THE PHARMACOLOGY AND TOXICOLOGY OF PROTEINS

Jeffrey L. Winkelhake and John S. Holcenberg, Organizers
February 21 - 27, 1987

Plenary Sessions Page

Poster Sessions

Proteins, Peptides and the Pharmacologist: State of the Art

J 001 CONTROL OF PROTEIN PASSAGE THROUGH THE MICROVASCULAR TO TISSUES. Dept. of Physiology and Biophysics, Univ. of Neb. Coll. Med., Omaha, NE 68105-1065. William L. Joyner, Ph.D.

Transvascular exchange of macromolecules across the microvascular barrier proceeds via porus and non-porus pathways. The major mechanisms determining the characteristics of transvascular exchange through porus pathways are diffusive and convective transport. Diffusive transport is a dissipative process that depends upon the difference of protein concentration and the permeability-surface area properties of the membrane. Convective transport is dominated by the balance of hydrostatic and osmotic gradients, the protein concentration and the hydraulic and reflection coefficients of the restrictive barrier; thus it is a process of ultrafiltration and solvent drag. Non-porus pathways may include vesicular mechanisms and some type(s) of carrier-mediated transport. All of these mechanisms can be modulated by physiological, pharmacological and pathological factors in a time-and spacial-dependent manner. Transport characteristics and their modulation can be different in different vascular beds, newly developing vessels and vessels exposed to metabolic products. Alterations in the chemical composition of a macromolecule can modify drastically the permeability properties of the molecule. Thus, to facilitate the delivery of various solutes to specific sites, an understanding of the permeability properties of the microvessels in relation to that particular solute is mandatory.

J 001a GENETIC MODIFICATION OF PROTEINS, David F. Mark, Alice Wang, Kirston Koths and Leo Lin, Cetus Corporation, Emeryville, California 94608.

The ability to express high levels of foreign proteins in E. coli is an important factor in allowing us to study the pharmacological activities of many human proteins which previously can only be obtained in minute quantities from human tissue cultured cells. However, when these proteins are produced at high levels in a foreign environment, problems with protein folding (fibroblast interferon and Interleukin-2), correct disulfide bond formation (fibroblast interferon and Interleukin-2) and micro-heterogeneity (Interleukin-2 and tumor necrosis factor) caused by incorrect modifications of the proteins are often encountered. By genetic modification of the proteins, we have been able to overcome many of these problems. Furthermore, such modifications have also allowed us to gain some insight into the structure function relationships of these proteins.

J 002 CELLULAR UPTAKE OF SYNTHETIC CARBOHYDRATE LIGANDS: DEPENDENCE ON BRANCHING PATTERNS. Y. C. Lee and R. Reid Townsend, Department of Biology, Johns Hopkins University, Baltimore, MD 21218.

Modification of proteins with carbohydrates results in a tremendous enhancement in their uptake by specific cells. As the sugar/protein ratio increases linearly, the binding affinity increases logarithmically (cluster effect). The effect of sugar clustering is even more evident with branched oligosaccharide ligands. Synthetic branched oligosaccharides or those derived from glycoproteins assume a limited number of conformers which serve to position the binding determinants for interaction with complementary receptor faces. By correlating the binding affinity and three-dimensional structure of a collection of oligosaccharides, the following requirements for binding to the hepatic Gal/GalNAc lectin were determined. A single lactosamine branch possessed an affinity of K_d= 1 mM. Clustering of two lactosamines around a trimannosyl core to form a biantennary structure (inter-Gal distance of 25 Å) increased affinity 1000-fold (K_d=2.4 uM). A ligand with lactosamines linked β(1,2) and β(1,4) to Man was found to have an even higher affinity of K_d=0.27 μM and an inter-Gal distance of 15 Å. Three Gal's arranged as a triantennary structure resulted in a affinity of 1 nM. Changing a single linkage in a branch of a triantennary structure from Galβ(1,4) to Galβ(1,3) increased the inter-Gal distance from 15 Å to 18 Å and decreased the affinity by 70-fold.

Ligands possessing the same K_d may show different efficiencies of net ligand uptake as a result of exocytosis. Uptake of a synthetic ligand with an off-rate of k_{-1} = 0.1 min^{-1} was efficiently internalized but more rapidly uncoupled from receptor and subsequently released from the cell.

Biochemical Pharmacology: Receptors and Glycoprotein Trafficking

J003 DELIVERY OF MONOCLONAL ANTIBODIES TO LYMPH NODES VIA THE LYMPHATICS. John N. Weinstein, David G. Covell, Jacques Barbet, Robert J. Parker, James L. Mulshine, Robert J. Parker, Andrew M. Keenan, O. Dile Holton, Jorge A. Carrasquillo, Renee R. Eger, Steven M. Larson, Susan M. Sieber, and Christopher D.V. Black, National Institutes of Health, Bethesda, Md 20892.

Subcutaneous injection provides an efficient way to deliver monoclonal antibodies via the lymphatics to normal cell targets in lymph nodes [1] and to early lymph node metastases [2]. Possible applications of the technique include "immunolymphoscintigraphy," "immunolymphotherapy," and regional "immunomodulation." Quantitative analysis of the pharmacology (for antibodies reactive with H-2K and Lyb 8 antigens on normal lymph node cells) indicates a pattern of "sequential overflow" along the lymph node chains. Large lymph node metastases may be penetrated only partially by the antibody [3], or lymph flow to the nodes may be prevented altogether. A quantitative analysis of the former problem will be offered.

There is a second type of access problem: Some lymph node groups are not accessible from subcutaneous injection sites. Particularly important are the lymph node groups that drain the lung and constitute sites for metastasis of lung cancers. It seemed to us likely that antibody injected through a fiberoptic bronchoscope into lung parenchyma would pass along the pulmonary lymphatic vessels to those nodes. Our preliminary studies in dogs demonstrate efficient, selective delivery by this approach. Analogous endoscopic injection techniques may be useful in other settings such as that of carcinoma of the colon and rectum.

Preliminary findings with respect to immunolymphoscintigraphy in melanoma have not been encouraging, but those using T101 antibody in patients with T-cell lymphoma have yielded strongly positive results [4]. Uptake in the regional lymph nodes exceeded 20 percent of injected dose, and most of that uptake appeared to be antigen-specific. However, the antibody binds to normal T-cells, hence the uptake was not necessarily tumor-specific.

1. Weinstein, et al., Science 218:1334, 1982.
2. Weinstein, et al., Science 222:423, 1983.
3. Weinstein, et al., in Site-specific Drug Delivery (E. Tomlinson and S.S. Davis, eds), John Wiley, N.Y., 1986, pp. 81-91.
4. Keenan, et al., J. Nucl. Med., in press.

J004 PHARMACOKINETICS AND PROTEIN THERAPEUTICS, Leslie Z. Benet, Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94143-0446.
When an understanding of how an individual patient will absorb and eliminate a drug is coupled together with knowledge of the pharmacologic effects of a given amount of the drug, a particular dose can be selected that will result in clinical efficacy and minimal toxicity. Such considerations have been defined adequately for many classical drugs; however, this approach has not been used as yet for the new peptide and protein therapeutic agents. Yet, regulatory agencies do require information concerning drug disposition which can be best described using pharmacokinetic principles. A large number of pharmacokinetic parameters may be determined in defining a new drug substance. However, certain critical parameters are of primary importance: clearance, volume of distribution, half-life, and bioavailability. To describe drug kinetics, either for therapeutic uses or for a basic understanding of drug disposition, one presupposes the ability to measure the "active drug" substance in various biological fluids. Almost all pharmacokinetic work with protein drugs up to the early 1980's was based on bioassay methods. The variability inherent in such methodology probably restricts our ability to adequately describe the disposition of these compounds. The recent implementation of more sensitive analytical methodology, such as the ELISA and IRMA procedures, may alleviate this variability problem. However, the specificity of such methods is often unknown, which leads directly to a concern generally recognized for classical drugs, but little investigated for proteins. That is, are metabolites being measured, and/or should they be measured? When analytical methodology is sufficiently sensitive most protein drugs show multicompartment pharmacokinetics, as do most classical drugs. Since these proteins are often naturally occurring at much lower concentrations, must we be concerned with the tissue distribution of these agents at therapeutic doses as reflected by multicompartment kinetics, and more specifically must we measure the accumulation of these substances at particular tissue sites? If the answer to this question is yes, then we face the even more difficult problem of convincing ourselves and the regulatory agencies, that studies in animals may yield meaningful results that can be extrapolated to man. This question is made more difficult when we attempt to carry out such studies with human specific protein in animals, or extrapolate data from animal specific protein to humans. At present pharmacokinetics is limited to the elucidation of parameter values which can be given to regulatory agencies to satisfy a specific criteria related to the understanding of drug disposition and the linearity of drug kinetics. Hopefully, in the future, when more experience has been obtained with protein drugs, the therapeutic use of pharmacokinetics may be invoked in the development of rational drug dosing regimens.

Pharmacokinetics: General Principles

J005 DRUG DELIVERY/PHARMACOKINETICS USING PEG-MODIFIED PROTEINS, Abraham Abuchowski, Enzon Inc., 300 C Corporate Court, South Plainfield, NJ 07080.

The greatest obstacles in the use of peptides and proteins for therapeutic purposes are short circulating life (even on initial injection), low stability and the development of immune complications after repeated use. These problems are even associated with the use of recombinant proteins in therapy.

The modification of proteins with polyethylene glycol (PEG) results in adducts with increased circulating lives, increased stability under physiological conditions and decreased to non-existent imunogenicity. The process involves the covalent coupling of strands of PEG to the amine groups of the protein.

Modification of proteins with PEG appears to be a general process. Over forty proteins have been modified. In every case, the circulating life was increased many fold. In the case of superoxide dismutase (SOD), unmodified SOD has a half life of 6 minutes in the mouse. The half life of modified SOD is approximately 2 days. The stability of such modified proteins is also greatly increased.

The immunogenic response to PEG-modified proteins as determined by the enzyme linked immunosorbent assay (ELISA) is generally less than a tenth of a percent of that seen following immunization with the unmodified protein. The antigenicity of proteins is also reduced following modification with PEG.

In spite of their extended circulating lives, PEG-protein adducts display less toxicity than the unmodified protein in acute, subacute and subchronic toxicology studies. Therapeutic studies in man are ongoing.

J006 RESIDUALIZING LABELS FOR DETERMINING SITES OF PROTEIN UPTAKE AND CATABOLISM IN VIVO, John W. Baynes and Suzanne R. Thorpe, University of South Carolina, Columbia, SC 29208.

Identification of the sites of uptake and catabolism of proteins with long half-lives in the circulation is difficult because the rate of delivery of the proteins to cells for degradation is slow, compared to the rate at which catabolic products are released from the cells. To circumvent this problem we have prepared both radioactive and fluorescent glycoconjugate labels for proteins which do not alter the kinetics or mechanism of protein catabolism, but which accumulate in cells following degradation of the carrier protein. Dilactitol-^{125}I-tyramine (DLT) and dilactitol-ethylene-diamine-fluorescein (DLF) are examples of this type of residualizing label. Autoradiography and fluorescence microscopy of tissues from rats injected with DLT- and DLF-labeled albumin showed that fibroblasts were a major site of albumin catabolism in peripheral tissues. Fibroblasts containing both radioactive and fluorescent degradation products have also been isolated from rat skin. Cultured fibroblasts containing fluorescent degradation products have been characterized by flow cytometry. These residualizing labels provide a novel approach for determining the eventual distribution of proteins and immunological reagents in the body and for measuring the uptake of proteins by cells in culture.

