed aqueous spaces. Lipid-soluble drugs can be incorporated into the lipid and water-soluble drugs into the aqueous compartments (1). Over the last several years we have explored the possibility that liposomes bearing an appropriate antigen or antibody could be "targeted" for particular cell types. Liposomes bearing the dinitrophenyl hapten (2,3) bound to cells with high specificity in several experimental systems but were internalized in significant numbers only when receptor-mediated endocytosis was possible. Methotrexate (MTX) encapsulated in the liposomes escaped the phagolysosomal apparatus after endocytosis and reached its site of action in the cytoplasm. We have also coupled immunoglobulin (and other proteins) to the liposomes covalently (4) by means of a heterobifunctional cross-linking reagent, SPDP. Liposomes bearing covalently coupled monoclonal antibody bind with high specificity and, in some cases, deliver their contents to the interior of the cell.

To avoid some of the obvious difficulties of ligand-mediated targeting in vivo, we wanted an approach which did not require that the liposome bear an immunogenic ligand, cross endothelial barriers, or interact directly with target cells. We therefore designed "temperature-sensitive" liposomes with a lipid phase transition temperature (T_m) of about 42°C — a temperature accessible by mild local hyperthermia (5,6). These liposomes are quite stable below T_m but are broken down at T_m by serum components (principally the lipoproteins) within at most a few seconds, therefore exposing local tissues to higher concentrations of free drug. When injected i.v. in mice with subcutaneous L1210 tumors, they delivered 14 times as much ^{3}H-MTX to tumors heated to 42°C as to unheated tumors in the same animals. Heating the tumor did not increase accumulation of MTX in other organs, suggesting that an increase in therapeutic index could be achieved. With therapeutic levels of MTX, we obtained a 4- to 16-fold greater cell kill than could be explained by the separate effects of heat and liposome-entrapped MTX (7). Since the strategy is fundamentally local, temperature-sensitive liposomes do not address the problem of widespread metastases.

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Free Radical Injury: A Basis for Selective Tumor Kill

1026 SELECTIVE ENHANCEMENT OF TUMOUR RESPONSE: MECHANISMS OF RADIATION- AND CHEMO-SENSITIZATION, Gerald E. Adams and Ian J. Stratford, Department of Physics, Institute of Cancer Research, Clifton Avenue, Sutton, Surrey SM2 5PX, England.

Many chemical agents potentiate the anti-tumour effect of both radiation and cytotoxic drugs. The main rationale for selective radiation sensitization in tumours lies in the differential enhancement of the radiation sensitivity of hypoxic cells which occur in most solid tumours and which are believed, in some cases, to adversely influence tumour response to radiotherapy. Hypoxic cell sensitization can occur by at least two mechanisms: a) a fast-free radical process whereby lethal radiation damage to DNA is substantially increased but only in the absence of oxygen; this process is virtually instanteneous; b) a much slower, probably non-free radical process, in which the cell's capacity to repair radiation damage is reduced or eliminated. The efficiencies of both types of radiation sensitization depend mainly but not exclusively on the electron-affinic properties of the sensitizers. Progress in mechanistic and applied studies which have led to the development of radiation sensitizers superior to misonidazole will be discussed.

Recently it has been shown that some nitroimidazole radiation sensitizers can increase the anti-tumour effect of various cytotoxic drugs, particularly alkylating agents. While some potentiation of normal tissue toxicity can occur under some conditions, there is now abundant evidence that the enhancement of anti-tumour effect is considerably greater. Like radiation sensitization, chemopotentiation correlates with the electron-affinic properties of the drugs but, unlike radiation sensitization, the phenomenon appears to be entirely due to slow metabolic processes. The mechanisms are complex but evidence is strong that inhibition of repair processes are involved and that a pre-requisite for at least part of the chemopotentiation is anaerobic cellular metabolism of the drug. Experimental evidence indicates that the basis of the differential anti-tumour effect lies partly in the rôle of this hypoxia-mediated process. While misonidazole itself shows potentiation, new agents, particularly nitroheterocyclics containing an alkylating function in the structure, are more effective.

Factors influencing the efficiency of chemopotentiation, including drug structure, tumour type, drug sequencing, etc., will be discussed.

"Radiation and Cytotoxic Drugs", Conference Proceedings, Key Biscayne (1981). Int. J. Rad. Oncol. Biol. Phys. (in press).

Preparation of Monoclonal Antibodies Specific for Human Tumor Cells

1027 HUMAN MELANOMA/CARCINOMA CROSSREACTING ONCOFETAL ANTIGEN IDENTIFIED BY A MOUSE MONOCLONAL ANTIBODY, Shuen-Kuei Liao, Peter B. Dent, Bryan J. Clarke and Pak C. Kwong, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.
We have produced a hybridoma by fusion of mouse p3-NS1-Ag 4 myeloma cells with splenocytes of a BALB/C mouse immunized with cultured human melanoma cells (CaCL 78-1). Doubly cloned cultures secreted an IgG1 monoclonal antibody (140.72) which identified a melanoma/carcinoma crossreacting antigen. Direct and blocking the mixed hemadsorption assays were used to test for reactivity against a large panel of cultured cell lines and fresh tissues. 140.72 antibody reacted with 18/20 melanoma lines, 5/14 carcinoma lines (2/4 breast, 1/5 lung, 2/2 colorectal, 1/1 squamous cell) and 1/1 fresh breast carcinoma and 2/2 lung carcinomas, but failed to react with any of 33 other cultured lines (4 neuroblastomas, 5 glioblastomas, 5 retinoblastomas, 3 sarcomas, 1 teratoma, 9 fibroblast lines, 11 hemopoietic lines) or 4 xenogeneic tumor lines tested. Blocking assays confirmed the results of direct testing. Furthermore, it did react with 10/11 tissue homogenates prepared from different fetuses of 10-14 weeks gestation. The antigen involved was shed by reactive lines. Reactivity of 140.72 was abolished by absorption with highly purified carcinoembryonic antigen (CEA). Thus the structure recognized by 140.72 monoclonal antibody appears to be a melanoma/carcinoma cross-reacting oncofetal antigen. Further characterization of the identified antigen and its relation to CEA will determine its potential utility in clinical diagnosis, prognosis and therapy.

Monoclonal Antibodies as Carriers for Cytotoxic Modalities

1028 AFFINITY THERAPY: ANTIBODIES AS CARRIERS FOR PLATINUM ANTICANCER DRUGS

Esther Hurwitz, Rina Kashi and Meir Wilchek, The Weizmann Institute of Science, Rehovot, Israel

Anti-tumor antibodies have been used in our laboratory as carriers of a number of chemotherapeutic agents in an attempt to increase their specificity towards tumor cells and thereby reduce their general toxicity. In this study platinum salts were complexed with anti-tumor reactive immunoglobulins. The platinum-complexed anti-tumor Ig's retained their specific antibody binding activity. The inhibition of DNA synthesis by platinum salts (K_2PtCl_4 and cis-diaminedichloroplatinum II) and platinum-Ig complexes was studied in two tumor cell lines. The platinum-Ig complexes were more effective inhibitors of DNA synthesis when the Ig had a binding affinity towards the test tumor cells. We suggest two possible mechanisms of action: (i) the antibodies may bring the platinum to the cell surface and promote the internalization of the metal through their cell surface antigen(s); (ii) platinum may be dissociated from its antibody complex on the cell surface and enter the cell via channels for ions which are opened through cell stimulation by specific antibodies, and interact with the DNA inside the cells. Our studies also suggest a possible mechanism for the action of platinum in general: attachment of platinum to tumor-cell surface antibodies may take place *in vivo* and thereby promote its delivery to the pertinent cells. Recent studies *in vivo* in which antibodies and platinum salts were injected separately may support this hypothesis.

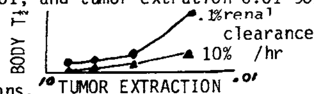
1029 MONOCLONAL ANTIBODIES TO TUMOR-ASSOCIATED ANTIGENS (TAAs) DEFINE HETEROGENEITY OF MAMMARY TUMOR CELLS, Patricia Horan Hand, Marianna Nuti, David Colcher, Yoshio A. Teramoto and Jeffrey Schlom, National Cancer Institute, NIH, Bethesda, MD 20205

Murine monoclonal antibodies, prepared against human metastatic mammary tumor cells, demonstrate differential expression of several TAAs among various mammary carcinomas and within a given tumor mass. Using the immunoperoxidase technique on serial sections of 45 primary mammary carcinomas, a spectrum of antigenic expression of TAAs was observed: 13 percent of the tumors reacted with all of a panel of five monoclonal antibodies, while 20 percent of the mammary tumors scored negative with all five antibodies. The remaining 30 tumors could be divided into eleven additional groups based on their differential reactivity with some, but not all, of the monoclonals. A heterogeneity in antigenic expression of mammary tumor cell populations within a given tumor mass was also observed. With respect to: (a) expression of TAA in one area of a tumor mass and not another, and (b) a "patchwork" effect in which antigen was expressed on cells immediately adjacent to cells which scored negative. Heterogeneity of TAA expression was also observed in established mammary tumor cell lines. An increase in cell surface binding of one of the monoclonals was associated with cells in S-phase, whereas a decrease in binding was observed at high cell density. Consistent with this finding, MCF-7 mammary tumor cells obtained from four sources could be differentiated from each other by their pattern of surface TAA expression. Human mammary tumors, derived from transplantation into athymic mice, also demonstrated this heterogeneity of TAA expression.

1030 EVALUATION OF RADIOLABELED MONOCLONAL ANTIBODY PARAMETERS NECESSARY FOR CANCER IMMUNORADIOTHERAPY, S.J.DeNardo, H.H.Hines, K.L.Erickson, G.L.DeNardo, U of Cal. Davis

The developement of effective immunoradiotherapy for cancer requires not only specific monoclonal antibodies to well chosen tumor associated antigens and appropriate radionuclides with short ranged energy deposition, but also an effective combination of other variables: 1) antibody fragment size giving the best invivo kinetics; 2) tumor extraction rates; 3) tumor residence time for the target antigen; 4) excretion rate of the radionuclide from the body; 5)type of attachment of the nuclide to the antibody fragment;6) length of physical $T\frac{1}{2}$ of radionuclide

Previous studies in our laboratories demonstrated that selected tumor specific monoclonal antibodies could be produced against P-51 murine melenoma, radiolabled with I-131, and used to deliver tumorcidal, currative radiotherapy. Thirty C-57 Black mice, carrying P-51 tumors were used to demonstrate the radiolabeled antibody invivo organ distribution,kinetics, and tumor extraction rate. Five mice were given the injections calculated to deliver 10,000 rads to the tumor, which resulted in longterm clinical cure. The radiation burden to the liver and whole body must be reduced significantly before human therapy is realistic. For this reason we have developed computor methods of optimizing the variables and evaluating the relative effects. When physical decay varied 0.5-8 days,renal clearance 0.1-0.001, and tumor extration 0.01-90% then high tumor extraction rates make renal clearance less significant, but at realistic tumor extraction rates,renal clearance and nuclide $T\frac{1}{2}$ become important for tumor verses body dose.Predictive computor programs are now being initiated to select the more effective labeled antibody modifications.



Mechanism and Clinical Use of Estrogen Antagonists

1031 THERAPEUTIC APPLICATIONS OF AUGER AND ALPHA EMITTING RADIONUCLIDES, William D. Bloomer, William H. McLaughlin, Ralph R. Weichselbaum, and S. James Adelstein, Harvard Medical School, Boston, MA 02115

We have developed strategies for the potential use of Auger and alpha emitting radionuclides in cancer therapy.

The Auger effects from ^{125}I are singularly damaging if localized within DNA as the thymidine analogue ^{125}I-iododeoxyuridine (^{125}IUdR). Recent work with ^{125}I-labeled intercalating agents and steroid sex hormones extends these observations by showing toxicity with ^{125}I in sites other than the DNA backbone. We have compared the cytotoxicity of ^{125}IUdR with ^{125}I-iodotamoxifen (^{125}ITAM). Toxicity is critically dependent upon the presence of specific hormone receptors and subcellular localization. When clonogenic survival is expressed as a function of the nucleic acid and protein subcellular fraction, ^{125}ITAM is just about as toxic as ^{125}IUdR.

We have also investigated the efficacy of ^{211}At tellurium colloid for treatment of experimental malignant ascites. This α-emitting radiocolloid can be curative without undue normal tissue toxicity. By comparison, negatron emitting ^{32}P as colloidal chromic phosphate had no antineoplastic activity. The most compelling explanation for this striking difference is the dense ionization and short range of action associated with α-emission. These results have important implications for the development and use of α-emitters for the treatment of human tumors.

1032 STRUCTURAL REQUIREMENTS FOR BINDING OF ANTIESTROGENS TO A SPECIFIC HIGH AFFINITY SITE IN MCF 7 CELLS, L.C. Murphy, C.K.W. Watts and R.L. Sutherland, Ludwig Institute for Cancer Research (Sydney Branch), University of Sydney, N.S.W. 2006. Australia.

Studies in this laboratory have revealed the presence of a high affinity, intracellular binding site in cytosol of some estrogen target tissues, which has narrow specificity for synthetic non-steroidal antiestrogens but is distinct from the estradiol binding site of the estrogen receptor. Further studies were undertaken to define in more detail the structural requirements for binding to this site and to investigate the cytotoxicity of compounds with weak or strong affinity for the site. Relative binding affinities were assessed in MCF 7 cell cytosol using a competitive binding technique with tritiated tamoxifen. Antitumor activity was assessed by the ability to inhibit the growth of MCF 7 cells in vitro. Several analogues of the common antiestrogens tamoxifen and clomiphene were tested. The alkylaminoether side chain was shown to be a major structural determinant for binding to the antiestrogen binding site (AEBS). Removal of this side chain or its replacement by a methoxy group eliminates binding. Changes in the length of the side chain, in the number and size of the alkyl substituents on the terminal amino group, and conversion of the ether linkage to an amine all influenced binding affinity. Analogues lacking the basic amino group had little or no affinity for the antiestrogen binding site. Preliminary data suggest that compounds having reduced affinity for the AEBS have reduced antitumor activity in vitro even when their affinities for the estrogen receptor are similar. It is concluded that there is some correlation between affinity for the AEBS and antitumor activity in vitro and further investigation of the role of AEBS in mediating antiestrogen action is warranted.

1033 EFFECTS OF ANTIESTROGENS ON THE CELL CYCLE KINETICS OF CULTURED HUMAN BREAST CANCER, C.K. Osborne and D.H. Boldt, University of Texas Health Science Center, San Antonio, TX 78284

Clinical trials have been initiated to evaluate the effects of combined chemo-hormonal therapy in breast cancer. Little is known about the potential interactions of these therapies, which might influence the net result of their combined use. Since cell cycle kinetics play a role in determining chemosensitivity, we have studied the effects of antiestrogens on the kinetics of MCF-7 human breast cancer cells. Tamoxifen (TAM) inhibits proliferation of MCF-7 cells. Using autoradiographic techniques, TAM results in a time-dependent decrease in the thymidine labeling index (TLI), indicating fewer cells in S-phase. By 72 hrs fewer than 10% of cells are in S-phase compared to 20-30% in controls. Partial rescue of TAM-inhibition is observed with a simple change to TAM-free media. The rescue is dramatic when 17β-estradiol (E_2) is added. An increase in the S-fraction is evident and by 24 hrs, 60% of the cells are in S-phase. To determine the effect of antiestrogen and E_2 rescue on the other phases of the cell cycle, DNA histograms were obtained using flow cytometry. TAM results in a time and dose-dependent decrease in the fraction of cells in S and G_2 + M phases, with a concomitant increase in the G_1 fraction. E_2 rescue results in a decrease in the G_1 fraction and a rapid increase in the fractions in S and G_2 + M. In summary, antiestrogens inhibit proliferation of human breast cancer cells by invoking a transition delay in the G_1 phase of the cell cycle. E_2 rescue relieves this block and allows a synchronous cohort of cells to enter S-phase. These data may have important implications for the design of clinical trials using combined chemoendocrine therapy.

1034 EFFECTS OF TAMOXIFEN ON THE CELL CYCLE KINETICS OF HUMAN MAMMARY CARCINOMA CELLS IN CULTURE,
R.L. Sutherland, I.W. Taylor, R.R. Reddel, R.E. Hall, Ludwig Institute for Cancer Research (Sydney Branch), University of Sydney, N.S.W. 2006. Australia.
The effects of tamoxifen on cell proliferation and the cell cycle kinetic parameters of two estrogen receptor positive (MCF 7, T 47D) and an estrogen receptor negative (BT 20) human mammary carcinoma cell line were investigated in exponentially growing populations of cells maintained in RPMI 1640 medium supplemented with 10 μg/ml insulin and 5% charcoal stripped fetal calf serum. Following drug treatment viable cell counts were made under phase-contrast on a haemocytometer and the percentage of cells in the G_0/G_1, S, and G_2 + M phases of the cell cycle were calculated from DNA histograms generated by flow cytometry. Tamoxifen caused a dose -dependent inhibition of cell growth in all three cell lines. This growth inhibition was accompanied by a dose-dependent decrease in the percentage of S phase cells and a concomitant increase in the proportion of G_0/G_1 phase cells. Simultaneous treatment of MCF 7 and T 47 D cells with a 10 fold lower dose of estradiol completely reversed the growth inhibitory and cell cycle effects of tamoxifen at doses below 5 μM. At higher doses of tamoxifen estradiol augmented the cytotoxic effects of tamoxifen whilst at the same time it partially reversed the depletion in S phase cells. Studies with cells synchronized by mitotic selection or centrifugal elutriation illustrated that tamoxifen acted early in G_1 to inhibit the progression of cells through the cell cycle. It is concluded that 1. tamoxifen is a cell cycle phase specific agent and 2. much of the antitumor activity of tamoxifen *in vitro* is not mediated through the estrogen receptor since similar kinetic events occur in both receptor positive and negative cell lines.

Molecular Analysis of Hormone Resistant Variants

1035 FUNCTIONAL RECEPTOR IN A HUMAN LYMPHOID LINE RESISTANT TO GLUCOCORTICOID-MEDIATED CYTOLYSIS,
Robert Zawydiwski, Jeffrey M. Harmon and E. Brad Thompson, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205
Studies to date on lymphoid cells resistant to glucocorticoid-mediated lysis indicate the biochemical lesions almost always involve defects at the glucocorticoid receptor level. We have isolated and characterized a clone (CEM-C1) of the human acute lymphoblastic leukemic cell line CEM which is insensitive to steroid concentrations that saturate receptors. Analysis of whole-cell binding data revealed that CEM-C1 receptors have kinetic parameters (R_o, K_d and nt) comparable to sensitive clone CEM-C7. Therefore, resistance to cytolysis is not due to impaired steroid absorption, affinity or nuclear localization. Furthermore, the physico-chemical properties of CEM-C1 receptor, as assessed by ion exchange chromatography on DEAE- and DNA-cellulose, are indistinguishable from wild-type receptor. On the basis of these criteria, receptor in the resistant clone appears identical to that in its sensitive counterpart. Moreover, glutamine synthetase induction is evidence for functional receptor in CEM-C1. Both the fold increase and steroid concentration dependence of enzyme activity were found to be similar to that observed in CEM-C7. Our results suggest that CEM-C1 may contain a post-receptor defect and that this clone would be suitable for complementation tests in somatic cell hybrids between appropriate dexamethasone-resistant phenotypes. Also, in the clinical setting patients may be found whose leukemic cells are steroid-resistant without showing any of the known receptor defects associated with such resistance.

1036 PURIFICATION OF HUMAN GLUCOCORTICOID RECEPTOR AND PRODUCTION OF ANTI-RECEPTOR ANTIBODIES.
Jeffrey M. Harmon[1], Howard J. Eisen[2], S. Stoney Simons[3], and E. Brad Thompson[4]. [1]Dept. of Pharmacology, USUHS, Bethesda, MD 20814, [2]Developmental Pharmacology Branch, NICHD, NIH, [3]Laboratory of Chemistry, NIAMDDK, NIH, [4]Laboratory of Biochemistry, NCI.
Glucocorticoid receptors were purified from the human lymphoblastoid cell line IM9 by a modification of the 2-stage DNA-cellulose chromatographic procedure of Eisen and Glinsman (Biochem J. 171:177, 1978). Purified preparations contained 4-6pmol receptor per ug protein (1500 fold purification) which eluted from Sephacryl S-300 with an apparent M_r of 5.6-6.0nm. This was indistinguishable from the apparent M_r of receptors in crude cytosol, indicating the presence of intact receptor. Based on a molecular weight of 90K daltons for the native receptor, the purity of our preparations was calculated to be ∿40%. Two New Zealand white rabbits were immunized at 4-week intervals with 50-80pmol purified receptor. The presence of antibody in the serum was detected by adsorption of ^{3}H-triamcinolone acetonide (^{3}H-TA) receptor-antibody complexes onto Protein A containing *Staphylococcus aureus* membranes and by a shift to higher apparent M_r in the elution of ^{3}H-TA labeled receptors from S-300. Sera from both rabbits contained IgG immunoreactivity after the 2nd immunization; maximum response was seen after three immunizations. These antibodies recognize both mouse and rat glucocorticoid receptors. After immunoprecipitation of ^{3}H-dexamethasone-mesylate (^{3}H-DM) labeled cytosol a single band of 90K daltons was resolved by SDS-gel electrophoresis. This band corresponds to the only cytosol protein to which the binding of this electrophilic affinity label is competable. Our results therefore suggest that these antibodies are highly receptor specific.

Hormone-Drug Interactions in Clinical Chemotherapy

1037 A PHASE II EVALUATION OF TAMOXIFEN, PREMARIN, METHOTREXATE (MTX) AND 5-FLUOROURACIL (5-FU) IN STAGE IV BREAST CANCER, J.C. Allegra, T.M. Woodcock, S.P. Richman, K.I. Bland, J.L. Wittliff, University of Louisville, Louisville, Ky. 40292
Complete remissions (CR) in patients with advanced breast cancer using either endocrine or chemotherapy are infrequent. Breast tumors are known to be heterogenous with respect to estrogen receptor (ER) status and the low CR rate may be secondary to this biochemical heterogeneity. Laboratory experiments using breast cancer cells in long-term tissue culture revealed that: 1) Tamoxifen is cytotoxic; 2) Estrogen stimulates the growth of ER positive cells and can rescue cells from Tamoxifen's effect; 3) Sequential MTX-5-FU is synergistic in rapidly growing breast cancer cells. Based on this, a phase II protocol was designed using Tamoxifen 10 mg po bid for days 1-10. This was followed by Premarin 0.625 mg po bid on days 11-14 and on day 14 the patients were given MTX 200 mg/m^2 IV followed in 1 hour by 5-FU 600 mg/m^2 IV. The patients were rescued with Leucovorin 10 mg/m^2 24 hours later. The cycle repeats every 18 days. Thus far, 28 patients have been entered and 22 are currently evaluable for response. Their median age is 49. All had an ECOG performance status of 2 or less. 64% were ER positive and 25% had received prior endocrine or chemotherapy. 18 patients were post menopausal and 10 premenopausal. 42% of patients had visceral dominant disease. Overall response rate was 73% and 12 of 22 (55%) attained a CR, and 4 of 22 (18%) had a partial remission. Toxicity was minimal. Median nadir WBC counts were 5,500 and median nadir platelet counts were 252,000. Nausea occurred in 25% of patients but there was no vomiting, hair loss, rash, diarrhea or mucositis. In summary, this combination hormonal-chemotherapy regimen is highly effective with a high CR rate and minimal toxicity.

1038 THE USE OF CORTICOSTEROIDS IN TIMED-SEQUENTIAL TUMOR THERAPY, Lewis M. Schiffer and Paul G. Braunschweiger, Allegheny-Singer Research Corporation, Pittsburgh, PA 15212,
A group of in-vitro techniques to measure changes in solid tumor cell proliferation, after perturbation by agents such as steroids, drugs and x-ray, has led to rational ways to sequence agents. The techniques are: ^{3}HTdR labeling index, DNA synthesis time and an estimate of tumor growth fraction, the PDP assay. Analysis of the kinetics of tumor growth showed that corticosteroids caused an inhibition of tumor growth by a dose dependent G1 block of cycle progression. Following release of the block, by cessation of corticosteroids, there was resumption of cycle progression. This ultimately results in an enriched cohort of cells in S phase. Together with the potential recruitment of cells from G0 phase, these cycling cells become amenable to cycle specific chemotherapeutic agents. We present results from 3 programs: A) Animal studies showing improved chemotherapy results when cytotoxic drugs are administered at the rebound peak of S phase following corticosteroid suppression of proliferation, and the drug and dose-dependent nature of this phenomenon, B) Animal studies showing there is a correlation between glucocorticoid receptor levels and anti-proliferative effect of the steroid. This relationship may predict the optimum time for sequencing chemotherapy following administration of a given amount of corticosteroid, C) The use of corticosteroids, in a manner similar to A) prior to use of cytotoxic drugs, in a pilot clinical program in metastatic breast cancer. Supported by CB-43899, CB-75083, CA-26020 and CA-27233.