J007 PEPTIDYLATION EFFECTING THE RECEPTOR AND TISSUE EFFECT SPECIFICITY AND ORAL ACTIVITY OF LOW MOLECULAR WEIGHT HORMONES

Kenneth L. Melmon
Manzoor Khan and Murray Goodman, Division of Clinical Pharmacology, Department of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA 94305 and Department of Chemistry, University of California, San Diego, La Jolla, CA 92093

We hypothesized that the pharmacologic effects of low molecular weight autacoids (catecholamine and histamine) might be preserved by derivatizing them at a site distant from the pharmacophore's receptor recognition moiety. Initially, the purpose of the derivitization was to alter the pharmacokinetics of the resulting congener derivative and conjugate. We did not expect that the derivatives would alter effects on tissue specificity; we expected only altered pharmacodynamics or tissue distribution based on altered in vivo pharmacokinetics and/or tissue distribution respectively.

A series of congener derivatives and conjugates made of the congener derivatives covalently linked to monodisperse oligopeptides, have unusual pharmacologic effects. Several of the derivatives are more potent than their progenitor, and some have surprisingly unprecedented receptor and tissue-effect specificity. For instance, norepinephrine attached to the amino end of the molecule to a branched alkene chain with a toluidide moeity has more inotropic and much less chronotropic effects than norepinephrene. The derivative's effect on heart and fat cells is far more pronounced than the progenitor but no effects on peripheral sites of catecholamine action are produced. An analogous compound of histamine loses its H_2, cardiac, and H_1 vascular activity and appears to become a specific H_1 agonist on murine natural suppressor cells. The dipeptide conjugate of the norepinephrine congener derivative becomes orally active with prolonged in vivo cardiac effects when compared to norephinephrine or isoproterenol. The changed tissue and effect specificity are so far not explained by altered in vivo pharmacokinetics. Nor can the difference of conjugates from progenitor be explained by anything other than the pharmacologically inert ligands (i.e., the peptidylation, etc.).

The ligands do not interact with classical receptors, nor do they prevent the pharmacophore from recognizing the receptors. All the effects of the derivatives are competitively blocked by classical blockers but after washing an antagonist from the preparation, the response to the agonist returns. The unusual effects and their duration seem attributable to the effects of the congener portion and peptide moeity of the conjugate that may be attracted to some undefined receptor microenvironment. Perhaps such derivatization of the surface active pharmacophores could impart similar tissue and effect specificity.

J008 CHEMICAL MODIFICATION OF α_2-MACROGLOBULIN AND ITS EFFECTS ON RECEPTOR BINDING

Salvatore V. Pizzo, Duke University Medical Center, Durham NC 27710.

α_2-Macroglobulin (α_2M), M_r = 720,000, is a plasma glycoprotein which is unique in its ability to bind and inactivate proteinases of each of the four major classes. We have published a three dimensional model of α_2M (Feldman SR, Gonias SL, and Pizzo SV, Proc. Natl. Acad. Sci. USA 82,5700-5704, 1985) which demonstrates that the molecule has D_2 symmetry with three C_2 rotational axes. It consists of four identical subunits which are organized into two functional domains or "half-molecules". These domains can be separated by limited reduction and carboxamidomethylation and they remain functional in proteinase binding. When α_2M is reacted with a proteinase, the inhibitor undergoes a major conformational change which physically traps the proteinase within one of its two binding pockets. α_2M subunits each contain a thiolester and direct reaction with CH_3NH_2 also induces a similar conformational change. By either mechanism of reaction, receptor recognition sites are exposed on each of the α_2M subunits. The receptor, $K_d \sim 0.5$nM, is present on most cell types including macrophages, hepatocytes and fibroblasts. Proteolytic degradation of α_2M with papain produces a carboxyl terminal fragment containing the receptor recognition site (α_2M fragment 1). This region of the molecule also binds a monoclonal antibody specific for the receptor recognition site. Reaction of either the native α_2M molecule or fragment 1 with cis-diehlorodiammineplatinum (II) (cisDDP) blocks receptor recognition. This reaction, which is reversed by treating the inhibitor with diethyldithiocarbamate, protects the receptor recognition site from destruction by H_2O_2. Amino acid analysis confirms the fact that H_2O_2 treatment destroys several methionine residues and that cisDDP treatment protects these residues. Only three methionine residues are present in the carboxyl termi of the α_2M subunits. It is concluded that one of these residues is at the α_2M receptor recognition site. This conclusion is supported by the fact that reaction of α_2M with cisDDP blocks binding of a monoclonal to the receptor recognition site. The binding of α_2M-proteinase complexes to macrophages is of major importance since this reaction regulates the ability of the cell to express Ia antigen, produce proteinases such as the tumoricidal enzyme cytolytic factor, and shuts off the respiratory burst of phorbol activated cells. Identification of the receptor recognition site has potential significance in production of an immunosuppressive peptide.

Pharmacokinetics: Chemically-Modified Proteins

J009 DELIVERY OF PEPTIDES AND PROTEINS VIA CONTROLLED RELEASE OSMOTIC SYSTEMS.
John W. Fara and Nigel P. Ray, ALZA Corporation, Palo Alto, CA. 94304

The availability of osmotic devices that allow for long-duration control over the rate of peptide administration in laboratory animals has opened up numerous methods, protocols, and models for delivering these substances to animals and potentially also to man. This presentation will focus on studies that have been done utilizing two of these devices: the implantable osmotic pump (ALZET®) for studies in animals, and the OSMET™ module for oral, vaginal, or rectal administration of drugs in clinical research.

In animals, osmotic pumps have been used extensively to deliver proteins, peptides and other short half-life agents which are difficult to deliver by other methods. These pumps allow for prolonged, constant delivery for up to 4 weeks following a single implantation procedure. With a short half-life agent, they assure drug presence without the need for repeated injections which cause stress-related physiological changes. Complete implantation of the infusion device permits free animal movement throughout the period of infusion. Osmotic pumps have been utilized as artificial organs, replacing or supplementing endocrine gland secretions, such as insulin. Additionally, they have been modified via catheter attachment for micorperfusion into soft tissue such as brain, into hollow organs, as the intestinal tract and the uterus, and into the systemic circulation as well as CSF.

Another important capability is the adaption of these osmotic systems to deliver at preprogrammed rates that vary over time. It is not yet clear which proteins or peptides are best given by a constant rate regimen and which might require a pattern of input. Researchers have devised various approaches to mimic circadian rhythms, to infuse agents to alter existing patterns, or to study what existing patterns may do to the test agent.

The same osmotic technology has been adapted to clinical studies with the OSMET™ modules. Designed for delivery over 8, 12, or 24 hours, these modules have been used in clinical investigative studies to deliver anti-inflammatories, antihypertensives, various receptor-blocking agents, and are now finding usefulness in evaluating peptide absorption across various mucosal surfaces.

J010 CONTROLLED RELEASE OF MACROMOLECULES FROM BIOERODIBLE HYDROGELS,
K.L. Smith and K.E. Thompson, Bend Research, Inc., 64550 Research Road, Bend, Oregon 97701.

A series of novel bioerodible polymers has been developed for controlled release of macromolecular drugs. The polymers are based on water-soluble polyester prepolymers that enable incorporation of labile drugs from aqueous solutions at ambient temperatures. By adjusting the chemical structure of the prepolymers and the crosslinking density, drugs can be delivered at constant rates for durations as short as a few days or as long as several months. The polymers appear especially promising for delivery of macromolecular drugs such as peptides, hormones, growth factors, and proteins.

Recent studies with these bioerodible polymers have focused on the effects on release kinetics of drug molecular weight and loading. We have found that for drugs with molecular weights higher than about 3000, drug release is controlled by bioerosion of the polymer, and is independent of molecular weight. At molecular weights below 3000, drug release is controlled by a combination of bioerosion and diffusion of the drug within the polymer. In separate studies, constant release rates have been obtained from systems with drug loadings as high as 20 wt%.

Pharmacokinetics: Controlled/Sustained Release Dosage Forms

J011 **REGULATION OF THE IMMUNE RESPONSE BY ALPHA$_2$-MACROGLOBULIN**, Bill Hubbard, John Yannelli and Greg Stelzer; Biotherapeutics, Inc., Franklin, TN 37064 and Duke University Medical Center

Alpha$_2$-Macroglobulin (α2M) is unique among the proteinase inhibitors; it is quite large, has 1:1 or 2:1 binding stoichometry, forms an irreversible bond with target enzyme(s) from all catalytic classes and it inactivates the proteinase through stearic hindrance via interiorization within the α2M molecule. Attendant with proteinase binding is a dramatic change of state (metastable to compact) which promotes rapid clearance of α2M from the periphery through the engagement of specific cell-surface receptors . We have investigated whether this compact or electrophoretically "fast" form (Fα2M) conveys additional biological information through somehow facilitating the sensing of the release of free proteinases. While this "proteinase sensing" probably can affect many physiological systems, we have focused thus far on the immune system *in vitro*.

We have observed immune suppression in cultures treated with Fα2M. The cause of the suppression or blockade appears to reside with the cells of the monocyte/macrophage (Mϕ) lineage, most probably as a result of directly blocking the early events of antigen presentation and other cellular collaborative functions. It is additionally possible that the Fα2M can perturb the immune response through the release of soluble mediator substances. We have evidence that Fα2M exerts a profound effect on many of the Mϕ effector functions with lesser or no consequences to the lymphocytic cells.

J012 CONVERSION OF ANTIGENS TO TOLEROGENIC DERIVATIVES BY CONJUGATION WITH MONOMETHOXY-POLYETHYLENE GLYCOL (mPEG), Alec H. Sehon, Univ. of Manitoba, Winnipeg, Canada.

In relation to developing new therapeutic procedures for the abrogation of IgE antibodies, which are responsible for common allergies of the immediate type, it was shown in this laboratory that coupling of an appropriate number of mPEG chains onto an antigenic molecule resulted in its conversion to a derivative capable of downregulating the IgE and IgG responses in nonsensitized mice and rats. Such derivatives possess the additional advantage of having a markedly reduced allergenicity as a result of the masking of some of the antigenic determinants of the original allergens by the long mPEG chains (3000 - 6000 D) and hence failed to induce anaphylaxis on intravenous injection. In view of the increasing therapeutic use in man of mouse monoclonal antibodies to human T cell markers for organ transplantation or to tumor antigens for immunolocalization or destruction of tumors, recently the procedure was extended to the synthesis of tolerogenic mPEG conjugates of xenogeneic monoclonal immunoglobulins. From the results obtained with diverse antigen systems in this and other laboratories, it may be concluded that: (i) tolerogenic mPEG conjugates induce suppressor T (Ts) cells and also inactivate or ablate the antigen specific, surface immunoglobulin positive B cells, and (ii) the suppressogenic capacity can be transferred to naive animals by splenic Ts cells of tolerized animals as well as by soluble factors of these cells. The possible mechanisms underlying immunological tolerance induced by mPEG conjugates will be discussed.

These studies have been supported by the Medical Research Council of Canada and the National Institute of Allergy & Infectious Diseases, N.I.H., Bethesda, MD.