1039 Adriamycin Sensitivity of Cultured Malignant and Nonmalignant Human Mammary Epithelial Cells: Helene S. Smith, Martha R. Stampfer, Adeline J. Hackett. Peralta Cancer Research Institute, Oakland; Donner Lab, Univ. of CA, Berkeley, California.

Using techniques previously developed to culture normal human mammary epithelium (Stampfer et al. In Vitro 16:415, 1980), we can now isolate, cryopreserve and culture human mammary cells from both normal and malignant specimens. By plating the cells with fibroblast feeder layers, a clonal assay suitable for quantitating drug sensitivity has been developed with plating efficiencies ranging from 1-40% (Smith et al. Cancer Res. 41:4637, 1981). With this assay, we have generated dose response curves for the chemotherapeutic drug adriamycin using cultures from breast carcinomas and nonmalignant tissues (reduction mammoplasties and mastectomy tissues peripheral to carcinomas). None of the tissue donors had undergone prior chemotherapy. We were able to detect heterogeneity in response to adriamycin among breast carcinoma cultures as well as among the nonmalignant specimens. The concentration of drug required for 50% survival varied from 2.4-79 ng/ml. The differences in sensitivity were unrelated to growth rates in culture. In some cases (both normal and malignant), we also detected heterogeneity in response to the drug among subpopulations within a single specimen. This intra-specimen heterogeneity could most easily be seen as a biphasic dose response curve when the data were plotted on semilogarithmic paper. These results raise many exciting research questions relating to the source of the heterogeneity as well as to potentila clinical applications.

1040 SYNERGISTIC MODULATION OF 5-FLUOROURACIL TOXICITY BY TAMOXIFEN, Chris Benz, Terrence Wu, and Ed Cadman, Yale School of Medicine, New Haven, CT. 06510
Modulation of 5-fluorouracil (FUra) metabolism and toxicity by methotrexate (MTX) and other antimetabolites occurs in many cultured tumor cell lines, including the estrogen receptor positive human mammary carcinoma, 47-DN. The growth rate of this cell line depends on exogenously administered insulin and estradiol, and can be reversibly inhibited by the antiestrogen, tamoxifen (TAM).
47-DN cell-cycle kinetics are altered by doses of TAM which suppress the synthesis of estrogen and progesterone receptors. In cloning assays, TAM is synergistic with FUra and sequentially combined MTX→FUra; in biochemical assays, TAM inhibits FUra intracellular accumulation and incorporation into RNA. This unique form of drug modulation may represent a form of "complementary inhibition," and supports clinical trials in breast cancer suggesting that TAM + FUra-containing chemotherapy is superior to chemotherapy alone.

1041 HIGH EFFICIENCY OF IMMUNOTOXINS POTENTIATED BY AMMONIUM CHLORIDE TO SPECIFICALLY KILL TUMOR CELLS
P. Casellas, H.E. Blythman, D. Carrière, O. Gros, P. Gros, J.C. Laurent, P. Poncelet, G. Richer, H. Vidal and F.K. Jansen. (Centre de Recherches Clin-Midy, 34082 Montpellier

To attempt passive cancer immunotherapy, immunotoxins have been synthetized consisting of an antibody against a cell surface antigen, linked by a disulphide bridge to the A-chain of ricin. The feasibility of this approach is supported by experiments which demonstrate in vitro the expected properties of specificity and cytotoxocity using four different models : an immunotoxin against : a) TNP-labelled cells ; b) mouse T-leukemia (WEHI-7 cell line) ; c) human T-leukemia (CEM cell line) ; d) human melanoma (SK Mel 28 cell line). However, although immunotoxins were able to kill the last plated target cell with high efficiency in vitro, the inhibition of tumor growth was only moderate, likely due in part to the short half-life and the slow kinetic of action of immunotoxins.

Nevertheless, ammonium chloride, which interferes with the internalisation process of certain macromolecules, strongly increases both in vitro kinetics and efficiency of immunotoxins. This potentiation by ammonium chloride was demonstrated in vivo as well.

Additional Poster Session I Abstracts

1042 INTERRELATIONSHIP OF THE INHIBITION OF DIHYDROFOLATE REDUCTASE AND PHARMACOLOGICAL PROPERTIES FOR A SERIES OF ANTIFOLATE COMPOUNDS, Michael R. Hamrell, Department of Pharmacology & Therapeutics and McGill Cancer Centre, McGill University, Montreal, Que. Can.
Studies on the relationship between methotrexate (MTX) resistance, drug uptake, and dihydrofolate reductase (DHFR) activity have indicated a good correlation between drug sensitivity and intracellular drug concentration in sensitive cells. In resistant cells however, this relationship changes and in many cases does not apply at all. New antifolate compounds have been extensively studied for use against MTX-resistant cells because of their unique pharmacological and physiochemical properties compared to MTX. A study was initiated to examine the amount of MTX and a series of antifolates required to inhibit DHFR and cause cytotoxicity in a MTX-resistant cell line. This cell line has a 7-fold increase in DHFR activity with normal transport. In addition, the resistant cell line has a DHFR with altered properties, that binds MTX less tightly than the parent cell line. Using this resistant cell line, I have compared the effectiveness relative to MTX, of a series of antifolates, to inhibit DHFR and kill cells. All the antifolates tested showed similar inhibition of DHFR activity in the parent cell line following a 3 hour exposure to drug. However, these antifolates showed a wide variation in ability to inhibit DHFR in the resistant cell line which was related to lipophilicity as well as the compound's structural similarity to MTX. The large differences in sensitivity seen, suggest that DHFR inhibition is a multifactorial phenomenom, which results from the interplay between achievable drug levels, target enzyme concentration, and drug-enzyme affinity.

1043 EFFECT OF CIS AND TRANS DDP ON THE MOLECULAR STRUCTURE OF CALF THYMUS CHROMATIN AND NUCLEOSOMES. H. Simpkins, L. Thompson, and M. Arquilla, Department of Pathology and Biological Chemisry, University of California, Irvine, CA 92717
Two fluorescence probes, one specific for single stranded guanine residues in DNA (terbium) and the other specific for the cysteine 96 in histone H3 (N-pyrene maleimide) have been employed to study the interaction of the highly antitumorgenic agent (cis-DDP) and its non-tumorgenic isomer (trans-DDP) with thymocyte nuclei and nucleosomes. It was found that in isolated DNA's from various sources including supercoiled plasmid DNA that cis DDP induced local unwinding of the helix; an effect which resulted in the guanine bases becoming accessible to the terbium probe and also to the dye acridine orange which showed orange instead of green fluorescence with the treated DNA's. These effects were not observed with trans DDP. However, in the DNA-protein complex the (nucleosomes) this effect on DNA was not observed but massive changes in accessibility of the cysteine 96 in histone H3 to the protein probe was detected at very low concentrations and short incubation times with cis and trans DDP. The latter drug reacted directly on the cysteine SH group to form H3-H3 dimers, whereas the former reacted near the probe's binding site, since no protein compositional change was observed only a massive change in fluorescence intensity.

The data shows the potential of these two probes to investigate molecular structural changes in nucleosomes caused by antitumor drugs; as well as showing that work with isolated DNA may show changes which are different to those observed in DNA-protein complexes.

1044 CYANATE OXIDATION PRODUCTS AS SELECTIVE INHIBITORS OF PROTEIN SYNTHESIS IN CULTURED TUMOR CELLS. L.C. Boffa, and V.G. Allfrey. The Rockefeller Univ. New York NY 10021
Sodium cyanate inhibits amino acids incorporation in a variety of animal tumor cells without effecting protein synthesis in the corresponding normal tissue of the tumor bearing animal. The drug has no effect when added to tumor cells in culture. However a rapid inhibition of protein synthesis in cultured tumor cells occurs when cyanate react with the drug metabolizing system of liver microsomes.
The active cyanate metabolite is most probably produced by an enzymatically catalyzed oxidative process. This has been suggested by the finding of an inhibitory effect of catalase on the production and accumulation of the active cyanate metabolite.
The mammalian hemoprotein lactoperoxidase has been reported to catalize the oxidation of the hydrogen peroxide at neutral pH [E.L.Thomas, Biochemistry 20:3273 (1981)].
We have detected similar oxidative compounds by reacting [14]-CNCO$^-$ in the lactoperooxidase system. The resulting metabolites appear to be active as selective inhibitors of protein synthesis in tumor cells grown in vitro, even in the absence of an added microsomal system to the assay. In fact, the inhibitory effects achieved with this system has the same characteristics of timing stability and extent as seen in the microsomal system.
Studies are in progress to isolate and characterize the active oxidative state of cyanate.

1045 PLASMIN-ACTIVATED ANTICANCER DRUGS, Philip L. Carl, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 and Michael J. Weber, Prasun K. Chakravarty, and John A. Katzenellenbogen, University of Illinois, Urbana, IL.
Many solid tumors possess elevated levels of specific proteases which are thought to play a role in such diverse aspects of tumor biology as invasiveness, metastasis, angiogenesis and chromosome instability. In particular plasminogen activator, a serine protease which activates the serum zymogen plasminogen to the active protease plasmin is elevated in many tumors and transformed cell lines. We have shown that by attaching a specific peptide to the primary amino group of several anticancer drugs, the drugs can be converted into inactive prodrugs which can be reactivated by tumor associated plasmin. In vitro these prodrugs show a seven fold improved selective cytotoxicity compared to the parent drugs when tested against normal and transformed chick embryo fibroblasts. The drugs are also active in vivo against the B16 melanoma - a murine tumor which produces elevated amounts of plasminogen activator. However we do not as yet have any evidence for an improvement in therapeutic index in vivo. Possible reasons for this difficulty will be discussed.

1046 FORCED SUICIDE AS A NEW MODE OF CANCER CELL ELIMINATION,
Shinichi Okuyama and Hitoshi Mishina, Tohoku Rosai Hospital, Sendai, Miyagi, 980, Japan

Cancer cells are eliminated by cell kill through radiotherapy, chemotherapy or immunotherapy, or by exfoliation through re-differentiation or surgery, a manual "exfoliation." A third and new mode of cancer cell elimination seems feasible by "suicide" or cell death from deficient handling of intrinsic toxic substances such as superoxide radicals. In mammalian cells, superoxide radicals are disposed of efficiently by superoxide dismutase (Cu-Zn SOD and Mn SOD). Cancer cells are solely dependent upon Cu-Zn SOD. Diet-induced hypocupremia reduces SOD activity (Russanov et al., 1979), and D-penicillamine-induced hypocuprozincemia induced an increase in the serum lipoperoxide levels as determined in terms of malondialdehyd (Okuyama and Mishina, in press), suggesting a probable inundation of superoxide radicals as the result of hypocuprozincemic inactivation of Cu-Zn SOD. An appreciable cancer cell lethality is inducible (Probable superoxide therapy of experimental cancer with D-penicillamine, Okuyama and Mishina, Tohoku J. Exp. Med., 135: 41, 1981). This type of cancer cell elimination would greatly help overcoming the log kill limitation of radiotherapy and chemotherapy without much hazard to the host patient because of the biological cancer cell selectivity and the low toxicity of such agents. This category of cancer cell elimination can probably be expanded to other metabolites and agents, demanding further intensive investigations.

1047 ISOLATED NUCLEOSOMES AS TARGETS FOR CYTOSTATIC AND CYTOTOXIC ALKYLATING AGENTS.
G. Yagil, Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

We have been studying in recent years the action of a bifunctional alkylating agent, bis(2-chloroethylamine) (nitrogen mustard, HN2) on isolated chromatin, as well as on its defined subunits, the nucleosomes (Yerushalmi and Yagil, Eur. J. Biochem. 1980, 103:237-246). A quantitative study of the interaction reveals that DNA in chromatin is up to 5 times as available to alkylation reaction as DNA free in solution. These findings have now been extended to nucleosomes and isolated core particles. Excessive reaction levels off when about 5% of all base pairs are substituted in intact chromatin (2.5% in mononucleosomes). Our interpretation is that a change in the conformation of the bases in DNA takes place when DNA is wrapped around the histone core, leading to increased reactivity.

These findings imply that isolated nucleosomes should be the preferred target substances when relative cytostatic or carcinogenic action of different agents is compared on the molecular level. We are presently using nucleosomes of defined sequence (constructed from histone cores and plasmid DNA fragments; Yagil and Van Holde, in preparation) to identify more closely the points of attack and obtain an improved target substance for assessing the action of cytostatic compounds of the alkylating class.

1048 ENZYME-MODULATED CANCER CHEMOTHERAPY BASED ON PHOSPHODIESTERASE.
K.C. Tsou, Y.Q. Chen and E.E. Miller, Departments of Pharmacology & Surgery, School of Medicine University of Pennsylvania, Philadelphia, PA 19104 U.S.A.

The presence and elevation of phosphodiesterase in tumor over normal tissue has been documented in several laboratories, but serious efforts to utilize the knowledge of these enzyme differences in cancer chemotherapy is still lacking. We wish to illustrate in this presentation both the 5'-nucleotide phosphodiesterase and cAMP phosphodiesterase can be target enzymes for enzyme-modulated cancer chemotherapy. Specifically, 2'oleoyl-2-aza-ε-3'5'-cAMP has been synthesized for glioma chemotherapy. This compound is 26% as effective as a substrate for cAMPDase. It also should have better lipid affinity than cAMP for glioma cells. When tested on glioma-26 cells in tissue culture it was found to be active at 10^{-3}M level (97% cell killed) and 10^{-4}M (86% cell killed). Aza 3'5'-cAMP (81%), 3'5'-cAMP (38%) and dibutyryl-cAMP (35%) are all less active. Preliminary study in a transplantable mouse glioma -26 tumor also showed that tumor size was significantly reduced at the 50 mg/kg dose for 5 days dose level in comparison to the control tumors. Fluorescent cytometric study shows that the drug is active as a S-phase specific inhibitor and that cAMPDase (low K_m) is associated with DNA synthesis in S-phase of cell cycles. Therefore this new fluorescent cyclic-AMP analog is also a useful labeling agent for monitoring proliferative cell population.
Supported by grant #CA 28770 from the National Cancer Institute.

1049 ANTICANCER ACTIVITY OF RHODAMINE-123, Samuel D. Bernal, Theodore J. Lampidis, Ian C. Summerhayes, and Lan Bo Chen, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

Rhodamine-123, a cationic laser dye, is selectively accumulated in the mitochondria of living cells. The mitochondria of certain carcinoma cells, especially those derived from lung, breast, colon and bladder, retain Rhodamine-123 longer than normal epithelial cells. We now report that the clonogenic ability of tumorigenic epithelial cells in-vitro is markedly reduced after treatment with concentrations of Rhodamine-123 that cause little or no reduction in clonogenic ability of non-tumorigenic epithelial cells. Rhodamine-123 was also effective in reducing tumor bulk and in prolonging survival of mice bearing Ehrlich ascites tumor, without producing marked toxicity.

1050 THE ROLE OF ESTROGEN RECEPTOR PROTEINS IN THE INHIBITION OF HORMONE RESPONSIVE BREAST CANCER CELLS BY AMINONUCLEOSIDE OF PUROMYCIN. Jolanta J. Cholon, Robert W. Lockwood and John Mallams, Departments of Pathology and Radiology, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, N.J. 07103.

Recent results in this laboratory indicate that aminonucleoside of puromycin differentially affects hormone responsive as opposed to hormone unresponsive human breast cancer cells. It selectively inhibits DNA synthesis and proliferation of hormone responsive but not hormone unresponsive cells. We, therefore, sought to establish whether this effect is exerted through the estrogen receptor mechanism present in hormone responsive cells. For experiments, cells were grown on medium containing hormone stripped serum supplemented with physiological concentrations of hormones with the exception of estrogen. The cells were then stimulated by the addition of estrogen and half the cultures were treated with aminonucleoside of puromycin. The cells were harvested at periodic intervals following aminonucleoside treatment, homogenized and assayed for estrogen receptor proteins by sucrose gradient centrifugation. The results show that aminonucleoside has no effect on estrogen receptor protein interaction indicating that the inhibition of hormone responsive cells by aminonucleoside is not mediated through estrogen receptor proteins. In conclusion, these results suggest that aminonucleoside of puromycin inhibits hormone responsive human breast cancer cells through a mechanism independent of estrogen receptor proteins, which is particular to these cells and which represents another characteristic distinguishing hormone responsive from hormone unresponsive breast cancer cells. Supported by a grant from the Foundation of CMDNJ.

1051 DIFFERENTIAL INHIBITION OF TWO STEPS IN REPLICON-JOINING AND DAUGHTER STRAND GAP REPAIR BY APHIDICOLIN, CYTOSINE ARABINOSIDE AND HYDROXYUREA, Helene Z. Hill, College of Medicine and Dentistry of New Jersey - New Jersey Medical School, Newark, New Jersey 07103

The effect of inhibitors of DNA replication, replicon joining (RJ) and daughter strand gap repair (DSGR) was studied in order to better understand the role of the latter two processes in response to chemotherapy. For these experiments, the mouse melanoma clonal line, B16CL4, was used. It has a high rate of DSGR relative to some other cell lines. Cells were pre-incubated with ^{14}C-dThd in order to label the parental DNA. They were then treated with UV (DSGR) or not (RJ), pulsed for varying lengths of time with ^{3}H-dThd and chased in inhibitor-containing non-radioactive medium. The cells were then washed and exposed to 2000 rads of 100kvp x-rays to prevent subsequent entanglement of DNA during centrifugation. The MW of the DNA was analyzed by alkaline sucrose gradient sedimentation and the amount of the ^{3}H-DNA cosedimenting with 60% of the high MW fraction of the ^{14}C-DNA was determined. The relative increase in the high molecular weight material during the chase was greater if the pulse was short than if the pulse was long, when any one of the three inhibitors was present during the chase. These results are interpreted to mean that, in the presence of these inhibitors, only those replicons which are poised to join at the end of the pulse are able to do so during the chase. Since aphidicolin is a specific inhibitor of DNA Polymerase α, it is surmised that these inhibitors affect chain elongation by polymerase α to a greater extent than they inhibit DSGR and RJ which must be performed by some other enzyme, possibly DNA Polymerase β. Experiments such as these can be used in the future to define the role played by chemotherapeutic agents which are inhibitors of DNA synthesis in the various steps of DNA replication and repair. When DNA replication inhibitors such as the 3 herein described are used in combination chemotherapy, they might better be combined with inhibitors of the joining step, rather than with each other.

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1052 POLYMERS OF ADRIAMYCIN AND THEIR CYTOTOXIC ACTIVITY. Zoltan A. Tokes, Kathryn E. Rogers, and Alan Rembaum. Department of Biochemistry and the Cancer Center, University of Southern California, Los Angeles, CA 90033 and Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91103.

A novel technique is introduced whereby a chemotherapeutic agent is covalently linked to large polymers or to solid surfaces. Such an approach creates a new type of interaction between the cell surface and the high density of covalently attached drug on the polymer surfaces. This interaction results in multiple and repetitious binding at the cell surface and consequently leads to increased cytotoxic activity. To demonstrate this approach, Adriamycin (Adr) was covalently coupled through Schiff's base condensation to polyglutaraldehyde microspheres. These drug-polymer complexes (Adr-PGLs) remain stable during incubation with cells and exhibit cytotoxic activity as measured by trypan blue exclusion and 51Cr release. Repeated use of the coupled Adr-PGLs in the cytostatic assays did not decrease their activity, indicating that these complexes can be recycled. A ten-fold increase in growth inhibition was observed with Adr-PGLs using drug resistant clones of CCRF-CEM, human leukemia cell lines. A one hundred-fold increase in cytotoxicity due to polymer attachment of Adr was observed with an established rat liver carcinoma cell line, RLC. Similarly, when carcinogen-altered drug resistant rat hepatocytes were treated for 24 hrs with 10^{-6}M free or polymer-bound Adr, a forty-fold increase in toxicity was observed with Adr-PGLs. However, normal rat hepatocytes remained more sensitive to the free Adr than the polymer-bound drug. These results illustrate the potential of polymer-drug complexes to overcome those forms of drug resistance which are caused by decreased drug binding and to enhance the general cytotoxic activity of anti-cancer agents.

1053 CANCEROSTATIC PROPERTIES OF THE PLANT TOXINS RICIN AND ABRIN, Oystein Fodstad, Aslak Godal and Alexander Pihl, The Norwegian Radium Hospital, Oslo 3, Norway.

Ricin and abrin belong to a group of extremely toxic plant proteins now receiving , much attention as they are used for conjugation to monoclonal antibodies for spesific targeting to tumor cells.The proteins as such inhibit cellular protein synthesis by inactivating the 60 S ribosomal subunit and are toxic to all eukaryotic nucleated cells. Preliminary data indicated that ricin and abrin may also have a role to play in cancer therapy on their own as they were shown to inhibit the growth of one mouse tumor. Recently we have found that the toxins indeed possess selective cancero -static properties. They have a significant but moderate activity against some murine tumors and on several human tumor xenografts in nude mice their growth-inhibiting effects are comparable or superior to those of the most commonly used chemotherapeutic agents. A selective potentiation was found in L 1210 mouse leukemia when ricin was used in combination with antitumor drugs belonging to several different classes. Results obtained in other tumors confirm that combination therapy with ricin or abrin is a particularly promising field. A very sensitive radio-immuno-assay has been developed for monitoring toxin blood levels and the toxins are now undergoing clinical phase-I trials.

1054 CAN XENOPUS OOCYTE BE A VALUABLE MATERIAL TO CONTRIBUTE TO A RATIONAL BASIS FOR CHEMOTHERAPY ? Pierre Pennequin and Josette Léger, Lab. Hormones, 94270 Bicêtre, France.

Xenopus oocytes maturation is studied with three different steroids : cortisol, testosterone and progesterone. Forces involved in melanosomes motion during maturation are under investigation. Effets of different types of drugs on maturation by natural steroids is studied : other steroids, cardiac steroids, anti-calcium drugs, diuretics, thiol reagents, cholinergic agonist and antagonists, anesthetics, emetine, chloroquine, insecticides. Using these drugs, differences in maturation by cortisol, progesterone, and testosterone appear. Total mRNA from Torpedo electric organ has been translated in Xenopus oocytes. Furthermore oocyte plasma membranes have been purified and used to prepare active immune serum.

Biochemical Modulation of Antitumor Chemotherapy

1055 EFFECT OF METHOTREXATE (MTX) ON THE DNA-DIRECTED CYTOTOXICITY PRODUCED BY EQUITOXIC DOSES OF FLUOROURACIL (FUra) AND FLUORODEOXYURIDINE (FdUrd) IN MURINE LEUKEMIA L1210 CELLS, R. Heimer, R. Dreyer and E. Cadman, Yale Univ. School of Medicine, New Haven CT 06510.

It has become recognized that FUra has at least two cytotoxic modalities described as DNA-directed and RNA-directed. 10μM FUra and 1.0μM FdUrd are equitoxic in tissue culture and in a soft agar cloning assay. While FdUrd cytotoxicity is reversed with thymidine alone, FUra requires both thymidine and uridine. We measured levels of FdUMP, the inhibitor of thymidylate synthetase (T.S.) and the metabolite involved in the DNA-directed toxicity. After 1 hr, [^{3}H]-FUra or -FdUrd produced similar total FdUMP levels: 914 and 807 fmoles/10^6 cells. We have measured the T.S.-FdUMP covalent ternary complex formed after 1 hr of [^{3}H]-FUra or -FdUrd and found 431 and 627 fmoles/10^6 cells respectively. Since FdUrd becomes FdUMP far more rapidly than FUra does, we measured the highest achievable levels of complex and found the maximum essentially equal after 4 hr of FUra and 30 min of FdUrd.

To evaluate the effect of MTX, cells were pretreated for 3 hr with 1μM MTX. We saw only modest changes in total FdUMP levels; a 10% increase for FdUrd and a 14% increase for FUra. Since MTX lowers tetrahydrofolate (FAH_4) pools and 5,10-CH_2FAH_4 is needed for ternary complex formation, we found, not surprisingly, less complex after MTX. MTX + FdUrd was 45.2% of control; MTX + FUra was 19.4% of control, even after 4h of FUra. Inhibition of T.S. by FUra after MTX is substantially decreased. Yet, MTX and FUra are synergistic in the cloning assay while MTX and FdUrd are less than additive. We conclude that the DNA-directed effect of FUra is an insignificant factor in the synergy of the combination MTX and FUra.

1056 THE INTERACTION OF METHOTREXATE (MTX) AND 5-FLUOROURACIL (FUra) IN HUMAN COLON XENOGRAFTS AND HOST NORMAL TISSUES, Janet A. Houghton and Peter J. Houghton, St. Jude Children's Research Hospital, Memphis, TN 38101.