Interference/Enhancement of Protein/Peptide Therapeutic Efficacy

J013 PREDICTORS OF RESPONSE TO METHIONYL HUMAN GROWTH HORMONE, PROTROPIN.
B. Sherman, J. Frane,*, A. Johanson*, Genentech, Inc.

Children with growth hormone deficiency (GHD) have been treated with pituitary growth hormone for over 25 years. Nevertheless, the major predictors of response to treatment have not been clearly identified. Seventy-nine children with carefully documented GHD have been followed during one to four years of treatment with Protropin, 0.1mg/Kg, IM, t.i.w. The mean growth rate was greatest in the first year, 9.9 ± 2.4 cm/year, and was 6.5 ± 1.6 cm/year in year four in the 17 patients treated for four years. There was no correlation of the growth response and the pretreatment growth rate. However, the first year growth rate was highly correlated with that in later years. Patients with idiopathic GHD, n=61, had a first year growth rate of 10.5 ± 2.4 cm/year which was greater than that of 18 patients with organic etiologies, 8.4 ± 1.9 cm/year, p+.002. Patients with organic etiologies also had significantly greater mean height, weight, height age and bone age. We used a variant of stepwise regression to explore the influence of idiopathic GHD. A model that accounted for 48% of the variability in the first year growth rate showed that a favorable response was best predicted by a lower basal somatomedin-C concentration, a lower height age, greater adiposity and greater maternal height. Thus, treatment with Protropin significantly increases the growth rate for up to four years, and there are etiologic, genetic, maturational and nutritional predictors of the growth response that can be used to guide therapy so the patients might achieve optimal growth rates and a near normal height.

J014 MONOCLONAL ANTIBODIES IN CANCER THERAPY, Brian F. Issell, Eric S. Groves and John D. Young, Cetus Corporation, 1400 Fifty-Third Street, Emeryville CA 94608.

A number of biological and pharmacological barriers exist to the effective therapeutic application of monoclonal antibodies (MAbs), either alone or as immunoconjugates. It is important to determine to what extent each potential barrier may be relevant and to examine ways of bypassing these barriers.

Tumor regressions have been observed with MAb based therapy. However, these responses have generally been partial and transient. The question of whether a sufficient quantity of MAb therapy is able to be delivered to the target site in order to permit a sustained effect will be addressed by reviewing organ distribution, imaging and pharmacokinetic studies. Assuming that adequate cancer cell epitope specificity exists for the MAb in question, then further mechanisms which may compromise the delivery of effective concentrations of antibody based therapy to the target site include the following: 1) containment within the vascular compartment; 2) uptake or trapping by non specific binding sites, such as within the reticuloendothelial system; 3) blocking of antibody binding sites by shed antigen; 4) antitherapy antibodies; 5) removal by proximal high affinity target cell epitopes; 6) antigen heterogenicity and modulation. Experimental data addressing the importance of these mechanisms will be reviewed and ways of improving selective delivery to the tumor site will be discussed. As an example of how immunoconjugates may differ from MAbs alone, we have found that immunotoxins are cleared from blood up to 4 times faster than their corresponding antibodies, and both have volumes of distribution approximately equal to the blood volume.

Even with effective delivery, a variety of further mechanisms may limit successful cytotoxicity. The possible events leading to cancer cell destruction by MAbs alone include: 1) complement dependent cytotoxicity; 2) antibody dependent cellular cytotoxicity; 3) growth factor and nutrient uptake interference; 4) vascular occlusion due to antibody-antigen complexes or other procoagulant effects. For antibody alone mediated mechanisms, blockade of activity can occur not only because of the failure of MAb delivery, but also because of failure of delivery of the secondary effector components, i.e. complement or effector cells. For MAbs used to deliver cytotoxic agents, i.e. immunotoxins, cell internalization and translocation to the ribosome by the toxin fragment are additional events necessary for cell killing. Experimental methods of enhancing some of these cellular cytotoxic mechanisms will be reviewed.

Clinical Pharmacology of Proteins - I

J015 DEFINITION OF IMMUNOMODULATORY EFFECTS OF INTERFERONS ALPHA, BETA, AND GAMMA, Ernest C. Borden, University of Wisconsin Clinical Cancer Center, Madison, WI 53792

Molecules which modify the host response to malignant disease have potential to augment effectiveness of other cancer treatment modalities. Therapeutic effectiveness of immunomodulatory molecules, suggested by studies in animal models over the last two decades, has now been confirmed in man. Interferons (IFN), purified to homogeneity, can induce regression in metastatic malignancy. Recombinant DNA technology has facilitated large scale production which has made use of pure molecules possible. It has also made possible specific, desired permutations in amino acid sequence.

We have defined the therapeutic and immunomodulatory effects of IFN in phase I and phase II trials. Augmentation of natural killer cell activity has been identified with IFNα and IFNβ. A low dose of IFNβ proved as effective in man as a high dose in augmenting both NK and K cell cytotoxicity. IFNβ and IFNγ enhanced expression of histocompatibility antigen proteins on peripheral monocytes, potentially making them more effective in antigen processing.

Our collaborative studies have identified activity of these molecules in multiple myeloma, hairy cell leukemia, renal carcinoma, breast carcinoma, and malignant melanoma. Studies are underway to begin correlation of the immunological changes with therapeutic response and to define effectiveness of IFNs in conjunction with other therapeutic modalities. In addition to their effects on virus diseases, it seems probable that IFNs will be a prototype for several immunoregulatory peptides which will impact on our treatment of malignant disease.

J016 INTERLEUKIN-2: CLINICAL PHARMACOLOGY AND IMMUNOLOGY IN PHASE II TRIALS Edward C. Bradley, Michael W. Konrad, Laura V. Doyle, Michael V. Doyle, Nitin K. Damle, Anne L. Childs, Susanne K. DeWitt, Sharon DeGroat, Brian F. Issell, Cetus Corp., Emeryville, CA 94608

Phase II trials of a novel recombinant interleukin-2 analog in patients with metastatic cancer have been performed and have allowed the correlation of pharmacokinetics and *in vivo* activation of lymphokine-activated killer cells (LAK) with clinical response. *In vitro* studies designed to determine the minimum concentration and duration of exposure to IL-2 necessary to result in optimal activation of LAK activity have been performed and results have also been correlated with clinical response. Antitumor activity has been seen in patients with melanoma, renal cell carcinoma, colon carcinoma, Kaposi's sarcoma, ovarian carcinoma, and lymphoma in patients treated with IL-2 with or without adoptive cellular therapy. Response rates appear to be dose and schedule dependent. Optimal clinical doses and schedules correlate with optimal *in vitro* LAK activation conditions, and the induction *in vivo* of LAK activity correlates with clinical response. These findings suggest that the *in vivo* induction of LAK activity, possibly at the tumor site, may be necessary for clinical tumor regression. Serum levels of shed IL-2 receptors rise promptly following IL-2 administration but may reflect a non-specific activation of lymphocytes which is unrelated to cellular mechanisms of tumor regression. This underscores the importance of identifying only those *in vitro* parameters of immune function which are functionally related to tumor regression as surrogate indicators of clinical response. Serum pharmacokinetics and *in vivo* LAK induction serve as useful tools for achieving the optimal clinical IL-2 dose and schedule.

J017 THE CLINICAL PHARMACOLOGY OF ACTIVASE (RT-PA), Elliott B. Grossbard, Department of Clinical Research, Genentech, Inc., SSF., CA 94080

Activase® brand of recombinant tissue plasminogen activator (rt-PA) is a fibrinolytic protein with a superior toxic: therapeutic ratio. Since Feb. 1984 over 2000 patients have been treated with rt-PA for a variety of thrombo occlusive disorders; over 1500 have been treated for acute coronary thrombosis. The clinical efficacy observed, as measured by angiography, has exceeded 70 per cent. Early trials were conducted with a predominately two-chain form of rt-PA (G11021), while recent studies have utilized a predominately one chain form (G11035). The pharmacokinetics of the two preparations were different in rabbits, primates, and humans. The more rapid clearance of G11035 necessitated an increase in the clinical dose utilized in the myocardial infarction studies. Studies of coagulation factors suggested that G11035 was more fibrinogen sparing. The principal toxicity of rt-PA is an exaggeration of the pharmacologic effect, i.e. bleeding. Since recent studies with a less effective fibrinolytic drug suggest that thrombolytic therapy can reduce mortality in myocardial infarction by 20 per cent, the introduction of rt-PA into the treatment of patients with acute myocardial infarction may be a major advance in the treatment of such patients.

Clinical Pharmacology of Proteins - II

J018 PROTEASE NEXIN-MEDIATED CONTROL OF PROTEASE ACTION AT CELL SURFACES. Joffre B. Baker, Michael P. McGrogan* and Chris Simonsen*. University of Kansas, Lawrence, KS 66045; *Invitron, 515 Galveston Dr., Redwood City, CA 94063.

A family of evolutionarily-related serine protease inhibitors called the serpins cumulatively account for several percent of the total protein in plasma. The reactive center (P_1P_1') residues of serpins resemble substrate cleavage sites, but lock the catalytic site seryl residues of targeted proteases into stable tetrahedral complexes. Deficiences in the plasma serpins cause a variety of pathological conditions including emphysema, thrombotic disorders and edema. Several newly identified serpins are secreted by cultured cells but are at trace or undetectable levels in plasma. One group of these are the fibroblast-secreted inhibitors called the protease nexins (PNs). PN-protease complexes are recognized by cell surface receptors that mediate their internalization and the lysosomal destruction of the bound proteases. Protease nexin I (PNI), a rapid inhibitor of trypsin, thrombin and urokinase, resembles the plasma thrombin inhibitor antithrombin III in that it has a high affinity for heparin and heparin vastly accelerates its inhibition of thrombin. In vitro, purified PNI inhibits the mitogenic response of fibroblasts to thrombin, and prevents the destruction of extracellular matrix that is caused by tumor cell secretion of urokinase (1). The latter inhibition is accompanied by a transient arrest of tumor cell proliferation. A thrombin complexing factor that is immunologically and electrophoretically similar to PNI is present on the surfaces of platelets where it may impose a thrombin threshold for platelet activation. Immunofluorescence experiments show that PNI antigen coats the surfaces of cultured human foreskin fibroblasts. Recent studies carried out with Daniel Hantai (Inserm, Paris) and Berry Festoff (Veterans Administration Hospital, Kansas City, MO) demonstrate that in rat skeletal muscle PNI antigen is localized to the neuromuscular junction. Dennis Monard and colleagues have recently cloned and sequenced the cDNA for a glial cell protein that causes neurite extension (2). Our recent cloning and sequencing of PNI cDNA shows that PNI and the neurite promoting factor are virtually identical. These and other findings implicate PNI in the control of cellular homeostasis, growth, and differentiation.

From cDNA sequence data the P_1P_1' residues of PNI have been tentatively identified as arginyl and seryl, respectively. The hinge region of PNI is apparently unique in having a lysyl residue where all other serpins have a glutamyl or glutaminyl residue.

1. Bergman, B.L., Scott, R.W., Bajpai, A., Watts, S. and Baker, J.B. (1985). Proc. Natl. Acad. Sci. U.S.A. 83:996-1000.
2. Gloor, S., Odink, K., Guenther, J., Nick, H. and Monard, D. Cell, in press.