Studies in a cultured human colorectal adenocarcinoma cell line (HCT-8) indicated that the endogenous level of 5-phosphoribosyl-1-pyrophosphate (PRPP) was elevated after exposure to MTX; this resulted in increased cytotoxicity when FUra was added subsequently (Cancer Res. 41:994, 1981). The effect of a sublethal i.p. administration of MTX (100 mg/kg) on 1) levels of PRPP in human colon xenografts maintained subcutaneously in immune-deprived CBA/CaJ mice, 2) PRPP concentrations in ileum and bone marrow from normal mice, 3) host toxicity of FUra, and 4) metabolism of [6-^{3}H]FUra (100 mg/kg), were examined *in vivo*.

Injection of MTX caused concentrations of PRPP to increase with time in three tumor lines ($HxELC_2$, $HxGC_3$, $HxVRC_5$), reaching a maximum of 305 to 456% of control between 14 and 24 hours after treatment. In contrast, no elevation of PRPP was observed in bone marrow, while in ileum, PRPP was maximally elevated (968% of control) at 4 hours, and remained above control levels (494% of control), 24 hours after drug treatment. When MTX preceded FUra by 24 hours, toxicity of FUra in normal mice was increased at least 4-fold and was characterized by gastrointestinal damage. This was accompanied by increased metabolism of [6-^{3}H]FUra and incorporation into the RNA of ileum (maximum 300% of control), while concentrations of FdUMP were minimally affected; in $HxELC_2$ tumors, RNA specific activity was increased to 192% of control (maximum), while in line $HxVRC_5$, no increase was observed. The percent increase in PRPP was dose-dependent, and at doses of MTX between 25 and 100 mg/kg, was greater in ileum than in tumors, 24 hours after treatment. It is concluded that the use of MTX in this system does not allow a selective increase of PRPP in tumors during the first 24 hours.

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1057 ENHANCEMENT OF ARA-C METABOLISM AND CYTOTOXICITY BY 2,3-DIHYDRO-1H-IMIDAZO [1,2-b] PYRAZOLE (IMPY) IN HUMAN PROMYELOCYTIC LEUKEMIA (HL-60) CELLS, Steven Grant, Kapil Bhalla, Frank Rauscher III* and Edwin Cadman,* Columbia University College of Physicians and Surgeons, New York, NY 10032 and *Yale University School of Medicine, New Haven, CT

The effect of pretreatment with IMPY, a long-acting ribonucleotide reductase inhibitor, on the metabolism and cytotoxicity of Ara-C was assessed in HL-60 cells. Logarithmically-growing cells were exposed to 3 mM IMPY (12 hr) followed by 10^{-6}M ^{3}H-Ara-C for 45 min. Total intracellular accumulation of Ara-C metabolites was 27.5 ± 4.8 pmol Ara-C/10^6 cells in IMPY-treated cells vs 14.0 ± 3.5 pmol Ara-C/10^6 cells in controls. HPLC analysis revealed an increase in both Ara-CTP generation (9.2 ± 1.4 vs 4.1 ± 1.0 pmol Ara-C/10^6 cells) and 4-hr Ara-CTP retention (4.2 ± 1.1 vs 1.8 ± 0.7 pmol Ara-CTP/10^6 cells) in cells exposed to IMPY. Co-incubation of cells for 12 hr with IMPY along with other ribonucleotide reductase inhibitors (e.g., deoxyadenosine, deoxyguanosine 10^{-4}M, thymidine 10^{-3}M) produced further increments in Ara-C intracellular accumulation. Inhibition of HL-60 colony formation in soft agar was 60% for 3 mM IMPY (12 hr), 82% for 10 nM Ara-C (continuous exposure) and 98% for sequential exposure to IMPY followed by Ara-C. Average inhibition of normal human marrow myeloid progenitors (CFU-GM) obtained from seven patients was 48% for 3 mM IMPY (12 hr), 72% for 10 nM Ara-C (continuous), but only 81% when the drugs were administered in sequence. IMPY enhances the metabolism and cytotoxicity of subsequently administered Ara-C in HL-60 in vitro. This biochemically-rational drug sequence deserves in vivo evaluation.

1058 MODULATION OF THE BIOCHEMICAL AND CLINICAL PHARMACOLOGY OF ARA-A BY DEOXYCOFORMYCIN.
Donald W. Kufe, Pierre P. Major, Ram Agarwal, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115.

We have employed cesium sulfate density gradient analysis to monitor the effect of deoxycoformycin (DCF) on the incorporation of 1-B-D-arabinofuranosyl adenine (ara-A) into cellular nucleic acids. The results demonstrate that ara-A is incorporated exclusively in DNA and that DCF enhances ara-A incorporation in DNA by two-three fold. The ara-A residues in DNA are detectable in internucleotide linkage and are labile in the presence of alkali. Further, we have demonstrated that there is a highly significant relationship ($p<0.0001$) between the incorporation of ara-A in the presence of DCF and loss of leukemic cell clonogenic survival. We have also administered five courses of ara-A and DCF to 2 patients and have studied the effects of DCF on the pharmacokinetics of ara-A. Ara-A was given by IV infusion over three hours at a dose of 300 mg/M^2/day for three days on the first two courses of treatment for each patient. On the initial course of treatment, patients received DCF (10 mg/M^2) 30 minutes prior to the ara-A infusion on days 2 and 3 only. However, on subsequent courses, DCF was administered on each of the three days. Ara-A was undetectable in plasma without DCF pretreatment, while ara-hypoxanthine (ara-Hx) reached levels of up to 13.4 µg/ml. DCF pretreatment resulted in detectable ara-A levels of up to 6 µg/ml and decreased ara-Hx levels. Further, the excretion half-life of ara-A increased during the three-day treatment schedule with DCF. There was no toxicity encountered in these patients and abrupt declines were observed in peripheral blast counts. These results demonstrate that DCF modulates both the clinical and biochemical pharmacology of ara-A.

1059 A ROLE FOR RATE OF 5-FLUOROURACIL (FUra) INCORPORATION INTO RNA IN MEDIATING FLUOROPYRIMIDINE CYTOTOXICITY.
R. Douglas Armstrong and Ed Cadman. Yale University School of Medicine, New Haven, CT 06510.

The incorporation of FUra into RNA (FUra-RNA) can contribute to fluoropyrimidine (F.P.) cytotoxicity. Numerous experiments have demonstrated that as the concentration of a F.P. is increased, a corresponding increase in FUra-RNA and RNA-directed toxicity results. However, there may be a more precise factor mediating RNA-directed toxicity than simply the level of FUra-RNA. Complete growth inhibition (G.I.) of Ehrlich ascites tumor cells results following a 4 hr exposure to either 1.0mM 5'-deoxy-5-fluorouridine (5'-dFUrd) or 0.01mM 5-fluorouridine (FUrd). Thymidine (dThd) supplementation will reverse 5'-dFUrd G.I. (suggesting that the toxicity was not RNA-directed), while FUrd G.I. was unaltered by dThd (suggesting toxicity was RNA-directed). In direct contrast to these results, a lower level of FUra-RNA resulted from FUrd compared to the FUra-RNA in cells exposed to 5'-dFUrd (FUrd: 4.2 pmol FUra/µg RNA; 5'-dFUrd: 11 pmol/µg RNA). However, FUra incorporation into RNA resulting from FUrd was found to occur at a much higher rate than the rate of FUra incorporation resulting from 5'-dFUrd (i.e. FUrd: $k = 0.021$ min^{-1} vs 5'-dFUrd: $k=0.007$ min^{-1}). These results suggest that RNA-directed F.P. cytotoxicity may reflect the rate of FUra incorporation (which determines the frequency of FUra for uracil substitutions per molecule of nascent RNA), rather than just the level of FUra in total cellular RNA. This concept would provide a rational means of developing drug combinations or administration regimens to either enhance or attenuate RNA-directed toxicity (depending on the result wanted) by altering F.P. metabolism and the resulting rate of FUra incorporation into RNA.

1060 COMBINATION CHEMOTHERAPY DIRECTED AT POTENTIATING THE INHIBITION OF RIBONUCLEOTIDE REDUCTASE,
Joseph G. Cory, Atsushi Sato and Gay L. Carter, University of South Florida College of Medicine, Tampa, FL 33612.

Ribonucleotide reductase (RR) catalyzes the key reaction in which the deoxyribonucleotide precursors of DNA are generated. RR has been separated into two components (the non-heme iron, NHI and the effector binding, EB), neither of which have enzyme activity alone. It has been possible to demonstrate that the known RR inhibitors are specific inhibitors of either the NHI or EB component. Hydroxyurea, guanazole, IMPY and the thiosemicarbazones are inhibitors/inactivators of the NHI component while Inox, 5'-deoxyinox, pyridoxal phosphate, dATP, dGTP and dTTP are inhibitors/inactivators of the EB component. Utilizing combinations of a NHI component inhibitor and an EB component inhibitor, potentiation of the inhibition of ribonucleotide reductase was observed. The addition of Desferal to either IMPY or hydroxyurea markedly potentiated the inhibition of ribonucleotide reductase activity and L1210 cell growth. Addition of EHNA, to a combination of deoxyadenosine and IMPY also potentiated the inhibition of cell growth. These studies show that rational combination chemotherapy protocols can be devised which are directed at potentiation of the inhibition of the ribonucleotide reductase site.

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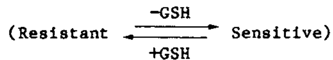
1061 CELL-SURFACE P-GLYCOPROTEIN IS ASSOCIATED WITH MULTI-DRUG RESISTANCE IN MAMMALIAN CELLS, N. Kartner*, J.R. Riordan† and V. Ling*, The Ontario Cancer Institute*, Hospital for Sick Children†, University of Toronto, Toronto, Canada M4X 1K9.
Mutations resulting in drug-resistance may limit successful cancer chemotherapy. A number of mutant mammalian cell lines have been isolated and shown to be multiply resistant to unrelated drugs as a result of reduced plasma membrane permeability. Previously we have isolated colchicine-resistant Chinese hamster ovary (CHO) cells which express such a membrane impermeability and have identified a 170K dalton surface glycoprotein (P-glycoprotein) which appears to be associated with this phenotype. In the present work, we examine the possibility that other multi-drug resistant lines also express this protein. This was found to be the case for a number of hamster, mouse, and human lines multiply resistant to antineoplastic drugs. Furthermore, an antiserum reacting with CHO cell P-glycoprotein cross-reacts with the presumptive P-glycoproteins of the other lines. Thus, it appears that multi-drug resistance may be universally mediated by some common mechanism which involves a conserved mammalian cell surface component, the P-glycoprotein. Such a protein could be a functionally important constituent of the normal cell surface. Its predominance in the drug-resistant cell may be due to overproduction as a result of gene amplification.
(Supported by NCI and MRC of Canada)

1062 INHIBITION OF GLUTATHIONE BIOSYNTHESIS BY S-n-BUTYL HOMOCYSTEINE SULFOXIMINE AND SENSITIZATION OF MURINE TUMOR CELLS RESISTANT TO L-PHENYLALANINE MUSTARD, David T. Vistica, Susan Somfai-Relle, Kayoko Suzukake, and Barbara Petro, Laboratory of Medicinal Chemistry and Biology, National Cancer Institute, Bethesda, Maryland 20205.

L-Phenylalanine mustard (L-PAM) resistant tumor cells can be completely sensitized to L-PAM by growth of cells in medium with a reduced concentration of the amino acid L-cystine to reduce the intracellular concentration of glutathione (Biochemical Pharmacology, in press). The present results indicate that the resistance is reversible and that the extent


(Resistant $\underset{+GSH}{\overset{-GSH}{\rightleftarrows}}$ Sensitive)

of the reversibility depends on the time interval needed to restore intracellular glutathione to pretreatment concentrations. During this period of intracellular glutathione replenishment, cells exhibit intermediate sensitivity to L-PAM. In an effort to apply these observations we have isolated sensitive and resistant tumor cells from CDF_1 mice and cultured them in medium containing S-n-butyl homocysteine sulfoximine, a potent inhibitor of γ-glutamylcysteine synthetase. These results indicate that treatment of L-PAM resistant cells with this inhibitor of glutathione biosynthesis results in a significant decrease in intracellular glutathione and nearly complete sensitization of the L-PAM resistant cell population. These observations served as the basis for in vivo studies which demonstrated that continuous intraperitoneal infusion of S-n-butyl homocysteine sulfoximine from an Alzet osmotic minipump prior to L-PAM administration resulted in an increase in the lifespan of tumor bearing animals.

Tumor Heterogenecity in Animals and Man

1063 TUMOR HETEROGENEITY AND DRUG RESPONSE, Bonnie E. Miller and Gloria H. Heppner, Michigan Cancer Foundation, Detroit, MI 48201.

It has been demonstrated repeatedly that tumors consist of subpopulations which differ in sensitivity to chemotherapeutic agents. This has suggested that the response of tumors to therapy is a function of the response, or lack thereof, of the individual subpopulations. We have shown that, although individual subpopulations may have characteristic sensitivities when they are growing in isolation, the sensitivities are changed when they are grown in each other's presence. With a mouse mammary tumor model system, we are investigating the mechanisms through which one subpopulation can influence the sensitivity of another. To date, three clases of mechanism have been seen. One type of mechanism is apparently dependent on the host. This is illustrated by the ability of a cyclophosphamide-sensitive subpopulation to enhance the sensitivity of a relatively resistant subpopulation through an effect on drug activation. A second type of mechanism is illustrated by the ability of a methotrexate-sensitive subpopulation to increase the sensitivity of another subpopulation in vitro. This interaction is mediated by a diffusable substance which is dependent on methotrexate metabolism. A third example of drug sensitivity interactions is the transfer of thioguanine sensitivity from sensitive to resistant cells. This interaction requires cell contact, and apparently occurs through the transmittal of thioguanine nucleotides across gap junctions. All three of these examples of drug sensitivity interactions show that tumor subpopulations do not respond independently to chemotherapy, but instead comprise an interacting system. (Supported by NCI Grant CA-27419 and the E. Walter Albachten bequest).

1064 CHEMOTHERAPEUTIC RESPONSE IN XENOGRAFTS: INTER– AND INTRA–TUMOR HETEROGENEITY. Peter J. Houghton and Janet A. Houghton, St. Jude Children's Research Hospital, Memphis, TN 38101.

A series of childhood rhabdomyosarcomas, each derived from a different patient, have been grown as xenografts in immune-deprived mice. The intrinsic sensitivity of tumors has been examined using classes of agents with different mechanisms of cytotoxicity that demonstrate cross-resistance in cells acquiring resistance to one class. Responses of tumor lines treated with a single maximally tolerated dose of vincristine (VCR), dactinomycin (DACT) or adriamycin (DOX) are shown. A tumor line intrinsically resistant to DACT (Rh12) or DOX (Rh14) may be very sensitive to VCR. This may indicate that the mechanism(s) responsible for intrinsic resistance and acquired resistance may be different. In order to determine whether the intrinsic sensitivity was a consequence of heterogeneous cell populations with different sensitivities, the rate at which resistance developed during VCR therapy (1.5 mg /kg q. 7d) was examined. From tumor RD a stable resistant line with an altered karyotype was selected readily. Resistant lines from Rh14 and Rh12 tumors have not been derived. These preliminary data suggest that initial tumor response is determined by heterogeneous populations with varying intrinsic sensitivities.

Tumor Line	VCR	DACT	DOX
Rh12	+++++	–	+++
Rh14	++++	–	–
Rh18	+++	+++	+++
RD	++	–	–

(Response: +++++ = cures → – = growth not inhibited)

Supported by grant CH-156 from the American Cancer Society and by ALSAC.

1065 ANALYSIS OF CELLULAR HETEROGENEITY IN HUMAN OVARIAN CARCINOMA; CHANGES ASSOCIATED WITH PROGRESSION IN AN UNTREATED PATIENT, Ronald N. Buick, William J. Mackillop, Jean-Pierre Bizzari and Steven S. Stewart, Ontario Cancer Institute, Toronto, Ontario, Canada.

Cellular heterogeneity associated with differentiation in human ovarian tumour populations has been studied. A composite picture of the cell renewal hierarchy in this malignancy has been developed through the identification of cells with a variety of functions and phenotypes within the tumour cell population. Those subpopulations characterized include stem cells (clonogenic cells with self-renewal potential), clonogenic cells (agar colony formation), proliferative cells (Labelling Index), cells expressing surface carcinoembryonic antigen, cells expressing surface NB/70K (a human ovarian differentiation antigen) and differentiated cells (those demonstrating accumulation of fat droplets histochemically). The relationship of these subpopulations has been analysed on the basis of cell density and volume characteristics. These procedures have been performed on seven consecutive malignant ascites samples (over a 9 month period) from an untreated patient with ovarian carcinoma. A progressive loss of cell differentiation features was seen with a concomitant shift of cells into clonogenic and stem cell subpopulations.

1066 SPECIFIC KARYOTYPIC AND TUMORIGENIC CHANGES IN CLONED SUBPOPULATIONS OF HUMAN GLIOMAS EXPOSED TO SUBLETHAL DOSES OF (BCNU). J.R. Shapiro, W-K.A. Yung and W.R. Shapiro. Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Five human gliomas were dissociated into single cells which were karyotyped and cloned (Cancer Res. June, 1981). Clones, cytogenetically identified as part of the original tumor, were expanded and treated with sublethal doses of BCNU. Control and treated clones were analyzed at passages 2, 8 and 12 to determine (1) clonal homogeneity, (2) retention or loss of specific chromosomes, and (3) growth potential in soft-agar and in the nude mouse brain. Clones near-diploid in chromosome number remained stable in vitro and retained their parental karyotype without producing new variants, while hyperploid (3n and 4n) clones were unstable, generating many new variant cells. In control cultures stable clones lost or gained few chromosomes, while unstable clones frequently lost chromosomes 1, 3, 9, 22, X and y, and/or gained chromosomes 19, 20 and 21. The modal chromosome number increased in 8 of 10 control clones and only 0.3% of the metaphases had structural rearrangements. None of the control clones grew in soft-agar or in nude mice. In contrast, BCNU (1 μg/ml for 1 hour) weekly for 5 weeks, altered both the chromosomal complement and the tumorigenicity of the clones. By passage 12, 6 of 10 clones showed a decrease in their modal chromsome number, loss of chromosomes 1, 2, 5 and 22, and structural rearrangements involving chromosomes 3, 10, 11, 21 and X. Five of 8 clones grew in soft-agar; of these, 2 grew in the nude mouse. Human glioma cells subjected to low dose BCNU developed marked changes in the distribution and structure of some chromosomes. The loss and retention of specific chromosomes may identify those important in tumorigenicity and drug resistance.

Analysis of Response Determinants in Man

1067 QUANTITATIVE REQUIREMENTS FOR CYTOXICITY OF 5-FU AGAINST HUMAN TUMOR CELLS.
John E. Byfield and Paula Calabro-Jones, Division of Radiation Oncology, University of California, San Diego, 92103.

We have determined the time-dose relationships for cell killing by 5-FU against 6 human epithelial tumor cell lines (Colon: HT-29/SP-1, LoVo/SP-1, Colo 205; Cervix: HeLa, Me-180-VCl; Ovary 2008). These have then been compared to the known human pharmacokinetics of 5-FU under bolus and infusional (24-120 hours) conditions. All of the tumor lines studies show a relatively uniform sensitivity to 5-FU, differences being greatest during pulse exposures. By comparing the sensitivity of the cells on a 60 minute exposure to the integral value for a bolus injection in man it can be seen that clinical responses to the drug would be very uncommon. However, similar data for clinically tolerable infusions >72 hours would predict a significantly enhanced response rate. Protection of human tumor lines by extracellular thymidine (T) must be clinically negligible since T does not protect any of the 6 lines studied at meaningful T levels. Similarly, serum uracil levels in man are well below those needed to protect. Overall the data strongly suggests that 5-FU infusions should significantly enhance the response rate to this drug. Coincident clinical studies by our group suggest 72 hours infusions may be optimal.

1068 DETERMINANTS OF SENSITIVITY TO FLUOROPYRIMIDINES IN GASTROINTESTINAL TUMOR CELLS
Wendy L. Washtien, Northwestern University Medical School, Chicago, ILL 60611

Five human gastrointestinal tumor cell lines were examined for their sensitivity to the fluoropyrimidines, fluorodeoxyuridine (FdUrd) and fluorouracil (FUra). The EC_{50} for FdUrd in these cell lines varied from 0.44 nM for SW 403 cells to 16 nM for HuTu 80 cells and in all cases was reversed by the addition of thymidine. The level of thymidylate synthetase in each cell line was determined by titration, using ^3H fluorodeoxyuridine-5'-monophosphate (FdUMP) as a specific probe. The concentration of FdUrd required for cytotoxicity in these cell lines correlated directly with the level of thymidylate synthetase in the particular cell line.

The cytotoxicity of FUra also varies among these cell lines: EC_{50} values range from 0.03 uM for SW 403 cells to 6 uM for HuTu 80 cells. For this drug, although a strong correlation exists between EC_{50} values for FUra and thymidylate synthetase levels in these cells, cytotoxicity cannot be completely reversed by the addition of thymidine, and in one cell line, thymidine appears to potentiate the cytotoxic effects of FUra. FUra metabolism was examined more closely in several of these cell lines to determine what other parameters are important in determining the differential cytotoxicity of FUra to these cells.

1069 CIRCUMVENTION OF ARA-C RESISTANCE IN MURINE AND HUMAN LEUKEMIA BY HIGH DOSE ARA-C: (Hi DAC): A MEANS FOR PHARMACOLOGIC "SELF-POTENTIATION", RL Capizzi, J-l Yang, F Griffin, E Cheng, D Sahasrabudhe, Y-c Cheng, Univ. North Carolina, Chapel Hill, NC 27514

Of the known pharmacologic determinants of ara-C action, some of the most significant include 1) phosphorylation of the nucleoside via dCyd kinase (dCydK) 2) inactivation via dCyd deaminase (dCydD) to ara-U, 3) duration of exposure (pharmacokinetics), 4) ara-CTP pool viz a viz dCTP pool, 5) incorporation of ara-CTP into DNA. All of these factors can be affected in a positive therapeutic way as a function of substantial increase in dose. A subline of L5178Y resistant to continuous exposure to 78uM ara-C has only 10% of original dCydK activity and negligible dCydD activity. In contrast, colon cancer HCT-8, has a high degree of dCydD activity. In both cell lines significant cytotoxicity is achieved with exposure to 0.1-1mM concentrations of ara-C. These levels are readily achievable in mice and man using gram dosages of ara-C. In leukemic mice, a bolus dose of 1 gm/kg results in mM blood levels and is curative without significant host toxicity. Studies of the pharmacokinetics of HiDAC (3 hr infusions of $3Gm/m^2$) in man reveals a triphasic decay with $T\frac{1}{2}$ α-10 min, β-29 min and γ-270 min. At 540 min the plasma level is still > 0.6uM, a value higher than that achieved during steady state from continuous infusions of 100-200 mg/m^2. Significant deamination of ara-C to ara-U was evident within 15 min with a peak level of 346 $\pm$ 146uM ara-U with a $T\frac{1}{2}$ β>6 hrs. The prolonged high plasma ara-U concentrations may account for the prolonged γ phase of ara-C in that ara-U serves as a competitive inhibitor of dCydD with a Ki of 5.6mM (Km of ara-C for dCydD is 125uM) providing a means for pharmacokinetic "self potentiation". Combination of HiDAC with sequential asparaginase in the treatment of refractory and high risk ANLL patients resulted in a 64% CR rate with tolerable toxicity, indicating a significant "dose effect" in these patients.

1070 THE USE OF NORMAL BONE MARROW CFU-GM TO ENHANCE THE PREDICTIVE ACCURACY OF THE HUMAN TUMOR STEM CELL ASSAY (HTSCA). Verena Hug, Benjamin Drewinko, Gary Spitzer, George Blumenschein, M. D. Anderson Hospital and Tumor Institute, Houston,TX 77030
In order to improve the HTSCA for the testing of newly-developed anti-cancer agents, we have expanded the assay system to include evaluation of drug effects on normal bone marrow CFU-GM. Myeloid progenitor cells are dose limiting host tissue cells for most cell cycle active chemotherapeutic agents. Determination of selective drug effects on tumor and myeloid progenitors allows the calculation of an in vitro "therapeutic index" for each drug. In our studies the dose range that inhibited bone marrow CFU-GM formation by greater 30% and less than 90% was considered to represent the range for relevant in vitro drug testing. Regression lines of dose-dependent bone marrow CFU-GM survivals with 3 anthraquinones derivatives, adriamycin, bisantrene and misantrene demonstrated that neither plasma levels nor clinically effective dosages can be utilized to extrapolate the dose range for in vitro testing. Drug sensitivity testing using the in vitro relevant dose range in 23 tumor samples changed the prediction of sensitivity by 50% (elimination of 5 false positives, addition of 1 false negative) and the prediction for resistance by 57% (elimination of 2 false positives and addition of 6 false negatives).