J019 PRECLINICAL PHARMACOLOGY OF ACTIVATED HUMAN PROTEIN C, Nils U. Bang, Scott C. Emerick, Hideki Murayama, S. Betty Yan, George L. Long, Cathy S. Harms, Carole A. Marks, Lawrence E. Mattler, Philip C. Comp, Naomi L. Esmon and Charles T. Esmon, Lilly Res Labs, Eli Lilly and Company, Indiana Univ School Med and Oklahoma Med Res Found, Indianapolis, IN and Oklahoma City, OK.

Although it is predicted from in vitro studies that activated human protein C (APC) may be an attractive antithrombotic agent because of its specificity of action (inactivation of factors Va and VIIIa), the antithrombotic properties of APC have not been demonstrated in vivo. In this study, we investigated the ability of APC to prevent accretion of radiolabeled fibrinogen (F) to preformed jugular venous thrombi in dogs and Rhesus monkeys. Highly purified plasma derived human PC was activated with rabbit thrombomodulin-bovine thrombin (T). Jugular veins were isolated bilaterally in 12 dogs (6 controls, 6 APC treated). Segments (5 cm) of all veins were clamped off and clots formed in the clamped off segments through the injection of .5 U bovine T. Stenosing ligatures were placed to prevent slippage of clots. Initially, dogs were given Amicar 200 mg/kg followed by 200 mg/kg/h for the duration of the experiments to block fibrinolysis. After release of the clamps, all dogs were given approximately 25 μCi [^{125}I] dog F immediately followed by APC treatment or vehicle infusion. In treated dogs, 84 μg (2 dogs), 126 μg (2 dogs), 168 μg APC/kg (2 dogs) was administered as an i.v. bolus followed by a constant infusion of 21, 31.5 and 42 μg/kg/h for 2 h. After 2 h infusions, all clots were removed, washed extensively in heparin saline and radiolabeled fibrin quantified. An estimate of bleeding was obtained through weighing gauze sponges before and after placement in surgical wounds every 30 min. APC infusions resulted in a 3-6 sec prolongation in the APTT with no changes in the prothrombin time, significant decrease in fibrin accretion onto thrombi compared to controls (controls: 4.5±2.1 mg, APC treated: 1.4±1.1 mg fibrin accreted/thrombus; P (by paired Student T test) <0.01. Bleeding from surgical wounds was minimal and no greater in APC treated than control dogs. Human PC antigen by ELISA ranged from .7 to 3.4 μg/ml. Surprisingly, 1 h after discontinuing the infusions, a fall of only approximately 20% of circulating antigen was noted. In Rhesus monkey experiments recombinant human APC was administered in a bolus of 60 μg/kg followed by 15 μg/kg/h for 2 h and no Amicar was administered. The experimental design was otherwise similar to dog studies. Fibrin accreted (mg/thrombus) were: controls: 55.4±33.4 (N=8), APC treated: 4.7±6.2 (N=10), P <0.001. In monkeys as in dogs, trivial prolongations in the APTT, no changes in the prothrombin time and no excessive bleeding in APC treated animals were noted. Thus, APC appears to be an active antithrombotic agent in dogs and monkeys and does not appear to cause increased blood loss during surgery.

J020 PROTEASE INHIBITORS PRODUCED BY VASCULAR SMOOTH MUSCLE CELLS (VSMC), Walter E. Laug, Childrens Hospital Los Angeles, Los Angeles, CA 90027.

Growth of endothelial cells (EC) on a preformed layer of VSMC resulted in a structure resembling rather myointimal proliferation than a real vessel wall. Free plasminogen activator (PA) activities were absent in the growth medium of such cocultures whereas pure EC cultures elaborated large amounts of these proteases. Two distinct inhibitors of PA separable, by heparin-sepharose affinity chromatography, were found to be produced by VSMC. These inhibitors resemble protease-nexin and the EC derived PAI-1, respectively. These inhibitors masked the PA activities elaborated by VSMC and demonstrated with specific PA gene probes. Thus, the net surplus of PA inhibitory activities produced by VSMC allowed for the neutralization of protease activities elaborated by EC. Despite the absence of free PA activities, VSMC were able to degrade interstitial tissue glycoproteins. In addition, VSMC degraded crosslinked tissue collagens despite their production of large amounts of tissue inhibitor of metalloproteinases. Thus, VSMC can degrade interstitial tissue proteins while inhibiting the degradative proteolytic activities of other cells. These findings are important for the understanding of progression of arteriosclerotic lesions from early to late stage.

J021 ATRIOPEPTIN: DISCOVERY AND IMPLICATIONS, Philip Needleman, Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

A potent, labile vasoactive, natriuretic-diuretic peptide was isolated from rat atria, purified and structurally characterized. Preparation of selective antisera, cDNA probes, and synthetic peptides has allowed characterization of the synthesis, storage, release, response, and therapeutic potential of the atrial peptides.

New Protein Therapeutics: Preclinical Studies - Cascade Network Proteins, Hormones and Enzymes as Drugs

J022 GLUCOSYL-B-GLUCOSIDASE DEFICIENCY: A MODEL FOR THE COMPLEXITY OF NOVEL THERAPY, John A. Barranger, Division of Medical Genetics, Childrens Hospital of Los Angeles, University of Southern California, Los Angeles, CA 90027.
Primary therapy for inherited disorders of metabolism has been attempted using organs, cells, gene products, and, perhaps within a few years, genes. A priori these approaches are limited by the incomplete understanding of the pathogenesis of any particular disorder. How extensively any approach will be limited by this lack of understanding is specific to the phenotype (ultimately the genotype) of any given disease. Primary therapy will not likely be successful for all cases of one disease (e.g., severe combined immunodeficiency) because of an underlying heterogeneity in genotype and consequently somewhat different pathogenesis. Moreover, what is successful for one disorder probably will not work for all disorders of even apparently similar types (e.g., lysosomal storage disorders). Attempts to reverse the diseases caused by a deficiency of glucosyl-B-glucosidase will be reviewed. Applications and limitations will be pointed out in light of the pathogenesis of the disorder and the molecular biology of glucosyl-B-glucosidase.

J023 APPROACHES TO COMBINED LYMPHOKINE AND CHEMOTHERAPY TREATMENTS FOR ANTITUMOR THERAPIES. Michael A. Palladino, Department of Molecular Biology, Genentech, Inc., South San Francisco, CA 94080
Recent advances in recombinant DNA technologies have made available sufficient quantities of biological response modifiers such as interferon-alpha, beta and gamma, interleukin-2 and tumor necrosis factor-alpha to allow for the examination of their clinical antitumor potentials. Results obtained from phase I and phase II clinical trials with some of these agents, however, have demonstrated that similar to the results obtained with chemotherapeutic agents, single drug therapy is generally not sufficient for the successful treatment of most cancers. We have, therefore, investigated the antitumor actions of interferon-gamma and tumor necrosis factor-alpha in combination with a variety of chemotherapeutic agents such as adriamycin and cisplatin. Antitumor efficacy was measured in vitro against syngeneic mouse tumors in syngeneic mice and human tumors implanted into the subrenal capsule of immunocompetent mice. The results demonstrated that as single agents, both tumor necrosis factor-alpha and interferon-gamma can exert significant antitumor effects in all three model systems. However, when the agents were combined with the particular chemotherapeutic agents, significantly greater antitumor effects were obtained. Increased toxicity of the various drug combinations as determined by decreased survival time or significant weight-loss was not detected. The results demonstrate that the antitumor efficacy of certain biological response modifiers can be significantly enhanced without additional systemic toxicity by their combination with chemotherapeutic agents.

Protein Pharmacokinetics/Pharmacodynamics and Delivery

J100 MONOCLONAL ANTIBODY PHARMACOKINETICS: LONG-TERM STABILITY OF THE ANTIGEN-BINDING PROPERTIES OF RADIOLABELED MONOCLONAL ANTIBODIES AND FRAGMENTS INJECTED IN NORMAL MICE, Jacques Barbet, Jean-Marc Le Doussal, and Michel Delaage, SA Immunotech and Centre d'Immunologie INSERM-CNRS, Marseille, France.

The Pharmacokinetics of homologous IgG monoclonal antibodies (mAbs) in mice are characterized by long half-lives and limited distribution. Available data are generally limited to relatively short period of observation (one week or less) and identification of radiolabeled proteins by crude techniques such as HPLC or trichloroacetic acid precipitation. We have studied in vivo long-term (2 months) stability of the antigen-binding properties of mAbs and of their radio-iodine labeling. Thus, mice were injected with a ^{125}I-labeled mouse IgG_{2a} mAb specific for human IgE antibody and sacrificed at selected time intervals. Distribution of radioactivity and binding properties were monitored in plasma and various organs. The basic results were: (i) after about a week, mAb clearance slowed down and significant amounts of intact radiolabeled antibody were still present in mouse plasma 72 days after injection, (ii) in most organs mAb immunoreactivity was maintained at pre-injection levels (85 %), and (iii) the specific activity of the radiolabeled mAb, as assessed by a sandwich immunoassay of anti-human IgE activity in mouse plasma, decayed at the same slow rate in vivo and in vitro. Similar conclusions were reached for F(ab')$_2$ fragments, but for a general acceleration of clearance and distribution. Thus, in the absence of antigen, radioiodinated homologous mAbs exhibit exquisite in vivo stability and, apart from some iodine uptake in the thyroid, products of their catabolism do not accumulate in the body: increased clearance and dehalogenation, or loss of immunoreactivity observed sometimes in clinical applications must result from antigen-binding, heterologous conditions or previous sensitization of the recipient, and these issues must be addressed for the development of in vivo immunodiagnostic and immunotherapy.

J101 ROLE OF VEROTOXIN-BINDING GLYCOLIPIDS IN THE HIGH AFFINITY BINDING OF α-INTERFERON TO ITS RECEPTOR, Amos Cohen, Gregory E. Hannigan, Zeev Estrov, Melvin H. Freedman, Brian R.G. Williams and Clifford Lingwood, Division of Immunology, Hospital for Sick Children, Research Institute, 555 University Avenue, Toronto, Ontario, M5G 1X8 CANADA.

The E.coli verotoxin exhibits selective cytotoxicity to primate epithelial cells. Of the lymphoid cells tested, only α-interferon sensitive cells such as hairy cell leukemia and Burkitt lymphoma cells were found sensitive to verotoxin. Analysis of Daudi Burkitt cell lymphoma mutants showed cross resistance between mutants selected for verotoxin and α_2-interferon resistance. Analysis ofverotoxin binding reveals two verotoxin binding glycolipid molecules, globotriosyl ceramide (GB_3) and galabiosyl ceramide, both terminating in the disaccharide galactose α1-4 galactose. Daudi cell mutants resistant to α_2-interferon as well as mutants resistant to verotoxin have reduced levels of GB_3 and undetectable amounts of the galabiosyl ceramide verotoxin binding glycolipids. Analysis of the binding kinetics of α_2-interferon to wild type and verotoxin resistant mutants reveals that the mutant cells lacking galabiose ceramide lost the high affinity component of interferon binding. These results suggest that either or both of these glycolipids serve as the functional receptor for verotoxin in Daudi cells and that these glycolipids associte with the interferon receptor protein in the creation of the high affinity binding of α_2-interferon.

J102 THE PHARMACOKINETICS OF MONOCLONAL IgG1, F(ab')$_2$ AND Fab' IN MICE, David Covell[1], Jacques Barbet[2], Oscar D. Holton[3], Christopher D.V. Black[1], Robert J. Parker[1] and John N. Weinstein[1], [1]National Cancer Institute, NIH, Bethesda, Maryland, 20892 [2]Centre d'Immunologie INSERM-CNRS, F-13288, Marseille. France. [3]Earl-Clay Laboratories Inc. Novato, CA 94947. The pharmacokinetics of an IgG1 and its F(ab')$_2$ and Fab' fragments following intravenous administration in mice have been studied by constructing a physiologically-based, organ-specific model to describe antibody biodistribution, catabolism and excretion. The antibody selected for study (MOPC-21) has no known binding sites in the body and therefore is useful for defining antibody metabolism by non-tumor tissues. When compared with whole IgG, the Fab' fragment a) is cleared from the body 35 times faster, b) has a larger total distribution volume, c) distributes more rapidly into this volume, d) produces higher interstitial:plasma concentration ratios, e) is catabolized principally by the kidney, f) is extracted from the circulation to a greater extent on each pass through the kidney, g) cycles through non-kidney interstitial spaces at least 7-fold fewer times per gram tissue weight before metabolism or excretion. The F(ab')$_2$ fragment has pharmacokinetic characteristics that fall between those of whole IgG and Fab'. These results 1) provide pharmacokinetic criteria for selecting whole IgG, F(ab')$_2$ or Fab' for various in vivo applications, 2) provide a framework for predicting cumulative tissue exposure to antibody labeled with different isotopes, and 3) provide a reference metabolic state for the analysis of more complex systems that do include antibody binding.

J103 RESPONSE OF RHESUS MONKEYS TO HUMAN MONOCLONAL ANTIBODIES: EFFECT OF SUBSTITUTING THE ANTIBODY, Paul H. Ehrlich, K. Elisabeth Harfeldt, James C. Justice, Zeinab A. Moustafa, and Lars Östberg, Sandoz Research Institute, Sandoz Pharmaceuticals Corp., E. Hanover, N.J. 07936.
We have shown previously that rhesus monkeys are, in general, tolerant to injections of human monoclonal antibodies. Multiple injections of one antibody, EV2-7, over 200 days resulted in no immune response in three monkeys. The same result was obtained with one monkey injected with a different monoclonal antibody, EV1-15. A second rhesus monkey injected with EV1-15 developed an anti-idiotypic response after the second injection. No anti-human IgG was detected in this monkey's serum within the limits of our assays. We reasoned that substituting EV2-7 for EV1-15 in this monkey would be possible since anti-idiotypic antisera against each antibody are not cross-reactive with the other antibody. This could be a model for switching to equivalent monoclonal antibodies in patients once an immune response has developed to an initial monoclonal antibody. However, soon after the initial injection of EV2-7 into the monkey with the anti-EV1-15 response, the EV2-7 was eliminated from the circulation. Therefore, even if an animal or patient has an immune response to a monoclonal antibody that is apparently totally anti-idiotypic, substitution of other monoclonal antibodies with non-cross-reacting idiotypes may not be a viable therapeutic strategy.

J104 MULTIPLE BOLUS INJECTION OF rt-PA LYSES CLOTS IN THE RABBIT JUGULAR VEIN MODEL AS EFFICIENT AS CONTINUOUS INFUSION, W.G. Eisert, H. Callisen, T.H. Müller
Thrombosis Research, Dr. Karl Thomae, Biberach, F.R.G.
Thrombolysis has been studied in vivo using a rabbit jugular vein model as described by Collen et al. Heparin was administered from the beginning of the operation. Clots of rabbit blood were allowed to age in situ for 1 or 4 hours before treatment. One hour after the two hour treatment interval, the clots were removed from the animal and their weight was determined. rt-PA treatment (0.7 mg/kg) was given either as continuous infusion (CI) over 2 hours or in fractionated doses; for intermittent infusion (IF) 0.09 mg rt-PA/kg was administered over 15 min. and this was repeated a further 3 times with 15 min. intervals between each administration in order to reduce the total rt-PA dose by 50 %. Four Bolus injections (BI; 0.09 mg/kg) at 30 min. intervals showed a reduction in clot weight (83.2 mg $\pm$ 11.9) when compared to IF (101.9 mg $\pm$ 5.8) and CI (99.2 mg $\pm$ 20.6) or saline treated controls (139.5 mg $\pm$ 27.9). Plasminogen or fibrinogen levels were not altered during these treatments, antiplasmin was reduced only during CI, although this was not significant. These data indicate that clots can be efficiently lysed by repeated bolus injections without noticeable fibrinogenolysis. Despite the short half-life, rt-PA bolus injections may be an effective and safe thrombolytic regimen to start or to preceed extensive treatment in coronary care units.

J105 DISPOSITION OF THE MONOCLONAL ANTIBODY-VINCA ALKALOID CONJUGATE, KS1/4-DAVLB (LY256787), IN FISCHER 344 RATS AND RHESUS MONKEYS, R. M. Goodwin and M. E. Spearman, Lilly Research Laboratories, Indianapolis, IN 46285.
The plasma pharmacokinetics, tissue distribution, metabolism, and elimination of the monoclonal antibody-vinca alkaloid conjugate, KS1/4-DAVLB, were studied in Fischer 344 rats and Rhesus monkeys. Plasma concentrations of KS1/4-[^{3}H]-DAVLB radioequivalents and functionally immunoreactive LY256787 were quantitated. The peak plasma concentrations were 196 and 1540 µg equivalents/ml of plasma in male rats dosed with 10 and 100 mg/kg, respectively, and 1100 and 1170 µg equivalents/ml in male and female monkeys. The plasma half-lives were 145 hr by radioactivity (153 hr by ELISA) in male rats dosed with 10 mg/kg, 62 hr (63 hr by ELISA) in male rats dosed with 100 mg/kg, 92 hr (81 hr by ELISA) in male monkeys dosed with 40 mg/kg, and 90 hr (79 hr by ELISA) in female monkeys dosed with 40 mg/kg. The correlation between the radiometric and ELISA values suggests that the majority of the circulating LY256787 is functionally immunoreactive. Plasma terminal volumes of distribution were relatively small suggesting that a large portion of the dose remained in the circulatory compartment for an extended time. Biodistribution studies in male rats demonstrated the greatest concentrations of LY256787 radioequivalents in the blood, lymphatic, lung, spleen, kidney, and gonad tissue. The primary route of excretion was fecal. Elimination half-times ranged from 150-250 hr. In rats and monkeys, the data are best described by a two-compartment pharmacokinetic model. The data in these studies correspond well to other pharmacokinetic data in the P3/UCLA human lung adenocarcinoma tumor-bearing nude mouse xenograft model in which LY256787 targeting has been found.

J106 AUGMENTATION OF MONOCLONAL ANTIBODY (MAb) BINDING TO HUMAN TUMOR CELLS THROUGH THE THE INCREASE IN CELL SURFACE TUMOR ANTIGEN EXPRESSION BY RECOMBINANT HUMAN LEUKOCYTE INTERFERON. J.W. Greiner[1], F. Guadagni[1], S. Pestka[2], and J. Schlom[1]. [1]Laboratory of Tumor Immunology and Biology, NCI/NIH, Bethesda, MD 20892; [2]Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Human IFN- α is a multigene family. We compared IFN- αA,B,C,D,F,I,J and K for their ability to growth inhibit human breast and colon carcinoma cells as well as to augment the expression of surface antigens. A high degree of heterogeneity with respect to these biological parameters was found among the IFN-α species. The A and B species were most potent in cell growth inhibition and in augmenting of MAb binding to cell surface antigens such as HLA, carcinoembryonic antigen (CEA) and a high-molecular weight tumor-associated glycoprotein, termed TAG-72. In contrast, the D and J species were virtually inactive in altering the expression of these or any other surface antigens. IFN- αD and - αJ were shown to interact with the interferon receptor by their ability to interfere with the antigen augmentation activities of IFN- αA or - αB. In vivo studies demonstrated that IFN-αA administration increased the amount of tumor antigen in colon tumor extracts and enhanced the localization of a ^{125}I-labeled anti-CEA MAb. IFN- αD treatment resulted in significant plasma interferon levels, yet, failed to alter either the antigen content or the in vivo localization of MAb. In conclusion, the IFN-α family exerts a wide range of biological actions. These findings are important for identifying those interferon species interferon that are capable of augmenting tumor antigen expression, and thus, may be earmarked for consideration in further study of the enhancement of MAb binding to human carcinoma cell populations.

J107 A SYNTHETIC PEPTIDE SELECTED IN CHOLERA TOXIN SEQUENCE IS IMMUNOGENIC ORALLY AND INDUCES LOCAL PROTECTION, Anne Guyon-Gruaz, Sylvie Pedoussaut, Agnes Delmas, Gerard Milhaud and Pierre Rivaille, U.A.163 C.N.R.S., Faculte de medecine St.Antoine, 27 rue Chaligny 75571 Paris Cedex 12 FRANCE.

Free synthetic peptides are poorly immunogenic in the absence of adjuvant and/or coupling to larger carriers. We postulated that peptides containing tetrapeptides unique to a protein would be highly immunogenic and could be used for immunization without carrier and adjuvant. Thus we chose, in the cholera toxin (CT) B subunit sequence, the hydrophilic peptides: 30-50 which contains the Arg 35 proposed to be involved in the binding of CT to its receptor and 50-75 which encompasses the 50-64 peptide shown to elicit neutralizing antibodies.

Since CT B subunit is known as a potent oral immunogen, which does not induce oral tolerance, a second aim of this study was the development of oral immunization using these synthetic peptides.

The two peptides, synthezized by the solid phase method, were injected intraperitoneally in a free form, whithout adjuvant: both peptides elicited antibodies cross-reacting with CT. The anti-50-75 antibodies neutralized CT biological activity. Moreover oral administration of these two peptides induced the production of seric antibodies and did not elicit oral tolerance. Mice orally immunized with the 50-75 were partially protected against the CT activity at the mucosal level.

These two peptides are potential synthetic carriers for oral immunization.

J108 PHARMACOKINETICS OF RECOMBINANT HUMAN COLONY STIMULATING FACTOR-1 IN RATS, Robert Halenbeck, Dick Bell, John Young and Kirston Koths, Cetus Corporation, 1400 Fifty-Third St., Emeryville, CA 94608.

We have characterized the pharmacokinetics of recombinant human colony stimulating factor-1 (rCSF-1) in rats. rCSF-1 was purified from supernatants of SV40-infected CV-1 monkey cells which had been transformed with a plasmid containing the human CSF-1 gene. The purified rCSF-1 was shown to be a dimeric, disulfide-linked glycoprotein, with an apparent native MW of approximately 65 kilodaltons.

The concentration of rCSF-1 in rat plasma was measured using a radioimmunoassay (RIA). Following intravenous administration, rCSF-1 was rapidly cleared from the circulation with an alpha phase of 2 min and a beta phase of approximately 20 min. Molecular sieve HPLC analysis showed that RIA-detectable rCSF-1 did not appear to be complexed with macromolecular serum components. Following intraperitoneal administration, peak rCSF-1 levels were reached in under 1 hr, 7% of the peak concentration was detectable after 5 hr, and over 20% of the injected material was absorbed into the bloodstream.

rCSF-1 was recovered in high concentrations in the urine as measured by RIA, and the excreted protein was shown to be biologically active using a colony-forming bioassay. Molecular sieve HPLC and Western analysis of the urine samples suggested that the rCSF-1 is excreted as an unproteolyzed, dimeric protein.