1071 PREDICTION OF RESPONSE TO THERAPY FOR ACUTE NONLYMPHOCYTIC LEUKEMIA (ANLL), H.D. Preisler, M.D. and Azra Raza, M.D., Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263
Remission induction therapy for ANLL requires the administration of intensive chemotherapy which always produces toxicity but does not always result in therapeutic benefit. The ability to determine in advance the drug sensitivity of leukemic cells would permit the avoidance of drugs which would produce only toxicity. The most effective method to date for measuring in vitro drug sensitivity is the measurement of the ability of drugs to kill the clonogenic leukemic cells (LCFUc). For previously untreated patients one can recognize the majority of patients who will benefit from ara C/adr therapy. 15 of 17 patients whose leukemic cells did not clone in vitro entered CR while only 37 of 57 patients whose cells cloned in vitro entered CR ($p=0.08$). In vitro sensitivity to ara C/adr was measured in 26 patients whose cells cloned in vitro. 13 of 14 patients in whom >30% of their LCFUc were killed by ara C/adr entered CR while only 5/12 patients entered CR if < 30% of their LCFUc were killed ($p=0.009$). The LCFUc system has several limitations: the cells of 25-30% of patients will not clone in vitro, the method requires at least 1 weeks time, and the small numbers of drug resistant cells, which probably ultimately cause relapse are not detectable. To this end we are developing flow cytometric methods for measuring drug resistance. This approach permits the interrogation of thousands of cells/minute and is capable of detecting rare drug resistant cells. A comparison of the LCFUc system and flow cytometry with respect to recognizing anthracycline resistant cells will be presented.

Liposome Encapsulation: Determinants of Localization and Drug Uptake in the Whole Animal

1072 LIPOSOME-ENTRAPPED ADRIAMYCIN: IN VIVO TISSUE DISTRIBUTION AND ANTITUMOR ACTIVITY A. Gabizon, A. Dagan, Y. Barenholz and Z. Fuks, Dept. of Radiation & Clinical Oncology, Hadassah University Hospital and Dept. of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel.
Neutral and negatively charged liposomes containing adriamycin (ADM) were tested for their *in vitro* cytotoxicity, *in vivo* tissue distribution and antitumor activity. Entrapment of ADM in negatively-charged liposomes containing phosphatidyl-serine (PS) or cardiolipin (DPG) was 3 to 4-fold higher than in neutral phosphatidyl-choline (PC) liposomes with or without cholesterol (Chol). In the presence of human serum for 1 hr, PC: Chol and PS:PC:Chol liposomes retained more than 80% of the drug while DPG:PC:Chol retained less than 40%. *In vitro* studies with primary cell cultures of a murine lymphoma showed that the cytotoxic activity of ADM was fully preserved with all the liposome preparations tested at the lowest active concentration of free ADM (10^{-7}M). Tissue distribution studies after i.v. injection into mice indicated that the levels of ADM increased severalfold in liver and spleen. In addition, the hepatic clearance of ADM was significantly slowed down by liposome drug delivery. The concentration of ADM in the heart was markedly diminished by delivery with PS:PC:Chol and PC:Chol liposomes but only slightly reduced when DPG:PC:Chol liposomes were used. The *in vivo* antitumor activity of liposome entrapped ADM was tested on a metastatic murine lymphoma. PS:PC:Chol liposomes containing ADM, given as a single i.v. injection, were at least as effective as an equivalent dose of free ADM in prolonging survival. These results indicate that liposome delivery may offer an efficient means of restricting cardiac uptake of ADM while preserving its antitumor therapeutic activity.

1073 TARGETING OF LIPOSOME ENCAPSULATED DRUGS BY COVALENTLY COUPLED MONOCLONAL ANTIBODIES,
Jacques Barbet, Patrick Machy and Lee D. Leserman, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cédex 9, France.
The targeted delivery of drugs in liposomes has been proposed as a possible improvement for chemotherapy and particularly for anti-neoplastic therapy. Monoclonal antibodies directed against tumor cells are excellent candidate as liposome-targeting ligands. However, until recently, this approach has been limited by the lack of means to fix these antibodies to liposome surfaces where they can mediate specific interactions with cells. Our newly developed technique for the covalent coupling of monoclonal antibodies to small, sonicated liposomes was used to study the specific delivery of methotrexate (MTX) to cellular targets in vitro. Liposomes containing MTX were coupled to monoclonal antibodies against determinants expressed by murine lymphocytes. These were incubated with lipopolysaccharide or Concanavalin A induced blast cells from CBA (H-2^k) or C57BL/6 (H-2^b) spleens. Only spleen cells that bind the monoclonal antibody used as the targeting ligand showed evidence of drug effect, demonstrating that the interaction of the liposomes with the cells was specific. With regard to the different determinants and antibodies, the effect of the liposome encapsulated MTX was not correlated with the number of liposomes bound to cells. These techniques thus provide a simple quantitative assay for the "internalization potential" of the target antigen for any monoclonal antibody, and may be useful for the design of targeted chemotherapy.

1074 ALTERED DISPOSITION AND INCREASED THERAPEUTIC ACTIVITY OF CHEMOTHERAPEUTIC AGENTS DUE TO LIPOSOME ENTRAPMENT.
Eric Forssen, John Todd, and Zoltan A. Tokes, Department of Biochemistry and the Cancer Center, University of Southern California, Los Angeles, CA 90033, U.S.A.
The antitumor activities of two chemotherapeutic agents, doxorubicin (Dxn) and methotrexate (MTX), entrapped in liposomes were investigated. Encapsulation of MTX in unilamellar liposomes increased its association with resistant CCRF/CEM leukemic cells fifty-fold compared to free drug, however cytostatic activity was not increased by a corresponding amount. Rat liver carcinoma cells were also able to take up the entrapped MTX at a much faster rate than free MTX. In contrast, the free drug was metabolized to its polyglutamate derivatives at a faster rate than entrapped drug, demonstrating that liposome encapsulation can alter the availability of a drug to the metabolic pathways within cells. Dxn liposomes nearly eliminate cardiac toxicity. Mice receiving liposome entrapped Dxn had peak cardiac tissue drug levels which were not substantially lower than those animals receiving free drug, indicating that liposomes can exert their protective effect by a mechanism other than the total exclusion of Dxn from cardiac tissue. Increased antitumor action has not been associated with greater tumor drug levels suggesting that liposomes mediate increased cytotoxicity by a mechanism other than higher local drug concentrations. In immune suppression tests, animals treated with two doses of free drug (5 mg/kg) following immunizations with xenogeneic red blood cells were able to produce minimal titers of hemagglutinating antibody. Liposome encapsulation however was able to prevent this immune suppression. These experiments indicate that the increased therapeutic effects seen with entrapped MTX and Dxn are not the direct results of an altered drug delivery to a specific tissue, but due to an altered drug disposition which may result in a reduced toxicity. (USPHS Ca 06481)

Biochemical Targeting: The T-Lymphocyte Malignancies

1075 CLINICAL AND BIOCHEMICAL SEQUELAE OF TREATING T-CELL MALIGNANCIES WITH 2'-DEOXYCOFORMYCIN,
John Smyth, Grant Prentice and Victor Hoffbrand, Edinburgh University and Royal Free Hospital, London, U.K.
2'-Deoxycoformycin (DCF) is a potent inhibitor of adenosine deaminase, whose activity is elevated in T-cell malignancies. In this phase II study we have treated 26 relapsed leukaemia/lymphoma patients with DCF (0.15 - 0.5 mg/Kg daily x 5). A 75% response rate was obtained in 12 patients with Thy-acute lymphoblastic leukaemia (7 complete remissions). Responses were seen in 4/6 T-cell lymphomas (1 complete) but only 1/5 common-ALLs responded. Remission duration ranges from 1 - 14+ months. The major toxicity was renal (38% patients). This was reversible in 6/10 patients but there were 4 deaths from renal failure. Conjunctivitis and minor elevations of serum aspartate transaminase levels were seen in 31% and 54% patients respectively. Determination of blast cell deoxyribonucleotides showed a positive correlation between clinical response and intracellular accumulation of dATP with decreased concentrations of the other three deoxyribonucleotides. Furthermore 2-hour in vitro exposure of pre-treatment blasts to DCF (10^{-5}M) plus deoxyadenosine (10^{-4}M) demonstrated a positive correlation between the accumulation of dATP and subsequent clinical response, suggesting the potential to predict response. The latter is particularly relevant to the further evaluation of this highly effective but nephrotoxic agent.

1076 DEOXYNUCLEOSIDE METABOLISM IN T/B HYBRID LYMPHOBLASTS: Joanne Kurtzberg and Michael S. Hershfield, Departments of Medicine and Biochemistry, Division of Rheumatic and Genetic Diseases, Duke University Medical Center, Durham, North Carolina 27710.
2'-Deoxycoformycin (dCF), a potent inhibitor of adenosine deaminase (ADA), has been effective in the treatment of T-cell, but not B-cell leukemias, a finding that appears to be related to the ability of T, but not B-lymphoblasts to trap deoxyadenosine (dAdo) as dATP. Both increased ability of T-cells to phosphorylate dAdo, and of B-cells to degrade deoxyribonucleotides could explain this distinction. However, differences in activities of dAdo kinases and cytoplasmic deoxyribonucleotidases in T- and B-cell lines are not sufficient to account for the 100-fold difference in their capacity to accumulate dATP. To further evaluate the basis for this phenomenon we have developed human B-cell/T-cell hybrid cell lines, and evaluated 1) sensitivity to the cytotoxic effect of dAdo 2) ability to accumulate dATP, and 3) ability to degrade intracellular dATP after expansion of this pool by prior incubation with dAdo. We fused CEM(T) cells with either WI-L2 (B) cells, or with a double mutant derived from WI-L2 that lacked both enzymes necessary to phosphorylate dAdo (adenosine and deoxycytidine kinases = AK^{-}-dCK^{-}). The concentration of dAdo required for 50% growth inhibition of CEM, WI-L2, and several CEM/WI-L2 hybrids were respectively, 0.35μM, 45μM, and 15-35μM. The hybrids were intermediate in their capacity to accumulate dATP, and in their capacity to catabolize an expanded dATP pool. CEM/AK^{-}-dCK^{-} hybrids were resistant to dAdo. Our results thus far indicate that "B-ness" is dominant over "T-ness", and are consistent with the operation in B-cells of a mechanism, possibly enhanced nucleotidase activity, that diminishes the ability to accumulate dATP from dAdo.

1077 RAPID DEVELOPMENT OF FUdR RESISTANCE AND ITS REVERSION BY HAT IN HUMAN T CELLS. A. Piper & M. Dyne, Ludwig Institute for Cancer Research, Sydney University. Aust. 2006.
Development of drug resistance is a major problem in chemotherapy. Studies with cell lines and animal tumors indicate that drug resistance mutation frequencies are usually $<10^{-5}$. However, in the human leukemic T-cell line CCRF-CEM development of FUdR resistance, associated with loss of thymidine kinase, occurs at a much higher frequency ($>10^{-2}$). Outgrowth of FUdR resistant cells occurs after 3-5 days exposure to an initially cytotoxic FUdR concentration (3μM). Cellular thymidine kinase declined steadily after 2 days exposure to FUdR and was undetectable after 6 days. The FUdR-resistant cells were approx. 400 times more resistant and resistance was maintained during 3 months growth without FUdR. However, exposure of FUdR-resistant cells to HAT (hypoxanthine 10^{-4}M, aminopterin 10^{-7}M, thymidine 10^{-5}M) resulted in reversion at a similar high frequency, outgrowth of FUdR-sensitive cells occurring within 3-4 days exposure to HAT. The high frequency of development and of reversion of FUdR resistance suggests that the changes in thymidine kinase expression, though stable and inheritable, are not due to gene mutation. Whilst it is possible that the wild-type CEM cells contain a stable population of FUdR-resistant cells, it is unlikely that reversion in HAT would be similarly due to a stable population of FUdR-sensitive cells, since these should have been eliminated, or if not cycling, continually diluted during the growth in FUdR to select resistant cells. Alternatively, development of FUdR resistance and its reversion in HAT could occur via stable and inheritable changes in gene expression. Such changes may be involved in drug resistance in vivo and may be expected to be as readily reversible as they are acquired, provided the appropriate selection pressure can be applied.