J109 CLINICAL STUDIES OF MONOCLONAL ANTIENDOTOXIN ANTIBODY XMMEN-0E5. W. Scott Harkonen, Ronald Mischak, Regina Alivasatos, Donald Kennedy, and Richard Greenberg, University of California, San Francisco, CA, XOMA Corp., Berkeley, CA 94710, and St. Louis University, St. Louis, MO 63104.

Nine patients with documented or suspected gram-negative sepsis were studied to determine the safety, pharmacokinetics, and immunogenicity of XMMEN-0E5, a murine IgM monoclonal antibody directed against the core lipid A component of bacterial endotoxin (L. Young, Clin. Res. 1984; 32(2):518A). Antibody was administered by a single intravenous infusion of 2-4 hours duration at doses ranging from 0.1 mg/kg to 15 mg/kg. Five patients subsequently had blood cultures positive for gram-negative bacteria; one patient had Torulopsis septicemia; two patients were culture negative; and one patient had gram-negative meningitis. No evidence of antibody-mediated toxicity was observed and all bacteremic patients survived their acute illnesses. Linear regression analysis revealed serum t1/2 values of 9.6 hours at the 0.5 mg/kg dose level; 9.03 hours at the 2 mg/kg level; 9.22 hours at the 7.5 mg/kg level; and 18.08 hours at the 15 mg/kg level. No difference in clearance was observed between bacteremic and non-bacteremic patients. The volume of distribution of the antibody was approximately 6 liters. Human anti-mouse antibodies were detected by enzyme-linked immunosorbent assay (EIA) one to three weeks after treatment in patients that received doses of 2 mg/kg or greater, but not in patients that received less than 2 mg/kg. This study indicates that XMMEN-0E5 is well tolerated at doses up to 15 mg/kg. Pharmacokinetic findings indicate that at doses less than 15 mg/kg, multiple infusions may be necessary to sustain therapeutic serum levels in the treatment of gram-negative bacteremia.

J110 PHARMACOKINETICS OF GENETIC AND DRUG INDUCED ALTERATIONS OF TISSUE PLASMINOGEN ACTIVATOR, Glenn R. Larsen, Mark Metzger, Yitzak Blue and Kim Henson, Genetics Institute, 87 CambridgePark Drive, Cambridge, MA. 02140.

We have developed a mammalian expression system which secretes high levels of tissue plasminogen activator (t-pa) into culture medium. T-pa is a serum glycoprotein involved in the proteolytic activation of plasminogen to plasmin, which catalyzes the dissolution of fibrin clots. The clinical efficacy of t-pa for use in myocardial infarct patients has been demonstrated. One potential drawback, however, has been the extremely short half-life of the protein which is approximately 7 minutes in man. We have established an *in vivo* model to evaluate the pharmacokinetic effect of genetic and drug induced alterations of t-pa. The effect of these alterations on *in vivo* clearance rates will be presented.

J111 DECAPEPTYL: RELEASE FROM MICROCAPSULES AND TESTOSTERONE SUPPRESSION IN THE DOG, G.Nicolau, A.P.Tonelli, J.R.Lawter, B.M Silber, R.A.Lanc, M.Lanzilotti, A.R.Dente, W.W.McWilliams, A.Yacobi, Medical Research Division, American Cyanamid Company, Pearl River, NY 10965.

Decapeptyl (CL 118,532), D-Trp^6-LH-RH) (D) is an LH-RH analog which has demonstrated effectiveness against the growth of steroid dependent tumors. The release of D and the relative bioequivalence from three microencapsulated formulations (F1, F2 and F3) intended for once-a-month intramuscular dosing, was investigated in beagle dogs. Plasma concentrations of D and testosterone were determined. Statistically significant differences in the total amount of drug released from the formulations were not present. However, the time course of D release was different, as was the profile of testosterone suppression, for the different formulations. Testosterone concentration profiles showed and initial elevation, lasting for 1-5 days followed by depression and total suppression lasting from 30 to 60 days postdose (greatest in F1 and F3). A positive correlation was established between the amount of D released on day 1 and testosterone suppression. The cessation of testosterone suppression was associated with the lack of detectable decapeptyl concentrations.

J112 ENZYMATIC PROTEOLYSIS OF THE PLATELET MEMBRANE GLYCOPROTEIN IIb/IIIa COMPLEX AND EXPOSURE OF FIBRINOGEN RECEPTORS. S. Niewiarowski, E. Kornecki, H. Lukasiewicz, A. Eckardt, R. Egbring and Y.H. Ehrlich. Temple Univ., Phila., PA, USA Univ. of Vermont, Burlington, VT., Univ. Hospital, Marburg, F.R. Germany.

Previous investigations from our laboratories indicate that the proteolytic enzymes: chymotrypsin (C), porcine pancreatic elastase (PE), and human granulocyte elastase (HGE) incubated with human platelets cause exposure of fibrinogen binding sites, resulting in a spontaneous platelet aggregation upon addition of fibrinogen. It is well known that fibrinogen receptors are associated with glycoprotein IIb (GPIIb) and glycoprotein IIIa (GPIIIa) on the platelet membranes. The purpose of the present studies was to characterize the proteolytic state of GPIIb and GPIIIa on the surface of platelets incubated with these enzymes, and to correlate such proteolysis with the exposure of fibrinogen receptors and fibrinogen-induced platelet aggregation. Incubation of platelets with C, PE, and HGE resulted in the formation of a 66kDa component derived from GPIIIa and caused an increase of a band corresponding to 66kDa dimer. Treatment of platelets with an excess of C or over prolonged periods of time resulted in degradation of GPIIb and 66kDa component derived from GPIIIa concurrent with a loss of platelet reactivity with fibrinogen. We propose that partial proteolysis of GPIIIa on the platelet surface may result in a change of its conformation and facilitate exposure of fibrinogen receptors and that at least some fibrinogen bindings sits are associated with a 66kDa component derived from GPIIIa.

J113 A HUMAN MONOCLONAL ANTIBODY:THE CYTOTOXICIC ACTIVITY IN VITRO AND TUMOR GROWTH INHIBITORY ACTIVITY IN VIVO. Kazuoki Osumi[1],Junzo Nagao[2] Hideo Yuasa[2],Hideki Fujimoto[1],Hideaki Hagiwara[2],Yoshihisa Kodera[3] and Ryuzo Ohno[3],Cent.Res.Lab.Mitsubishi Petrochem.Co.Ltd.[1],Hagiwara Inst.Health[2],1st. Dept.Int.Med.Nagoya Univ.Sch.Med.[3]

A human x human hybridoma termed CLNH11 was produced by fusing 6TG resistant human B lymphoblastoid cell line(UC729-6) with lymphocyte obtained from a patient with squamous cell carcinoma of cervix[4].CLNH11 secreted a human monoclonal IgG 1,κ (CLN-IgG) which bound to various human tumor lines, but not to normal fibroblast.CLN-IgG exhibited the activity of ADCC to human cervical carcinoma lines(ME-180,HeLa229) and a human lung carcinoma line(A549) which reacted with CLN-IgG,but not to a human bladder carcinoma line(HT-1376) which did not react with CLN-IgG.

Nude mice were injected with $5x10^6$ of ME-180 cells subcutaneously and were given s.c. injection of 1 to 5mg of CLN-IgG with tumor cells or i.v. injection within 1Hr tumor cells inoculation.The growth of tumor were completely surpressed for 14 days in CLN-IgG given group,but not in control group and in nonreactive human IgG given group.After 14 days the growth of tumor was observed.The growth of relapsed tumor was surpressed again with i.v. injection of CLN-IgG on day 30.

4) Hagiwara et.al. Molec.Biol.Med. 1,245 ('83)

J114 A PHARMACOLOGICALLY IMPROVED STREPTOKINASE- POLYETHYLENE GLYCOL COMPLEX, Shrin Rajagopalan, Steven L. Gonias and Salvatore V. Pizzo, Duke University, Durham NC 27710.

A series of new, covalent polyethylene glycol (PEG)-streptokinase adducts were synthesized and characterized. PEGs with average molecular weights of 2000, 4000, and 5000 were activated with carbonyldiimidazole and coupled to the protein under standardized reaction conditions. Steady-state kinetic analyses demonstrated comparable K(m) values for the activation of plasminogen by streptokinase and by PEG-derivatized streptokinase, while k(cat) values were somewhat decreased after PEG-modification. Nonetheless, when plasminogen was incubated with the streptokinase derivatives and plasmin generation was studied as a function of time, the PEG-derivatives displayed activity comparable to native streptokinase. A possible explanation for this data was that PEG-modification protected streptokinase in activator complex from being degraded. Improved pharmacologic properties for PEG-streptokinase included greatly diminished reactivity with anti-streptokinase antibodies and an increased circulatory half-life in mice. Plasmin in complex with modified streptokinase also displayed an increased circulatory half-life in mice. Activator complexes containing native and PEG-modified streptokinase were compared in terms of their ability to bind to and lyse fibrin bound to polystyrene tubes. It is suggested that PEG-streptokinase complexes may be useful in the treatment of thrombotic disorders.

J115 DIFFERENTIAL ENHANCEMENT OF THE ACTION OF INDIRECT IMMUNOTOXINS. J. K. Weltman, P. Pedroso and S-A. Johnson. RIH-Brown University, Providence, RI 02902.
The lysosomotropic agents NH_4Cl and chloroquine (CLQ) differentially enhance the action of indirect immunotoxin (IIT) prepared either from ricin A (RA) or from pokeweed antiviral protein (PAP). Target H69c lung carcinoma cells, which had been incubated with mouse anti-H69c Ig, were treated with IIT, which consisted of anti-mouse Ig (anti-MIg) disulfide-linked either to RA or to PAP. Incorporation of H^3-leucine into cellular protein was measured after 18 hours at 37°. Inhibition of protein synthesis caused by IIT in the presence of the lysosomotropic agents is given below (mean inhibition ± SD; n=5):

IIT	RPMI-1640	CLQ (0.1 mM)	NH_4Cl (20 mM)
anti-MIg-RA	37 ± 5%	97 ± 1%	93 ± 1%
anti-MIg-PAP	18 ± 6%	85 ± 1%	10 ± 10%

Both CLQ and NH_4Cl enhanced the inhibitory activity of the RA-based IIT, relative to inhibition in RPMI-1640 medium ($p < 0.0001$). CLQ enhanced the inhibitory action of the PAP-based IIT ($p < 0.0001$), but NH_4Cl did not. These results suggest that PAP-based IIT is processed differently from RA-based IIT in H69c cells. Inactivation of the PAP-based IIT may be independent of intralysosomal $[H^+]$. This work is the first example of a PAP-based IIT, and represents a considerable improvement over the previously available IIT in detection of anti-tumor antibodies because of a greater than 2-fold decrease in background toxicity. (See also BioTechniques 4, 224, 1986).

J116 SUSTAINED RELEASE OF A PEPTIDE FROM LIPOSOME FORMULATIONS, Annie Yau-Young, Michael Law, Joan Chow and Jan-Ping Lin, Liposome Technology Inc., 1050 Hamilton Ct., Menlo Park, CA 94025.
This study was conducted to examine the feasibility of using liposomes as sustained release delivery vehicles for biologically active peptides. We have encapsulated a peptide hormone (MW 5,000), P-18, in multilamellar and unilamellar liposomes with high encapsulation efficiencies. Full biological activity of the peptide was released when the P-18-liposomes were lysed. Radio-labeled P-18 and P-18-liposomes were injected intramuscularly and subcutaneously into rats. The half-life of clearance of P-18 from the site of injection was several minutes, while that of P-18-liposomes ranges from 6 hours to 90 hours depending on the formulation. Immunoreactive P-18 was detectable up to several days in plasma of rats which had received P-18-liposomes. P-18-liposomes retreived from the site of injection over a 7-day period demonstrated expected level of biological activity. Histopathological examination in rats showed no significant inflammatory response to multiple dosing of liposomes and P-18-liposomes relative to saline administration at the site of injection. Stability study of P-18 in liposomes demonstrated that liposome formulations can retain the activity of P-18 at concentrations where P-18 would be inactivated.

New Protein Therapeutics - Animal Models and Combination Proteotherapies and/or Proteins + Drugs

J200 SELECTIVE ABLATION OF B CELLS IN VIVO BY AN ALPHA EMITTER, ^{212}Bi, CHELATED TO A MONOCLONAL ANTIBODY. C.D.V. Black, R.W. Atcher, A. Lewis, O.A. Gansow, J. Barbet§, O.D. Holton,III, D.G. Covell & J.N. Weinstein. National Cancer Inst., NIH. Bethesda, MD 20892. §Centre d'Immunologie INSERM-CNRS, Marseille, France. An anti-murine B cell antibody (anti-LYB8.2) has been conjugated to the α-particle-emitting radionuclide ^{212}Bi using a bifunctional ligand, 1-(p-isothiocyanatobenzyl)-DTPA. Specific activities in the range of 1-12 μCi/μg protein were obtained. Specificity of the preparation for murine B cells was demonstrated in vitro by inhibition of ^3H-thymidine incorporation into B and T cells. B cells were killed at doses less than one tenth those required for equivalent killing of T cells. Subcutaneous injection into the footpads of mice caused a selective killing of almost all of the B lymphocytes (IC50 10-fold lower than T cells) in the lymph nodes draining the injection site; accessory cell function was unaffected. B lymphocytes in the nodes completely disappeared for several hours but recovered to 50 % of pre-treatment levels 24 hrs after injection. The cytocidal effects were blocked when the immunoradionuclide was co-injected with an excess of unlabelled α-LYB8.2. Splenic B and T lymphocytes were not affected by s.c. injection of ^{212}Bi-labelled antibody, but the B cells were selectively killed when the preparation was administered i.v. After i.v. injection the lymph node lymphocytes were not affected. These experiments provide a basis for the in vivo use of α-particle-emitting radionuclides in the treatment of lymphomas and lymph node metastasis, and perhaps in the control of tolerance and immunization.

J 201 PRECLINICAL DEVELOPMENT OF KS1/4-4-DESACETYL VINBLASTINE-3-CARBOXHYDRAZIDE (KS1/4-DAVLB-HYDRAZIDE) FOR SITE-DIRECTED THERAPY OF HUMAN ADENOCARCINOMAS, Thomas F. Bumol, Bennett C. Laguzza, Sonja V. DeHerdt, John E. Parrish, Eric L. Andrews, John L. Zimmermann, A. Leroy Baker, and Lynn D. Apelgren, Lilly Research Laboratories, Indianapolis, IN 46285.

Monoclonal antibody KS1/4 which recognizes a human epithelial/epithelial malignancy associated antigen has been studied as a site-directed therapy agent in preclinical efficacy and toxicology studies as the KS1/4-DAVLB-Hydrazide (LY203725) conjugate. This conjugate retains excellent antigen reactivity as measured by various serological techniques and high vinca alkaloid potency. KS1/4-DAVLB-Hydrazide can effect 100% tumor suppression in tumor initiation xenograft models of lung and colorectal adenocarcinoma origin (I.V. Rx, Days 2,5,8 after tumor implantation) at doses ranging from 0.25 mg/kg-1.0 mg/kg (vinca equivalent). Treatment of sixteen day established xenografts with KS1/4-DAVLB-Hydrazide have effected greater than 90% tumor regressions and KS1/4-DAVLB-Hydrazide treatment has resulted in long-term survival ($p<0.001$) and tumor free animals (>300 days) in an i.v. experimental metastases xenograft model. In all efficacy experiments, an excellent therapeutic index is maintained over free drug treatments. In addition, these effects are antigen mediated as demonstrated with experiments utilizing irrelevant myeloma-davlb-hydrazide conjugates or antigen negative xenograft targets. Preclinical pilot toxicology studies in rats and primates suggest an effective therapeutic window for human clinical trials. Comparisons of KS1/4-DAVLB-Hydrazide to a previously described conjugate, KS1/4-DAVLB, will be discussed.

J 202 EFFECT OF COMBINATION RECOMBINANT HUMAN TUMOR NECROSIS FACTOR (rTNF) AND INTERLEUKIN 2 (rIL-2) ON EXPERIMENTAL E. COLI INFECTION IN MICE. K.T. Chong, Cetus Corp., Emeryville, CA 94608

When administered prophylactically rIL-2 protects mice against lethal bacterial challenge (26th ICAAC, Abstract #768). In this study we combined rIL-2 and TNF to evaluate the effects of this combination of lymphokines in a similar bacterial infection model. Each lymphokine was administered as a single intraperitoneal dose, 4 hours before challenge with a lethal dose of a clinical isolate of Escherichia coli. IL-2 protection was dose dependent, showing maximal effect at the high dose of 2 mg/kg (specific activity, 3.9×10^6 u/mg); at this dose 90% of treated mice survived as compared to 0% for the excipient buffer control ($P < 0.001$). TNF alone was administered at various doses from 0.04 to 400 ug/kg. Unlike IL-2, TNF only induced a maximal protection of 40-50% survival (as compared to 0% for buffer control) at dose of 0.4-4.0 ug/Kg. In combination treatment, when IL-2 and TNF were administered as two simultaneous injections, significant ($P < 0.01$) survival (80%) was seen at the much lower IL-2 dose of 0.1 mg/kg and TNF dose of 0.4 ug/kg (20% survival for either TNf or IL-2 alone). Thus, combination of TNF and IL-2 is more effective at inducing host resistance to infection than the administration of TNF or IL-2 alone ($P < 0.05$). These studies indicate that the protective effect of IL-2 and TNF combination is synergistic and may have potential therapeutic benefit for the prophylaxis of bacterial infections in clinical and veterinary medicine.

J 203 Encapsulation Of Tumor Necrosis Factor (TNF) In Liposomes: Characterization And Effects On Bioactivity. R J Debs, R Philip, E N Brunette, L B Epstein, N Duzgunes, University of California, San Francisco, 94143.

Recent studies have demonstrated that TNF is an important mediator of the host immune response. It exerts direct antitumor and antimicrobial activity in vitro, as well as inducing activation of cell-mediated immunity. However, TNF can produce significant host toxicity, thus limiting its therapeutic index in vivo. The drug carrier system, liposomes have been shown to enhance cytokine induced macrophage activation, while reducing associated host toxicity. Targeted delivery of TNF to immune effector cells (as well as to tumors or sites of infection), may enhance efficacy while limiting toxicity. Therefore, we encapsulated recombinant human TNF (rTNF), Genentech, in phosphatidylglycerol/phosphatidylcholine/cholesterol or phosphatidylglycerol/cholesterol liposomes. Recovery of rTNF in liposomes ranged from 3 to 35% depending on both liposome phospholipid composition and mean diameter. Both hydrophobic and electrostatic interactions appear to influence the extent of TNF uptake by liposomes. The amount of rTNF recovered from liposomes, as measured by ELISA, was increased up to 6 fold by detergent lysis, suggesting that the majority of encapsulated protein is contained within the liposome. Preliminary studies indicate that liposome-encapsulated TNF is at least as effective as free TNF in inducing interleukin-1(IL-1) release from human monocytes, and cytolysis of murine and human tumor cell lines. rTNF synergizes with low dose recombinant human gamma interferon (Genentech) to induce IL-1 release. Further in vitro and in vivo studies of liposome-encapsulated rTNF are in progress.

J 204

Abstract Withdrawn

J 205 SEQUENCE-DEPENDENCE OF COMBINATION ANTI-TUMOR PROTEOTHERAPY WITH HUMAN RECOMBINANT INTERLEUKIN-2 AND TUMOR NECROSIS FACTOR Stacey Gauny, Robert Zimmerman and Jeffrey L. Winkelhake, Cetus Corp., Emeryville, CA 94608

Human recombinant Interleukin-2 and human recombinant Tumor Necrosis Factor exhibit marked synergy (cures) when administered in combination to BD2-F1 mice shortly after subcutaneous innoculations of tumor cells (B16 melanoma, L1210 leukemia, P815 mastocytoma, EL-4 lymphoma and Meth-A sarcomas were studied). Similar results are seen in an artificial metastasis model using intravenously-administered B16 melanoma cells. Optimal therapeutic efficacy (blockage of tumor takes) is observed when the IL-2 + TNF combination is administered for 14 days with TNF at a maximum tolerated dose (MTD = dose/schedule which gives 5-10% body weight loss with no deaths), while the IL-2 dose in the combination can be diluted to 1/100th of the MTD. Sequence studies show that TNF is also rate limiting in that it must be administered either prior to or with IL-2. That is, IL-2 cannot be given prior to TNF to obtain optimal synergy. A sequence of immune modulatory events is thought to be responsible since synergy is not seen when these two protein therapeutics are administered to Nude (T cell deficient) or NIH-3 (T and Lak cell deficient), but is seen with Beige mice bearing tumors. Concomitant administration of immunosuppressive doses of cytoxan (along with the TNF + IL-2) also ablates therapeutic efficacy with these tumor models.

J 206 Demonstration of a Protease Nexin-like Thrombin Inhibitor on the Platelet Surface. Robert S. Gronke and Joffre B. Baker. Department of Biochemistry, University of Kansas, Lawrence, Kansas, 66045.

Platelets possess a factor on their surfaces, which forms a SDS-resistant 77k Dalton complex with ^{125}I-thrombin. This factor, similar in several respects to the fibroblast secreted thrombin inhibitor protease nexin I (PNI), is termed platelet protease nexin (PNp). ^{125}I-thrombin-PNp complexes 1) bind tightly to anti-PNI antibody, 2) do not form if ^{125}I-thrombin has its active site blocked with diisopropylphosphofluoridate, and 3) do not appear on platelets in the presence of heparin. In a 30 s binding incubation with < 0.3 nM ^{125}I-thrombin, ^{125}I-thrombin-PNp complexes account for most of the specific binding to platelets. The dose dependence of the ^{125}I-thrombin reversible binding to platelet receptors can be determined by subtracting the contribution of the ^{125}I-thrombin-PNp to specific ^{125}I-thrombin binding. This generates a sigmoidal dose-dependence curve. It is possible that at low thrombin doses, PNp effectively captures most of the thrombin that collides with platelets, preventing thrombin from reaching platelet receptors. ^{125}I-thrombin-PNp complexes are found both associated with platelets and free in the binding medium. The free complexes do not bind back to platelets. Their formation thus probably occurs at the platelet surface followed by a sloughing into the binding medium. ^{125}I-thrombin-PNp complexes form on isolated platelet plasma membranes, indicating that the platelet plasma membrane contains PNp in stable association. In addition, thrombin at >[3nM] stimulates the expression of PNp. The latter PNp may come from platelet granules because its appearance is inhibited by drugs that block granule release.