1078 ENZYMES, INHIBITORS, AND LEUKEMIA, LYMPHOMA, Mary Sue Coleman and *Michael Grever, University of Kentucky, Lexington, KY, 40536, and *Ohio State University, Columbus, OH, 43210.
We have characterized enzymes which play unique roles in DNA metabolism of human lymphocytes and have developed new strategies of therapy for lymphoma-leukemia by exploiting properties of these enzymes. Those selected were human terminal deoxynucleotidyl transferase (TdT), adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP). Immunofluorescence or radioimmunoassays were developed for each. Both TdT and ADA were shown to be useful markers of cell type. In a trial of 71 patients with CML, we show ADA to exhibit a high correlation with disease course. Elevated ADA in buffy coat occurs concommitant with acceleration of CML (24/31) and may be useful in predicting onset of blastic transformation. Concurrent TdT assays in blast crisis identify lymphoid populations (30% of patients). We are currently testing selective inhibitors of these enzymes _in vitro_ and _in vivo_. Deoxycoformycin (dCF), a potent inhibitor of ADA, is being tested in _clinical_ trials. Thirty-four patients with hematologic malignancies have been treated and the biochemical consequences of subsequent perturbation of nucleotide metabolism were monitored in a variety of blood cells and body fluids. The toxicity of dCF, uniformly associated with depletion of ATP in cells and elevation of dATP, can be controlled by monitoring dATP levels in erythrocytes. Chronic administration of low doses of dCF is promising in treatment of CLL (7) and mycosis fungoides (4). Ablation of ADA activity by dCF in combination therapy with Ara A has been explored with encouraging results in refractory leukemia.

1079 DIFFERENTIAL SENSITIVITY OF HUMAN T AND B LYMPHOBLASTS TO CYTOTOXIC NUCLEOSIDE ANALOGS, Vernon L. Verhoef and Arnold Fridland, St. Jude Children's Research Hospital, Memphis, TN 38101

The selective cytotoxicity of purine deoxyribonucleosides for T cells is a currently proposed mechanism for the selective disfunction of T cells in immunodeficiency diseases. Cytotoxic nucleoside analogs might therefore be useful agents to selectively kill subpopulations of human lymphoid cells during lymphoproliferative diseases. We have compared the sensitivity of cultured T lymphoblasts with cultured B lymphoblasts to growth inhibition by ten cytotoxic purine and pyrimidine nucleosides and their analogs. Four such agents, adenosine, in combination with deoxycoformycin (dCF), 2'-azido-2'-deoxy-arabinosyladenine (2'-azido-2'-deoxy-araA) with dCF, pyrazofurin and 5-fluoro-deoxyuridine, are equally cytotoxic to T and B lymphoblasts and are termed Type I agents. Four nucleoside analogs, araA with dCF, 2-fluoro-araA, araC and 2'-O-nitro-araC, are 2-6 fold more cytotoxic to T cells than B cells and are termed Type II agents. Two deoxyribonucleosides, deoxyadenosine with dCF and deoxythymidine are 28-50 fold more cytotoxic to T cells than B cells and are referred to as Type III agents. Type II and III agents, with the exception of deoxythymidine, were 7 to 2000 fold less effective against T-lymphoblast CCRF-CEM cells deficient in both deoxycytidine kinase and adenosine kinase whereas Type I agents were 1 to 1000 fold less effective against the double mutant. Phosphorylation via deoxycytidine kinase was uniquely associated with cytotoxicity of Type II and Type III agents. The 5'-nucleotide derivatives of seven of these agents were tested as substrates for a partially purified preparation of a soluble deoxynucleotidase of CCRF-CEM cells. The results revealed a striking positive correlation between an agent's selectivity for T cells and the specificity of its 5'-phosphorylated derivative for the nucleotidase. The role of soluble nucleotidase in nucleoside analog metabolism in T and B cells is now being investigated. (Supported by grants from NIH CA-09346, ACS IN-99H, and by ALSAC.)

1080 MECHANISM OF ACTION OF DEOXYADENOSINE/ADENOSINE DEAMINASE INHIBITOR COMBINATIONS: LYMPHOTOXICITY IN G_1 AND G_0 PHASES OF CELL CYCLE. R,M, Fox and R.F. Kefford, Ludwig Institute for Cancer Research (Sydney Branch) University of Sydney, N.S.W. 2006. Australia.

ADA inhibitors are undergoing clinical trial in management of T. ALL and other lymphoid malignancies. Cultured T. ALL are sensitive to µM dAdo in the presence of ADA inhibitors (EHNA or deoxycoformycin). Using flow cytometry and BdUrd quenching of 33343 Hoechst DNA fluorescence we show dAdo (3µM) causes a G_1 block in T. ALL cells. Cells in S phase exposed to dAdo complete that S phase, pass through G_2 + M, enter and accumulate in G_1. This phenomenon is prevented by coincubation with dCyd which competes with dAdo for phosphorylation by dAdo kinase. By contrast dAdo (400 µM) induces an S phase block in resistant EBV transformed B cells. The dAdo sensitivity of T. ALL cells correlates with dATP accumulation but not with dAdo inhibition of S Adenosyl-homocysteine Hydrolase. Peripheral blood lymphocytes (T, non-T and B. CLL) in G_0 (by DNA flow cytometry) exposed to µM dAdo (in presence of ADA inhibition) elevate their dATP pools to a similar extent as T. ALL cells. Progressive cell death was observed after 24 hr. Peripheral blood lymphocytes (T and nonT) and T. ALL lack an ecto-adenosine triphosphatase which is present in EBV transformed B lymphocytes. This enzyme can degrade dATP (1-3 µmoles/10^6 cells/hr). Mitogen transformed B but not T lymphocytes express this enzyme activity. This specific toxicity of dATP for lymphocytes in G_0/G_1 is not compatible with inhibition of ribonucleotide reductase. This cycle specific toxicity is analogous to that produced by glucocorticoids or dibutyryl cyclic AMP. The exact biochemical mechanism of dAdo induced cytotoxicity remains to be determined.

Drug Transport and Antitumor Response

1081 A Binary Model of Alkylating Agent Transport into Cells: Clinical Implications. John E. Byfield & Paula Calabro-Jones, Univ. California, San Diego, Calif. 92103

Recent experiments in this laboratory have shown that the useful anti-cancer alkylating agents (AA) can be divided into two distinct classes: Carrier-dependent drugs (CDAA) and Carrier-independent agents (CIDAA).[1] CDAA are primarily water-soluble and enter into mammalian cells by normal membrane (transport) carriers such as amino acid, lipid precursor and probably sugar carrier proteins. Included among the CDAA are compounds such as nitrogen mustard, cis-dichloro diammine platinum, melphalan, activated cyclophosphamide etc. The model predicts that the fundamental requirement for membrane carrier transport dictates many, if not all, of CDAA properties such as high activity against rapidly proliferating cells, exclusion by the blood-brain barrier, tissue-receptor normal tissue toxicity (renal, pancreatic), relative sparing of non-proliferating marrow stem cells, and their capacity to develop membrane-related resistant tumor cells. Contrariwise, CIDAA are either lipid-soluble or amphipathic and show virtually the opposite set of physiological properties. Of interest is the further observation that at least one CDAA (phosphoramide mustard) shows a histogenetic selectivity against ontogenetic lymphoid products (B versus T cells) that also appears membrane receptor mediated. The usefulness of the model lies in: (a) its implications for the development of entirely new CDAA, (b) its potential for using schedule manipulations for suppressing resistant mutants, and (c) the obvious constraints it places on the potential for useful polychemotherapeutic manipulations using existing AA. Supported by the Irvin Stern Foundation.

1. Byfield, J.E. & Calabro-Jones, P.M., Nature (in press, 1981).

1082 Influence of Methotrexate (MTX) on Membrane Transport Systems in Growing Human Lymphocytes. K.J. Scanlon, L. Lachman, P. Wang-Iverson, M. Pallai and S. Waxman. Depts. of Neoplastic Diseases, Biochemistry & Medicine, Mt. Sinai Sch. of Med., N.Y. & Dept. Microbiol. Duke Univ. Med. Ctr., Durham, N.C.

Evidence is presented that the ASC amino acid transport system makes a major contribution for concentrating essential amino acids and other essential nutrients in growing lymphocytes. Furthermore, this system is very sensitive to inhibition by 2,4 diamino compounds (i.e. MTX). The expression of this system has been studied by stimulating resting lymphocytes with a mitogen and a growth promoting protein. Human lymphocytes, depleted of monocytes (<5%), were stimulated with variable concentrations of interleukin-1 (IL-1) and/or phytohemagglutinin (PHA). In the presence of suboptimal doses of PHA, IL-1 can elicit a dose response curve for optimal cell growth, maximum DNA synthesis and maximum Na dependent A and ASC amino acid transport at 72 hrs after stimulation. In contrast to data obtained from unstimulated lymphocytes, IL-1/PHA stimulated lymphocytes exhibited enhanced non-specific Na dependent ASC transport. Methionine, which is transported primarily by the A and L systems, was blocked partially by MeAIB (<20%) or BCH (<20%), while methionine uptake by the sodium dependent ASC system was blocked by AIB (80%) or threonine (67%). MTX has also been shown to inhibit this Na dependent ASC system thus limiting the uptake of essential nutrients. This data suggests that PHA and a monocyte derived, human growth promoting protein alters the lymphocyte membrane so that the cells are able to concentrate essential nutrients by an amino acid transport system that is sensitive to MTX.

1083 PERMEABILITY PROPERTIES OF MULTIPLE DRUG-RESISTANT CHO CELLS.
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Variants of CHO cells selected for resistance to colchicine and cross-resistant to a diverse range of anti-neoplastic drugs take up reduced amounts of them because of reduced membrane permeability (Biochim. Biophys. Acta 373, 242, 1974; *ibid* 455, 152, 1976). Although the presence of a particular plasma membrane glycoprotein (P-glycoprotein) correlates strongly with resistance and decreased permeability (J. Biol. Chem. 254, 12701, 1979), the mechanism of the latter has not been elucidated. However, recent findings indicate that this permeability restriction is not limited only to drugs but seems also to apply to some physiological permeants. Thus the normal rate of leakage of K^+ ions from cells is reduced in the drug resistant variants. There is a corresponding diminution of the rate of active K^+ transport back into these cells. Furthermore, the rapid rate of K^+ loss from resistant cells mediated by gramicidin-D, to which these cells are also resistant, is reduced relative to that from normals. Gramicidin-S, to which the mutant cells are not more resistant than the sensitive wild type, results in the same rate of K^+ loss from both. Cross-resistance and reduced permeability to melphalan which enters cells via an amino acid transport system suggests that it is also restricted (Cancer Res. 41, 393, 1981). These suggestions of a rather generalized membrane change are being tested further. Although fluidization of membrane lipid strongly inhibits ^3H colchicine uptake, a difference in bulk lipid fluidity between resistant and sensitive cells has not been detectible using lipid soluble esr and fluorescent probes. (Supported by MRC and NCI of Canada)

1084 POLYCATIONIC DRUG CARRIERS: EXPLOITING THE LYSOSOMAL ENVIRONMENT FOR THE INTRACELLULAR RELEASE OF ACTIVE DRUG, Hugues J.-P. Ryser and Wei-Chiang Shen
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Conjugation of methotrexate (MTX) to poly(L-lysine) and other basic peptides enhances the membrane transport of MTX and overcomes drug resistance in cultured transport-deficient cells. These conjugates are transported through endocytosis and intracellularly broken down to release active drugs inside lysosomes. Due to its indigestibility, poly(D-lys) is not a pharmacologically effective carrier even though its cellular uptake is comparable to that of poly-(L-lys). It can, however, be converted into a useful carrier if a lysosome-digestible linkage is inserted between the drug and the polypeptide. Thus, MTX-triglycine-poly(D-lys) causes strong cytotoxicity ($ID_{50} = 2 \times 10^{-8}M$) in MTX-resistant cells. Similar results were obtained by attaching MTX to HSA and subsequently linking MTX-HSA to poly(D-lys). The cellular uptake of MTX-HSA-poly(D-lys) is 50-fold higher than that of MTX-HSA, but only one third that of MTX-poly(D-lys). Even so, a complete growth inhibition of MTX-resistant cells is achieved with $3 \times 10^{-8}M$ MTX-HSA-poly(D-lys), while MTX-HSA, MTX-HSA plus poly(D-lys) or MTX-poly(D-lys) are not inhibitory. These results indicate that peptides and proteins can serve as digestible spacers to liberate active drugs from carriers under the action of lysosomal enzymes. Other properties of lysosomes can also be exploited for intracellular drug release. Linking daunomycin and poly(D-lys) through a cis-aconityl linkage creates a pH-sensitive bond that liberates free drug at lysosomal pH (B.B.R.C. 102, 1048, 1981). Such linkages can release unaltered drug and be adapted to lysosomotropic macromolecular carriers other than polycations, such as antibodies, glycoproteins or peptide hormones.