J207 EFFICACY OF A MURINE MONOCLONAL ANTIBODY PF1/D-4-DESACETYLVINBLASTINE-3-CARBOXHYDRAZIDE CONJUGATE (PF1/D-DAVLBHYD) IN A SQUAMOUS CARCINOMA XENOGRAFT MODEL, David A.Johnson, B.C.Laguzza, A.L.Baker, M.C.Butowski, C.L.Nichols, and D.V.Fix, Lilly Research Laboratories (MC931), Indianapolis, IN 46285

The murine monoclonal antibody PF1/D has been reported to be preferentially reactive with squamous cell carcinoma (Fernsten et al Cancer Research 46:2970, 1986). We wished to determine whether this antibody would be effective as a DAVLBHYD conjugate in treating a human squamous carcinoma derived cell line grown as a nude mouse xenograft.

Antibody was purified from ascites using standard Protein A affinity techniques. SDS-PAGE and Coomassie blue staining indicated greater than 95% purity, though two light chains were detected. Subsequent immunoblot analysis revealed the presence of two heavy chain isotypes (IgG3 and IgG1) and analytical chromatography in three systems demonstrated the presence of molecular heterogeneity. These results are consistent with the secretor status of the original fusion partner and suggest the possible presence of hybrid molecules.

Antibody was conjugated to DAVLBHYD using techniques detailed elsewhere (Laguzza et al, In Preparation). Antigen binding capacity post conjugation was minimally reduced. When tested in a 3 day established tumor model using a 3 dose protocol, the conjugate proved superior to free antibody or an irrelevant conjugate at suppressing tumor growth, resulting in 100% inhibition at a 2 mg(vinca content)/kg dose level. These data suggest that antibody PF1/D, as a DAVLBHYD conjugate, may have therapeutic potential.

J208 MONOCLONAL ANTIBODY - 4-DESACETYLVINBLASTINE-3-CARBOXHYDRAZIDE CONJUGATES: DESIGN AND PREPARATION, Bennett C. Laguzza, Cynthia L. Nichols, Stephen L. Briggs, A. Leroy Baker, Thomas F. Bumol, David A. Johnson, and James J. Starling, Lilly Research Laboratories, Indianapolis, IN 46285.

4-Desacetylvinblastine-3-carboxhydrazide [DAVLBHYD, R. A. Conrad et al, J. Med. Chem. 22, 391, (1979)], a potent cytotoxic agent, has been coupled to a variety of murine monoclonal antibodies (MoAbs) selective for human solid tumors. The rationale and chemistry of construction of these MoAb - DAVLBHYD conjugates will be described. The native MoAbs' antigen recognition characteristics, as well as other biochemical and physical properties are well retained in the conjugates. These conjugates exhibit significant anti-tumor activity in vivo against human solid tumor-nude mouse xenografts, with increased efficacy over free DAVLBHYD.[1]

1. See Bumol et al, D. A. Johnson et al, and Starling et al, this symposium.

J209 **NEUTRALIZING ANTI-HUMAN C5a MONOCLONAL ANTIBODIES.** James W. Larrick, Tracy Deinhart, Jeff Wang and Stephen Kunkel, Cetus Corporation, Palo Alto, CA 94303, and University of Michigan, Ann Arbor, MI 48109. Human C5a (hC5a)is generated by both the classical and alternate pathways of the complement cascade. C5a causes activation and aggregation of granulocytes by binding to high affinity receptors. Such aggregation produces tissue damage in clinical conditions including pulmonary dysfunction and leukostasis in hemodialysis, burn injury, post-pump syndrome and the adult respiratory distress syndrome (ARDS). Because polyclonal anti-C5a antisera reduced mortality in a primate model of ARDS (JCI 77:1812), we have generated a panel of anti-hC5a murine monoclonal antibodies. Eleven of 12 monoclonals positive in the initial ELISA screen immunoprecipitated radiolabelled hC5a; 10 of the antibodies neutralize the ability of hC5a to cause neutropenia in rabbits. One of these antibodies, 10F7, blocks hC5a-induced polarization and hC5a-induced chemiluminescence of human neutrophils. Based on competitive immunoprecipitation assays using zymosan-activated sera, the monoclonal antibodies are weakly cross-reactive with C5a from guinea pig, dog, monkey and rabbit. At least two epitopes on hC5a are identified by these antibodies. Antibody 10F7 has an affinity of $3.2 \times 10^{9} M^{-1}$. The other monoclonals have somewhat lower Ka's. One or more of these antibodies may have therapeutic potential in syndromes of complement activation such as ARDS.

J210 IN VIVO EFFICACY OF DRUG CONJUGATES DERIVED FROM MONOCLONAL ANTIBODIES OF THREE DIFFERENT ISOTYPES WHICH BIND THE HUMAN TUMOR ASSOCIATED ANTIGEN, KS1/4, James J. Starling, Ronald S. Maciak, N. Ann Hinson, Cynthia L. Nichols and Bennett C. Laguzza, Lilly Research Laboratories, Indianapolis, IN 46285.
Three monoclonal antibodies (Moabs) of different isotypes have been isolated which bind to the human tumor-associated antigen originally defined by the KS1/4 MoAb (Varki, et al, Cancer Res., 44:681, 1984). These MoAbs were designated L1-(IgG2b), L2-(IgG1), and L4-(IgG2a) KS. Binding specificity, immunoprecipitation, and competitive binding analyses indicated that these MoAbs recognize the same epitope. These antibodies have been studied as MoAb-drug conjugates against a variety of human tumor targets grown in vivo as nude mouse xenografts. The MoAb-drug conjugates were constructed using ascites-produced, protein A-purified, antibodies conjugated to 4-desacetyl vinblastine-3-carboxhydrazide. Efficacy was determined using various dosing protocols on 2 to 21 day established tumors of lung, pharynx, colon, prostate, and skin origin. Control experiments included using dual flank antigen positive and negative tumors, free MoAbs, free drug, and mixtures of MoAbs and drug. These studies indicated that significant tumor growth suppression and actual tumor regression could be achieved by the MoAb-vinca conjugates and that this activity was antigen mediated. The drug conjugates were much more efficacious than free drug or free MoAbs administered either singly or in combination with each other. Each of the antibody isotypes used in these studies was equally effective in suppressing tumor growth as drug conjugates.

J211 TOXICITY AND IMMUNOGENICITY OF MONOCLONAL ANTIMELANOMA ANTIBODY RICIN A CHAIN IMMUNOTOXIN IN RATS, John B. Stoudemire, Ronald P. Mischak, and W. Scott Harkonen, XOMA Corporation, Berkeley, CA 94710.
This study was performed to assess the subacute toxicity and immunogenicity of an antimelanoma monoclonal antibody-ricin A chain conjugate (XOMAZYME®-MEL) in rats. The test groups received intravenous injections of the immunotoxin at a dose of 1 or 5 mg/kg/day for 14 consecutive days. The control group received normal saline. Animals from each group were sacrificed on days 8, 15, and 22 for evaluation of the pathology, hematology, serum chemistry, and antibody responses. Toxicities observed in the group receiving immunotoxin at a dose of 5 mg/kg/day included transient weight loss, peripheral edema, leukocytosis, hypoalbuminemia, and elevated liver enzymes. Histologic findings included hepatocyte vacuolization, focal myocardial and skeletal muscle degeneration, and proteinaceous casts in the renal tubules. Toxic effects were minimal in the group receiving immunotoxin at a dose of 1 mg/kg/day. Antibodies to the murine immunoglobulin and ricin A chain components of the immunotoxin were detected in the sera of both treatment groups. The development of antibody titers appeared to be temporally related to a reduction in the systemic toxicity. Data on the toxicity and immunogenicity of this conjugate will facilitate the design and the interpretation of human clinical trials of immunotoxins.

J212 CORTICOTROPIN-RELEASING FACTOR: ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES. Edward T. Wei and Juliann G. Kiang. University of California, Berkeley CA 94720.
The pharmacological treatment of pain has long relied upon the narcotic analgesics and the non-steroidal anti-inflammatory analgesics. The narcotic analgesics are effective against both mild and severe pain and are thought to act on receptors located in the brain and spinal cord. The non-steroidal anti-inflammatory analgesics are effective against mild pain and act via inhibition of prostaglandin synthetase. In recent years, a third mechanism for designing drugs to control pain and inflammation has been proposed. A number of neuropeptides have been discovered in peripheral nerve that may affect the algesic (pain-producing) and inflammatory processes. Agents designed to block or enhance the actions of these neuropeptides may have therapeutic value and, unlike the narcotics, would be non-addicting because they do not cross the blood-brain-barrier. We have recently found that corticotropin-releasing factor (CRF), a 41-residue peptide that regulates the secretion of proopiomelanocortin from the pituitary, has profound inhibitory effects on inflammation and nociception in various bioassays. CRF, at μg/kg doses administered to anesthetized rats, blocked the edema and protein extravasation produced by thermal injury and by chemical irritation to the skin and tracheal mucosa. For example, CRF injected 30 μg/kg i.v. to anesthetized rats, prevented the swelling and protein extravasation evoked by 10 N HCl applied to the pawskin. The actions of CRF were striking and obvious to the naked eye of the lay person. The bioassay data on the efficacy of CRF and its possible mechanism of action will be described. This research is supported in part by USPHS Grant No. DA-00091, DA-03777 and the University of California Toxic Substances Teaching and Research Program.

J213 ROLE OF FREE RADICALS IN TUMOR CELL SENSITIVITY TO RECOMBINANT HUMAN TUMOR NECROSIS FACTOR: IMPLICATIONS FOR MECHANISMS OF ACTION, Robert J. Zimmerman, Anthony Chan, Phoebe Landre, Jeffrey L. Winkelhake, Cetus Corporation, Emeryville, CA 94608

The intracellular glutathione levels of 3 human tumor lines and 5 mouse tumor lines were determined in order to investigate the role of radical scavengers in tumor cell sensitivity to recombinant human tumor necrosis factor (rhTNF). Both in vivo and in vitro, correlations were found between high glutathione levels and tumor cell resistance to rhTNF, and on the other hand, low glutathione levels and rhTNF sensitivity. The transplantable murine fibrosarcoma, Meth A, a TNF^S line in vivo, was less sensitive to rhTNF and host toxicity was reduced, when the hosts were pre-treated with uric acid, a major radical scavenger in humans and other primates. Conversely, pretreatment of the tumor-bearing host with BSO, an inhibitor of GSH biosynthesis, resulted in an increased sensitivity of Meth A to rhTNF. The data are consistent with the hypothesis that tumor cell sensitivity to rhTNF is dependent on free radical scavenging capacity. Whether these radical species are generated by host immune effector cells that have been activated by rhTNF, or a direct consequence of rhTNF binding, internalization and metabolism in target cells, or a combination of these 2 activities, are areas of ongoing investigation. We hypothesize that activation of the arachidonic acid cascade is involved in the mechanism of action of rhTNF, which can produce lipid peroxidation and other associated radical species, as well as the biologically active metabolites of the lipoxygenase and cyclooxygenase pathways.

NOTES: