177P ROBUST AND SIMPLE SOFTWARE / HARDWARE TO RECORD MUSCLE CONTRACTIONS IN THE PRACTICAL CLASS

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The kymograph is simple to use but lacks precision and sensitivity offered by mechanical transducers. Commercial systems which might replace the kymograph target the researcher rather than the student. Our objective was to create a recording system which is intuitive and easy to run in a busy practical class.

Hardware: The hardware consists of a robust almost unbreakable piezoelectric transducer, a bridge amplifier, an interface card (Data Translation DT302) and computer.

Software: The software displays the record on the computer screen as well as controlling the gain, acquisition rate and zero offset. On opening the application, it self zeroes the input, and begins the data acquisition. It runs on 20 work-stations each running under Windows 95/98 and networked with Novell. The initial settings are can be configured on one computer and these settings are read by the other machines on start up from a configuration file on the server. This file also contains a preset list of protocols, and stores other settings which allow data saving and replay, facilities that are password protected.

Security features: To obtain a hard copy, each student at a work-station has to enter their name on the screen and then one copy is printed out for each name. To ensure that the record used for the write-up is the student's own work, the printout is date stamped and shows the student name. The printout also shows event marks and user entered text describing the protocol.

Help: The software comes with a help file explaining the function of the controls, information related to the experiments and a video file showing the dissection and installation of the preparation.

Usage: The system has been exposed to around 2000 students during the past two years. The students range in ability from diploma nursing to second year science students. It has been used to rabbit gut contractions, frog heart, frog gastrocnemius muscle and vitalograph recordings for mostly 2 hr classes.

178P FOLATE-MEDIATED DELIVERY OF THERAPEUTIC AND IMAGING AGENTS TO CANCER TISSUE *IN VIVO*

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The vitamin folic acid (FA) enters cells either through a carrier protein (termed the reduced folate carrier) or via receptor-mediated endocytosis facilitated by the folate receptor (FR). Because folate-drug conjugates are not substrates of the former, they penetrate cells exclusively via FR-mediated endocytosis. Overexpression of FR on cancer cells allows tumour-selective target-ing of folate-drug conjugates to malignant tissues, including cancers of the ovary, cervix, endometrium, kidney, breast, brain, lung and colon. FR expression appears to be even further upregulated in cancers that are resistant to standard chemotherapy in higher grade and later stage neoplasms. Thus, precisely those cancers that are most difficult to treat by classical methods are most easily targeted by folate-mediated therapy.

Folate-mediated tumor targeting has been exploited to date for delivery of the following molecules and molecular complexes: (i) protein toxins; (ii) chemotherapeutic agents; (iii) genes; (iv) oligonuceotides; (v) ribo-zymes; (vi) radioimaging agents; (vii) MRI contrast agents; (viii) liposomes with entrapped drugs; (ix) radiotherapeutic agents; (x) immunotherapeutic agents; and (xi) enzyme constructs for prodrug therapy. In all cases, in vitro studies demonstrate a significant improvement in potency and cancer cell specificity over the non-targeted form of the same agent. Where live animal studies have been conducted, they also reveal improved response rates. Results of both preclinical and clinical studies of several folate-targeted drugs will be presented.

Recent applications of folate targeting to the diagnosis and treatment of various inflam-matory diseases will also be described. During clinical evaluation of our folate-targeted imaging agent, it was observed that arthritic (but not normal) joints, as well as malignant issue, were readily visualised. We have subsequently learned that macro-phages express the folate receptor, and that the receptor is unable to bind either folic acid or its drug conjugates until the macrophage is activated. As a consequence, normal macrophages are refractory to folate-drug conjugates, but activated macrophages take them up avidly.

Based on studies in animals, we envision applications of folate targeting in the treat-ment of diseases caused by activated (but not resting) macrophages, including rheuma-toid arthritis, ulcerative colitis, Crohn's disease, psoriasis, osteo-myelitis, multiple sclerosis, graft versus host disease (e.g. organ transplant rejection) and athero-sclerosis.

Leamon C.P. & Low P.S. Drug Discovery Today, 2001, 6: 44-51

Reddy J.A. & Low P.S. Critical Reviews in Therapeutic Drug Carrier Systems, 1998, 15: 587-627.

179P ANTI-CANCER PROTEIN THERAPY: TRANSDUCTION OF TUMOUR SUPPRESSOR PROTEINS *IN VIVO*

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Our laboratory is focused on understanding the molecular basis of cancer, specifically the consequences that occur due to deregulation of tumor suppressor functions during G1 cell cycle progression. The ultimate goal of our work is to use this knowledge to devise anti-cancer strategies that specifically kill tumor cells *in vivo* by delivery of tumor suppressor proteins. Indeed, due to evolutionary pressures, tumor suppressor proteins perform very specific functions inside cells and therefore can selectively kill tumor cells while leaving the surrounding normal cells (and tissues) unharmed. However, in general, we are currently restricted to delivery of small molecules that are less than 600 Daltons. This bioavailability size limitation has restricted the development of novel drugs and remains a bane of the pharmaceutical industry. Unfortunately, tumor suppressor proteins, while maintaining specificity for their intracellular targets, are 20x or even 100x larger in size and, therefore, currently undeliverable.

To address the delivery problem, our laboratory further developed a strategy, termed 'protein transduction' or 'protein therapy', that allows for the delivery of large, biologically active proteins and enzymes in excess of 100,000 Daltons into 100% of cells. The strategy involves the generation of an N-terminal fusion protein that contains the TAT protein transduction domain (PTD). The PTD delivers the cargo, in our case a tumor suppressor protein, to all cells by a process that is independent of receptors, transporters or endocytosis and instead relies on passive diffusion. We have recently shown that proteins can be transduced into most, if not all, cells and tissues in mouse models, including crossing the blood-brain barrier. Thus, protein transduction has great potential to deliver novel anti-cancer therapies.

Recently, our laboratory has been working on specific killing of peritoneal tumors *in vivo* by reconstitution of tumor suppressor function via protein transduction. Several tumor types, notably ovarian, breast, colon, stomach, pancreatic and lymphomas, invade or metastasize to the peritoneum (abdominal cavity) with devastating consequences to the patient. There are currently no viable treatments for Stage IV peritoneal malignancies. Therefore, we have begun treating mouse models harboring peritoneal tumors with transducible tumor suppressor proteins. Early data suggest that targeting two independent pathways with transducible p53 and p27 tumor suppressor proteins results in a synergistic effect and marked reversion. Moreover, due to the absence of a size limitation, we are currently developing additional novel anti-cancer transducible proteins that may have even greater potent anti-cancer effects.

Although these studies require further investigation, they serve to demonstrate for the first time that transducible tumor suppressor proteins can modulation tumor biology *in vivo*. Indeed, our collective observations begin to open the door to the delivery of entirely new classes of pharmaceuticals in the intermediate molecule (1,000-5,000 Daltons) and large molecule (>5,000 Daltons) range to treat human disease.

180P UTILITY OF OLIGOMERS OF ARGININE TO ENHANCE TOPICAL DELIVERY AND ORAL BIOAVAILABILITY OF A VARIETY OF DRUGS

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CellGate transporters, either alone or when conjugated to therapeutic drugs, have been shown to readily enter all layers of the keratinized epithelia of the skin. When conjugated to cyclosporin A (CsA), they transport the drug effectively to T lymphocytes in the dermis, the cell type believed to be central in both dermatitis and psoriasis. A releasable conjugate between the transporter and CsA was designed and shown to release sufficient drug in the dermis to be efficacious in an animal model of contact dermatitis.

The transporters are also capable of entering effectively the nonkeratinized epithelia of the murine cheek, tongue and colon. They do not significantly enter the epithelia of the stomach or small intestine. *In vitro* experiments demonstrated that CellGate transporters rapidly and efficiently crossed the luminal membrane of monolayers of transformed colonic cells, but did not continue across the cell to exit into the basolateral chamber. However, CellGate transporters were shown to modify both the amount and rate of uptake of cyclosporin A (CsA) and taxol in the bloodstream after either direct injection into the ascending region of the rat colon or incubation in the oral cavity.

The data in these experiments were consistent with a mechanism in which conjugation of CellGate transporters results in a dramatic increase in both the water solubility of the drugs and the rate they enter squamous or columnar epithelia. The conjugates were not transported across the epithelia into the blood stream. Rather, increases in the levels of the drugs observed in the bloodstream appeared to be due to the hydrolysis of the conjugates in the epithelia, and the subsequent diffusion, or transport, of the released parent drugs across the basolateral epithelial membrane without the aid of CellGate transporters.

181P FUNCTION OF Rho GTP-BINDING PROTEINS IN ACTIN DYNAMICS DURING PHAGOCYTOSIS

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Phagocytosis is a universal cell function, which exploits ubiquitous and mostly conserved cell machinery to bind (generally in a receptor-dependent way) and internalize particulate material over 0.5 μm in diameter. Among a variety of dedicated surface proteins, the receptors for the constant region of immunoglobulins (FcRs) mediate removal of pathogens opsonised by specific anti-bodies. A trademark of phagocytosis, conserved from single-celled amoebae to most metazoan cell types, is the exquisitely localized actin polymerization that takes place right underneath the phagocytic target. Actin polymerization proceeds from signal transduction downstream of the phagocytic receptors. Even though the precise signalling cascades linking ligated phagocytic receptors to actin polymerization are not fully understood as yet, it has become clear that Rho GTPases control the cytoskeletal rearrangements during particle uptake in mammalian professional phagocytes. Rho GTPases are also necessary for complementary receptor-mediated phagocytosis as well as apoptotic cell uptake in *Caenorhabditis elegans* and in macrophages.

Actin assembly underneath Ig-opsonized particles requires Cdc42 and Rac1, two members of the Rho family GTPases that control the formation of actin-rich filopodia and membrane ruffles, respectively. During FcR-mediated phagocytosis, dominant-negative mutants of Cdc42 and Rac1 block particle entry, while they have no effect on initial adhesion of the phagocytic target to their receptors, indicating that GTPase function is needed downstream of ligated receptors. Recently, we have shown that Cdc42 and Rac1 seem to control different steps in the phagocytic process, namely pseudopod emission for Cdc42 and phagosome closure for Rac1, at least in the case of FceRI-mediated phagocytosis. The role of the third prototypical RhoGTPase, RhoA, in FcR-mediated phagocytosis is still unclear.

The recruitment of active forms of these proteins to the plasma membrane via the rapamycin system has confirmed the differential role of Cdc42 and Rac1 in phagocytosis. This experimental system uses a stably transformed phagocytic cell line expressing a plasma membrane receptor, termed CD25-FKBP2, consisting of two copies of a rapamycin-binding domain mutant form of Cdc42 or Rac (Cdc42V12 or Rac1V12) are further expressed in the original CD25-FKBP2 receptor-expressing cell line.

Upon clustering of the CD25-FKBP2 receptor with anti-CD25 antibody coated beads and rapamycin treatment, Cdc42V12-FRB or RacAV12-FRB can be induced to associate with the cytoplasmic region of CD25-FKBP2 with rapamycin acting as a bridge. Using this system, we have found that local recruitment of activated Cdc42 triggers the formation of actin-rich membrane protrusions, while in the case of activated Rac1, membrane recruit-ment leads to ingestion of anti-CD25 antibody coated beads used for receptor aggregation. Altogether, these findings confirm the ability of Cdc42 in inducing actin-rich pseudopods and the permissive role of Rac1 in particle entry.

The biological actions of the small GTPases are mediated by their GTP-dependent interactions with downstream effectors. Knowledge is starting to accumulate on the mechanism(s) by which effectors for the Rho-family GTPases mediate actin polymerization during phagocytosis. WASP/N-WASP (Wiskott-Aldrich Syndrome Protein) and Scar/WAVE (WASP family Verprolin-homologous) family proteins, key regulators of actin polymerization downstream of Cdc42 and Rac, control actin nucleation at the leading edge. During phagocytosis, we found that WASP co-localize with F-actin at forming cups. Moreover, WASP recruitment to the plasma membrane induces actin-rich membrane protrusions, similar to the pseudopods that formed next to clustered, GTP-bound Cdc42. We are currently analyzing the function of WASP family proteins in actin polymerization during phagocytosis.

182P EPITHELIAL POLARITY AND MORPHOGENESIS

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Spatial asymmetry is fundamental to the structure and function of most eukaryotic cells. A basic aspect of this polarity is that the cell's plasma membrane is divided into discrete domains. The best studied and simplest example of this occurs in epithelial cells, which line exposed body surfaces. Epithelial cells have an apical surface facing the outside world and a basolateral surface contacting adjacent cells and the underlying connective tissue. These surfaces have completely different compositions. Epithelial cells use two pathways to send proteins to the cell surface.

Newly made proteins can travel directly from the trans-Golgi network (TGN) to either the apical or basolateral surface. Alternatively, proteins can be sent to the basolateral surface and then endocytosed and transcytosed to the apical surface. We are studying the machinery that is responsible for the specificity and regulation of polarized membrane traffic in epithelial cells. I will discuss several recent results.

1. The polymeric immunoglobulin receptor (pIgR) transcytoses IgA from the basolateral to the apical surface. Trans-cytosis is stimulated by ligand binding. Binding of IgA causes dimerization of the pIgR, which leads to activation of a non-receptor tyrosine kinase, p62Yes. Mice knocked out for this kinase are deficient in IgA transport. We now find that the cytoplasmic tail of the pIgR interacts directly with rab3b, in a GTP dependent manner. Binding of IgA to the pIgR causes dissociation of rab3b. These results suggest that rab3b may be involved in the recycling of IgA-free pIgR to the basolateral surface. Binding of IgA and dissociation of rab3b may be involved in transcytosis of pIgR to the apical surface.

2. When epithelial cells, such as MDCK cells, are plated in a 3 dimensional collagen matrix, the cells form hollow, polarized cysts with the apical surface facing the lumen of the cyst. When collagen-grown cysts are stimulated with hepatocyte growth factor (HGF), the cysts develop branching tubules, providing a simple model system for studying tubulogenesis. The exocyst is an eight-subunit complex involved in targeting transport vesicles to specific regions of the plasma membrane. We have found that HGF treatment causes the exocyst to relocalize from the region of the tight junction to the growing tubule, indicating that new membrane is being directed to the tubule. Overexpression of a subunit of the exocyst, hSec10, causes the cysts to elaborate an increased number of tubules, indicating a direct connection between membrane traffic and tubulogenesis. Moreover, overexpression of hSec10 causes increased synthesis of a basolateral plasma membrane protein, as well as all major secretory proteins. This increased synthesis is mediated at the level of translation, not transcription, and apparently involves a direct physical interaction of hSec10 and Sec63b, a subunit of the translocon.

183P APPLYING TOGA™ TECHNOLOGY TO FIND BIOLOGICALLY RELEVANT GENES IN PEYER'S PATCH EPITHELIUM DEVELOPMENT

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New biotechnology approaches to gene discovery promise a more focused approach to development of new therapies; properly designed, these approaches can also suggest improvements in drug delivery. This presentation will focus on the use of TOGA technology as a method for comprehensive analysis of gene expression in biological samples.

TOGA analysis, in conjunction with the TOGA Portal software, is a powerful method for identifying nearly all genes expressed within a sample and for making accurate and reliable comparisons between different cells, tissues, or conditions. TOGA is uniquely well suited for identification of genes whose expression is limited to select tissues or specific cells.

The TOGA technology allows for the identification of expressed polyA mRNAs by amplifying and separating distinct expressed sequences on the basis of the location of a restriction enzyme site relative to the polyA addition site, and the 3' adjacent four nucleotides (comprising a "parsing" sequence). These sequence-dependent features constitute a "digital address" for any gene, and the presence of a peak at any specific address reveals the expression of a specific gene. The intensity of any peak is proportional to the level of gene expression, so the TOGA Portal software can be used to do direct comparisons of gene expression across different RNA samples from different cells or tissues. Regulated genes identified in this manner can then be further studied for their role in the biology of the experimental system.

In the Peyer's Patch epithelium, there are specialized cells, called M cells, that are efficient in sampling antigens within the intestinal lumen and transporting them across the epithelium to the underlying lymphoid follicles. The M cell development appears to depend in part on the induction by B lymphocytes. Our aim in these studies is to identify those genes specifically induced in M cells so that we may understand more about the biology of these cells and their role in the induction of immune responses in the Peyer's Patch.

In order to provide the most direct comparisons of differentiated M cells versus closely related non-M cell tissue, we generated TOGA samples from a number of different in vitro and in vivo systems. First, the co-culture of Caco-2 cells with RajiB cells can induce the Caco-2 cells to resemble M cells in some functional respects. Thus, we used samples from this model to identify the genes induced under co-culture conditions. Second, we were able to enrich for Peyer's Patch follicle associated epithelium (FAE) by microdissection of mouse tissues, so that the FAE enriched genes may be identified in TOGA analysis.

From nearly 15,000 identifiable peaks in the Caco-2 model, and over 19,000 peaks in the mouse tissue samples, we were able to identify over 150 peaks with regulated expression consistent with M cell representation within samples. Further analysis of expression within Peyer's Patch tissue by *in situ* hybridization and elimination of other candidates on the basis of gene identity will now allow us to focus on those genes most likely to be important to specific aspects of M cell function.

184P USE OF MIXTURE-BASED COMBINATORIAL LIBRARIES TO IDENTIFY LIGANDS THAT TARGET RECEPTORS IN THE GASTRO-INTESTINAL TRACT

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Various procedures exist for encapsulation of pharmaceutically active ingredients in biodegradable particulate delivery systems, which allow the active substance to pass through the stomach and survive to reach the target region of the gastrointestinal tract (GIT). In practice, bioavailability with these formulations is lower than with parenteral routes, primarily due to poor particle uptake across epithelial cell barriers. Lectins, naturally occurring proteins with affinity for sugar residues, have been investigated as promoters of bioadhesion in the GIT (Gabor *et al*, 1998; Foster *et al*, 1998). However, the use of lectins remains problematic because such large proteins are liable to degradation both in the gut and during preparation of formulations. This, together with potential immunogenicity and cytotoxicity effects, limits the use of lectins as targeting agents to deliver drugs to/across the GIT. The aim of our research is to identify small molecules, which mimic the function of lectins and, therefore, have properties applicable to targeted drug delivery.

Peptide and small molecule mixture-based positional scanning combinatorial libraries (Pinilla *et al*, 1992; Houghten *et al*, 1999) were screened in competitive assays with the fucose-binding lectin Ulex europaeus I (UEA-1). Individual compounds were synthesized from a number of libraries and their binding affinities were evaluated. Biotin-labelled lead compounds were then evaluated in whole cell assays, both in solution and adsorbed on to 0.3 mm streptavidin-coated polystyrene particles. The binding in solution to Caco-2 cells was measured by flow cytometry. The particle binding and/or uptake was assessed in Caco-2 cells *in vitro* and mouse intestinal loop models *in vivo* using confocal microscopy imaging.

From >40 peptide, peptidomimetic and organic molecule libraries screened, individual compounds exhibited IC_{50} values of 40-80 mmolar. Derivatives of these compounds subsequently exhibited IC_{50} values of 1-4 mmolar representing a sig-nificant enhancement over L-fucose, the control inhibitor (IC_{50}~700 mmolar). Biotin-labelled leads exhibited dose dependent binding to both Caco-2 cell membrane fractions and whole cells in solution. Fluorescent streptavidin-polystyrene par-ticles coated with the biotinylated lead compounds exhibited binding and uptake into M-cells comparable to UEA-1 coated control particles in a mouse intestinal loop model.

A number of leads which exhibit substantially higher activity than natural lectin substrates such as L-fucose, have been generated. The potential for use of these lectin mimetics in oral targeted drug delivery applications has been demonstrated using a model particulate system. The M-cell specific nature of the mimetics in the mouse intestinal loop model is of particular interest in the context of vaccine delivery to antigen presenting cells. Structure activity analysis of the mimetics is ongoing to identify other derivatives with enhanced activity as well as functionality determinants.

Gabor F *et al*, J Cont Release 1998:55;131-142

Foster N *et al* Vaccine 1998:16;536-541

Pinilla C *et al* BioTechniques 1992:13;901-905

Houghten RA *et al* J Med Chem 1999:42; 3743-3778

185P SCREENING PHAGE DISPLAY LIBRARIES *IN VIVO* FOR THE IDENTIFICATION OF INTESTINAL TARGETING LIGANDS

LM Higgins, GG Donnelly, I Lambkin, D O'Malley, J Dee, D Byrne, M Smith, D Brayden & D O'Mahony Elan Biotechnology Research, Dublin.

Oral delivery of vaccines giving rise to a mucosal immune response is highly desirable as many pathogens invade via mucosal surfaces. M-cells are professional antigen sampling cells that are found in the epithelium of the gut associated lymphoid tissue or Peyer's patch. The transcytotic capacity of M cells and the downstream processing of antigen sampled would suggest that targeting vaccines to M cells would enhance oral immunisation (Foster *et al,* 1998). However, to date no human M-cell marker has been identified as a target for delivery of vaccines and/or other drugs through the M-cell route.

The aim of this study was to identify novel targeting ligands for M cells of Peyer's patch using *in vivo* phage display library screening. The key advantage to this technique is that peptides are selected on the basis of inter-action with target receptors *in situ.* Phage display technology has been used previously for the identification of peptide ligands that target tumor vasculature (Arap *et al,* 1998) and vasculature of different organs (Rajotte *et al,* 1998).

A 12-mer peptide phage display library displaying up to 10" phage clones was administered into male Wistar rat intestinal loops that contained at least one Peyer's patch (n=5). Following a 2-hour incubation bound phage were amplified in *E. coli* and isolated by PEG precipitation. A total of four "bio-panning" cycles were performed. In addition, *in vitro* ELISA-based screening was performed to examine binding to intestinal PP and non-PP tissue homogenates of several species. DNA from one hundred phage clones with the highest binding affinities (OD >0.8) was sequenced to identify peptide insert. Peptides were synthesised for use in binding, uptake and immunohistochemical studies.

All clones selected demonstrated broad species specificity including human intestinal cell lines. There was no difference in binding of phage clones to PP compared with non-PP tissue. Thirty unique sequences were identified. Many of the clones were selected in more than one rat used in the screening cycles. Analysis of the peptide sequences revealed that several peptides contained common tripeptide motifs. Two peptides shared a tetrapeptide motif. Searches were performed using the Swissprot database to identify possible homologues for each peptide sequence. Several potential target receptors were identified, although needing confirmation. The 30 unique peptides were synthesised with various tags for use in binding and uptake studies aimed at designing vaccine delivery technologies. In addition stabilised versions of these peptides were synthesised. Immunohistochemistry was carried out on human intestinal tissue using synthesised peptides and binding to epithelium was demonstrated. Peptides with high binding affinities (kD <30uM) were conjugated to fluorescent-loaded particulate systems to examine drug delivery capabilities in cell and animal models.

We have identified multiple novel phage clones displaying peptides which mediate binding to intestine of several species. Some of these ligands show homology to potential target receptors/proteins of interest. Peptides were synthesised and functionality, as well as binding to human intestinal tissue, was demonstrated. The use of these ligands conjugated to drug delivery model systems is ongoing.

Arap A *et al* 1998. *Science.* 279:377-380

Foster *N tal* 1998. *Vaccine* 15:536-571.

Rajotte D *et al* 1998. *J. Clin. Invest.* 102:430-437.

186P EVALUATION OF TARGETED LIPID-PROTAMINE-DNA (LPD) COMPLEXES FOR GENE DELIVERY APPLICATIONS

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For *in vivo* systemic gene delivery, one of the main barriers is delivery of the gene payload to the required target tissue, while keeping transduction of other tissues to a minimum. One way to overcome this problem is through using targetting moieties on the surface of the gene delivery vehicle, which specifically recognise receptors on the surface of the target cells. We are currently using a lipid-protamine-DNA (LPD) system that has previously been used successfully as a vehicle for systemic gene delivery (Li *et al.* [1998] Gene Therapy, 5: 930-937).

We have incorporated pegylated lipids into this system in order to control surface properties of the LPDs and increase their circulation time, with the ultimate aim of increasing gene delivery to tumours. This inclusion of pegylated lipids decreases non-specific cell transduction *in vitro* in a dose dependent manner, a result that can be partially attributed to a decreased electro-static interaction with cell membranes. In order to restore LPD binding to cells and to specifically target LPDs to tumour cells, a number of lipid-targeting ligand conjugates have been included in the LPD formulations. We have used the following conjugates to target tumor cells: DSPE-PEG5000-LHRH (leutinising hormone release hormone) and DSPE-PEG5000-4CRGD (binds to integrins).

187P EXPERIENCES WITH GENE DELIVERY USING NON-VIRAL AND VIRAL SYSTEMS

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The field of gene therapy continues to have tremendous potential, the scope of which promises to expand with the flow of information arising from the explosion of genomics and proteomics work.

The delivery of genes to desired organs and cellular targets for therapeutic purposes continues to represent one of the greatest technical challenges for gene therapy. Currently, gene delivery is accomplished using systems which can be divided into two general types, viral and non-viral. Viral systems are based on a number of different RNA and DNA viruses each with its own inherent features. Non-viral systems are typically based on synthetic lipids or polymers. Dependent on the disease, therapeutic indication, length of desired gene expression, cellular target, and empirical data generated by preclinical experiments, a choice can be made as to which type of system is preferable.

A general overview of the characteristics of different systems will be presented. Our preclinical and clinical experience using Adeno-Associated Virus (AAV) vectors for the treatment of cystic fibrosis and rheumatoid arthritis will illustrate the use of a viral based system. The application of lipid-based systems will be illustrated by their use for the treatment of cancer through delivery of the Adenovirus E1A gene by local or intravenous administration.

Gene delivery systems have been developed which represent viable platforms by which certain diseases may currently be treated. Furthermore, new developments which can enhance the utility and efficacy of these technologies represent the future approaches that will allow the full potential of gene therapy to be attained.

188P *IN VIVO* PHAGE DISPLAY OF PEPTIDE LIBRARIES: FOR THE IDENTIFICATION OF NOVEL CHEMICAL LEADS AND THERAPEUTIC DRUG TARGETING

Elias Lazarides Targeted Molecules Corporation

Targeted Molecules (TMC) was founded in June 2000 to commercialize a patented technology platform discovered by Dr. Erkki Ruoslahti's laboratory at The Burnham Institute (TBI), La Jolla, CA. The key discovery is that blood vessels have a molecular signature that varies from organ to organ, and within the same organ from normal to disease tissue. Dr. Ruoslahti's laboratory developed a unique approach (*in vivo* panning using phage display) to identify peptides that home to particular vascular receptors with a high degree of specificity.

In vivo panning has several important advantages over traditional methods for identifying receptors and lead molecules.

- Since panning is performed in whole animals, this method eliminates the high experimental risk of translating *in vitro* results to an *in vivo* system.
- This process is rapid, requiring less than three months from initial panning to identification of an optimal homing peptide and characterization of the mouse/human receptor.
- Our system biology approach eliminates prejudice toward known receptors and enables identification of novel receptors or new binding sites on known receptors.
- Use of the phage provides unmatched screening efficiency by exposing vascular receptors to billions of peptide sequences simultaneously.

Primary applications of these homing peptides are: (1) the molecular targeting of therapeutics to specific sites of disease with both drugs (doxorubicin) and biologicals (TNFα), and (2) the identification of clinically relevant vascular receptors and drugable leads.

TMC's goal is to map the entire mouse, and by extension human, vasculature. We will compile this map ("Angiomics Library") by establishing a highly efficient industrial process for the identification of homing peptides and vascular receptors. The effort by TBI scientists has focused on angiogenic models of cancer, diseases of the retina (e.g. AMD), and arthritis. While continuing to work in these areas, we are also planning to search for peptides and receptors using models for a number of other important clinical conditions including diseases of the prostate, CNS and PNS, obesity, diabetes and ischemia.

TMC has an exclusive license to the first generation homing peptides discovered by TBI and for use of the *in vivo* panning method. The IP portfolio consists of 4 issued and 8 pending patents. The Company completed its first therapeutic targeting partnership in October 2000, with the Elan Corporation, focusing on the development of targeted particles (e.g., liposomes, nano-crystals) for the oncology market.

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Curnis F, Sacchi A, Borgna L *et al.* Nature Biotechnology, 18:1185-1190, November 2000.

189P TRANSCUTANEOUS IMMUNIZATION: TARGETING LANGERHANS CELLS WITH ADJUVANTS

Gregory M. Glenn. IOMAI Corporation; Walter Reed Army Institute of Research, USA

There has been recent intense interest in using the skin immune system for vaccine delivery. The skin is an attractive delivery target because it is densely populated with potent immune cells, Langerhans cells, which can orchestrate systemic immune responses. Langerhans cells sample antigen in the skin and migrate to the draining lymph node for antigen presentation. For an effective immune response to occur, the Langerhans cells must be activated by danger signals such as bacterial proteins, or other compounds that interact upon the cell machinery which is designed to alert the immune system upon challenge by micro-organisms. The activation results in an increased number of migrating Langerhans cells into the draining lymph node and an increased state of activation of these migrating cells. As a result, the Langerhans cells can induce potent immune responses that lead to effective immunity.

The danger signals required for activation of the immune system are compounds broadly known as adjuvants. The most basic and widely studied adjuvants are bacterial products such as LPS and exotoxins; how-ever, a large number of synthetic products have the same property. Among the bacterial exotoxins, cholera toxin and heat-labile enterotoxin from *E. coli* are well-described as potent adjuvants in many settings. There is a burgeoning literature regarding their use for immunization via the skin which complements an extensive literature on their use via mucosal routes. There are several choices among the exotoxins, including the native holotoxins, their β-subunits, mutants and chimeras. The mutant and chimera toxins have the advantage of decreased enterotoxicity, but appear to have varied concurrent attenuation of their adjuvant strength.

We have demonstrated that adjuvants can readily be delivered using simple patch systems. Recent clinical studies have confirmed extensive animal data that large proteins can be delivered into the epidermis to safely evoke a robust systemic immune response. The addition of an adjuvant such as LT to a protein such as tetanus toxoid results in high levels of anti-TTx IgG in the serum. The animal and human data have shown that adjuvants play a critical role in induction of robust immune responses when using the skin for immunization.

The ability of adjuvants to activate LCs for puposes of immunization is a fundamental vaccine delivery observation that opens up the skin for immunization. Other routes of vaccine delivery such as oral, nasal, rectal, or injection strategies have advantages and limitations. It appears that transcutaneous immunization will open the skin to vaccine delivery and will become an important consideration for both existing and new vaccines as well as for immunotherapy.

190P NONINVASIVE DELIVERY OF BIOPHARMACEUTICALS THROUGH THE SKIN BY CONTROLLED INFUSION THROUGH MICROPORS™ CREATED IN THE STRATUM CORNEUM

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A practical, reliable, and controllable noninvasive delivery of macromolecular drugs such as peptides, proteins, and vaccines, is highly desired and needed in the pharmaceutical industry. Many approaches have been researched; however, none are yet developed as products. The transdermal route has previously not been viable for molecules typically larger than 500 Daltons due to the limitations on permeation through the stratum corneum.

Altea has now developed a novel method to deliver macromolecular drugs through the skin by forming openings (MicroPors™) through the stratum corneum of 50-100 microns diameter and 50 microns deep. These MicroPors™ are formed by a painless, virtually instantaneous process involving the vaporization of a tiny area of the stratum corneum by the application of focused thermal energy. They are nontraumatic to the surrounding and underlying viable tissues, but allow access to the viable epidermis. The MicroPors™ remain open for days when kept occluded, and completely disappear in 7-10 days with normal rejuvenation of the stratum corneum. We have demonstrated delivery of biopharmaceuticals, including biologically-active insulin, in both a pulsatile or, in the case of insulin, on-demand bolus and a continuous infusion manner.

Insulin Delivery: MicroPors™ were formed using the Altea microporation system by the rapid (milliseconds) application of pulses of heat using microfilaments placed in contact with the subject's skin. An array of pores was made on the forearm of human subjects and a solution of lispro insulin was applied over this MicroPor™ matrix. Basal infusion was demonstrated from a 5 cm^2 patch pulsed with current (1 mA ave), achieving 10 μU/ml serum insulin after 75 min as quantitated using a lispro specific assay. On-demand delivery of biologically-active insulin was demonstrated by a decrease in blood glucose over a 60 min period that was comparable in kinetics and amount to that obtained with SC injection. These pharmacodynamic effects were quantitated either by fingerstick blood glucose or euglycemic clamp studies. No apparent insulin depot occurred at the skin administration site, as shown by a rapid decrease in serum insulin levels after cessation of the active delivery phase.

DNA Delivery: Plasmid DNA encoding green fluorescent protein (GFP) was applied over a MicroPor™ matrix formed on the skin of hairless guinea pigs. Expressed GFP was monitored *in vivo* at the skin application site with a fluorescent imaging system. The expression of GFP from the applied plasmid DNA was detectable after 19 hours at the local application site, and was still strong after 72 hours.

MicroPor™ delivery enabled the systemic delivery of biologically-active insulin in human subjects both in on-demand and basal dosing regimens. This could enable the development of a noninvasive insulin delivery system with dosing flexibility. In other studies, non-invasive DNA delivery and subsequent protein expression was demonstrated using a plasmid encoding for GFP. This technique should prove useful in optimising DNA vaccines or DNA delivery for gene therapy.

191P VACCINE DELIVERY ACROSS THE SKIN SURFACE USING NEEDLE-FREE POWDERJECT TECHNOLOGY

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Most current vaccines consist of liquid formulations of antigens, administered via intramuscular needle injection. PowderJect has developed novel technologies for vaccine delivery that offer potential significant benefits over these products. PowderJect has developed two distinct classes of novel hepatitis B vaccines, based on powder-form conventional proteins and DNA plasmids.

The outer layer of the skin, the epidermis, is rich in antigen-presenting cells, making it an appropriate target for vaccine delivery. PowderJect's powder injection technology administers vaccines to this immuno-competent tissue painlessly via a needle-free device, thereby eliminating the hazard associated with contaminated sharps. The device utilises a jet of helium gas to accelerate powder-form vaccines to high speed. This provides the vaccine particles with sufficient momentum to penetrate the stratum corneum, and to enter the epidermis. Research in laboratory models and humans has demonstrated powder injection's ability to achieve significant immune responses, suggesting that delivery to the antigen presenting cells enhances vaccine immunogenicity.

Hepatitis B DNA vaccines are coated onto gold microspheres and administered via powder injection. The gold provides the requisite particle density to penetrate the stratum corneum, and are sufficiently small to allow delivery directly into the epidermal antigen presenting cells. This approach has proved highly effective at inducing both antibody- and cell-mediated immune responses in human clinical trials. Clinical data show that the vaccine was well tolerated and elicited protective levels of antibody in all vaccinees: the world's first achievement of protective immunity with a DNA vaccine. The vaccine also induced CTL responses in all evaluable subjects and cell mediated Th1 immune responses in the majority of recipients. This ability to elicit the type of cellular responses that are believed to be relevant to immunotherapy is currently under clinical investigation. Additional clinical research in subjects who failed to respond to commercial vaccines will be presented.

Powder injection is also highly appropriate for administering conventional protein antigens. Antigen containing powders were formulated by drying the antigen in sugar excipients, grinding the material, and sieving to obtain a uniform sized particle fraction. Epidermal powder immunization (EPI) with particles in the size range of 20-53 um diameter delivers antigens to the extracellular space in the skin. EPI with influenza virus antigens elicited a higher antibody response and protected mice from challenge much more efficiently than subcutaneous needle injection. These immune responses can be enhanced with adjuvants without the toxicity usually associated with adjuvant use. Furthermore, the inclusion of very low doses of a bacterial enterotoxin in the formulation is able to stimulate a mucosal immune response in addition to a serum antibody response. We have also explored the delivery of protein antigens on gold particles. EPI with protein antigens coated onto gold particles in the 1-2.5 um diameter size range delivers the vaccine to the intracellular space of skin cells. This delivery modality induces both antibody and cell mediated immune responses similar to those observed with DNA vaccines coated onto gold particles.

192P DEVELOPMENT OF A FLOW-CYTOMETRY PROCEDURE FOR CHARACTERIZATION OF NOVEL LECTIN MIMETICS *IN VITRO*

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Over the course of the last ten years there has been much interest in the field of bioadhesion as a strategy to improve the bioavailability of pharmaceutically active substances administered orally. The binding characteristics of lectins, plant proteins with affinity for sugar residues, have been studied in intestinal cells as potential promoters of bioadhesion in the gastrointestinal tract (Gabor *et al*, 1998; Foster *et al*, 1998). However, lectins are prone to degradation both in the gastrointestinal tract and during drug formulation. There are also potential immunogenicity and cytotoxicity effects with these proteins that limit their usefulness as drug delivery agents. We have developed a small molecule, lectin mimetic compound. This mimetic has been shown to inhibit binding of *Ulex europaeus* I (UEA-1), a fucose-binding lectin, to membrane prep-arations of the human epithelial cell line Caco-2. The biotinylated form of the mimetic has also been shown to bind directly to these membrane preparations, and UEA-1 competes for its binding. The aim of this research is to develop a whole cell binding assay that can be used to study and compare the binding affinities of these mimetic compounds.

Caco-2 cells were analysed by flow cytometry for binding of both biotinylated UEA-1 and a biotinylated lectin mimetic using a streptavidin FITC probe. While clear positives were shown with biotinylated UEA-1 at concentrations as low as 1.0 μg/ml, concentrations of the mimetic of up to 65 mM were negative with this assay.

The sandwich protocol increased the fluorescent signal as evidenced by the ability to measure a biotinylated UEA-1 sample of 0.02 μg/ml. Previously, the lower limit had been 1.0 μg/ml with the streptavidin-FITC probe. The nucleic acid stain 7-actinomycin D (7-AAD), a fluorescent dye for the identification of dead cells, was used to exclude these cells from analysis as they were known to bind non-specifically to FITC-avidin D. Binding of the biotinylated lectin mimetic was demonstrated at both 50 μM and 10 μM concentrations.

A whole cell binding assay has been developed for the characterization of binding affinities of small molecule, lectin mimetics. This will allow structure activity analysis of these mimetics to identify those functional groups that enhance activity using whole cells in solution.

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Foster, N *et al*, Vaccine 1998:16(5);536-541

193P THE INTEGRATION OF POSITIONAL SCANNING LIBRARIES WITH BIOINOFORMATICS AND PROTEOMICS

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Positional scanning synthetic combinatorial libraries have been successfully used for the identification of high affinity ligands for antibodies, T-cells, G-coupled receptors and enzymes. The active peptides have resulted from the combination of the amino acids in the most active mixtures of the library, and may not necessarily correspond to sequences in proteins.

A biometrical analysis was developed in an effort to identify peptides within protein sequences from the data derived from the screening of the libraries. This new algorithm allows the systematic comparison of millions to trillions of peptides in the libraries with the millions of peptides in protein databases. The integration of positional scanning libraries with this biometrical analysis has been used to identify ligands from protein databases for many interactions examined with positional scanning peptide libraries.

Examples of its use for the identification of T-cell and G-coupled receptor ligands will be presented.

194P PHASE I STUDY OF s.c. AND i.v. ISIS 104838 (ANTI-TNF-ALPHA), A SECOND GENERATION ANTISENSE OLIGONUCLEOTIDE.

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ISIS 104838, a chemically modified phosphorothioate 20-mer antisense oligonucleotide (ASO), utilizes 2'-O-(2-methoxyethyl) modified nucleotides at the 3' and 5' termini, with a 10-base 2'deoxy gap (Baker 1999). The 3' and 5' MOE modified nucleotides resist exonucleases, prolonging T1/2g. MOE 'gapmer' ASOs are metabolized by cellular endonucleases, which cleave in the deoxy gap region into 2 fairly equal sized oligonucleotides. ISIS 104838 hybridizes to a TNFa exon 2 sequence. ASOs act by binding their target mRNA through highly specific Watson-Crick hybridization, offering a very high level of target specificity. TNFa mRNA duplexed with ISIS 104838 is cleaved by the ubiquitous nuclease RNase H (Crooke 1999). TNFa mRNA expression was inhibited by ISIS 104838 (IC_{50} < 1 uM) in LPS-stimulated THP-1 monocytes. Re-sponse was sequence-specific vs. control (scrambled; mismatched) ASOs.

IV administration of ISIS 104838 was studied in a double-blind, placebo-controlled study (CS1) in healthy males aged 18 and 45 years. Doses ranged from 0.1 to 6.0 mg/kg, infused over 60 minutes on Days 1, 8, 10, and 12. A second trial (CS2) assessed the safety and local tolerability of single 1 cc s.c. abdominal injections of placebo or ISIS 104838, at doses from 25 to 200 mg/mL, followed by 3 qod injections in new subjects. The multiple doses ranged from 0.1 to 6 mg/kg, using a maximum 1 cc volume and 200 mg/mL concentration.

Results: ISIS 104838 i.v. was well tolerated; 2 subjects (1 each at 4 and 6 mg/kg) developed skin rashes (pompholyx; palmar erythema/ flaking) after 2 infusions. Clinically silent post-infusion aPTTs increased transiently a maximum 33 seconds after 6 mg/kg i.v., due to a known indirect coagula-tion pathway effect (Sheehan 2001). Stimu-lated TNFa production by blood was suppressed ($p<0.02$). PK values are in Table1; the apparent terminal plasma elimination T1/2g was @14 days.

PK Table 1	N	Cmax	T1/2	AUC	Clearance
0.5 mg/kg	3	5 ug/mL	0.6 hr	8.4	62 mL/min
2 mg/kg	3	26 ug/mL	1 hr	50.4	39 mL/min
6 mg/kg	3	53 ug/mL	1.4 hr	121	49 mL/min

ISIS 104838 s.c. was well tolerated; a 6.0 mg/kg s.c. subject was withdrawn for reversible thrombocytopenia (191K falling to 113K). Injection site reactions were mild, with tenderness, redness or swelling around 12-24 hours. aPTTs increased 8 seconds after 6 mg/kg injections. PK values are in Table 2.

PK Table 2	N	Cmax	T1/2
50 mg/mL	3	0.9 ug/mL	3 hr
100 mg/mL	3	2 ug/mL	2.7 hr
200 mg/mL	3	5.5 ug/mL	4.7 hr

Conclusion: ISIS 104838 can be dosed at 1-4 week intervals either s.c. or i.v. ISIS 104838 has improved tolerability vs. unmodified phosphorothioate ASOs (Glover 1997).

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195P DRUG ADDICTION, REWARD DYSREGULATION AND ALLOSTASIS

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Neuropharmacologic studies with animal models of drug addiction have provided evidence for specific neurochemical mechanisms in subregions of the basal forebrain such as the nucleus accumbens and amygdala in drug reward and stress that become disregulated during the development of drug addiction and alcoholism. There are multiple neurotransmitter systems that converge on the region of the nucleus accumbens and amygdala that are involved in the acute positive reinforcing effect of drugs.

Current studies are aimed at exploring how these neurochemical mechanisms change with the development of addiction. Animal models have been developed for various stages of the addiction cycle including the motivational effects of drug withdrawal, craving, and protracted abstinence. A conceptual construct focused on allostatic changes in reward function that lead to vulnerability to relapse provides a heuristic framework by which to identify the neurobiologic mechanisms involved. Evidence exists for alterations in the same systems implicated in the acute reinforcing effects such as the dopaminergic, opioidergic, serotonergic, and GABAergic systems as well as the recruitment of neuropeptides such as corticotropin-releasing factor that are impli-cated in behavioral responses to stressors. These changes in the reward system to maintain hedonic stability are hypothesized to convey the vulnerability for development of alcoholism and relapse in recovering addicts (allostatic state).

Knowledge of the neuroadaptations in the reward system provoked by chronic drug administration may provide the key to understanding the mechanisms of addiction and vulnerability to relapse as well as provide insight into potential treatment.

196P INVOLVEMENT OF DIFFERENT OPIOID RECEPTORS AND PEPTIDES IN CANNABINOID DEPENDENCE

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Cross-interactions between opioid and cannabinoid systems in dependence and reward-related processes have been reported in many studies. Delta9-tetra-hydrocannabinol (THC), the active ingredient in marijuhana, can produce in rodents dependence and several motivational responses, including rewarding effects and dysphoria. We have recently established a model of tolerance and dependence to THC in mice as well as a model of place conditioning to evaluate both rewarding and aversive properties of THC in mice. These two models represent useful tools for the study of the neurobiological basis of THC actions.

We have used these behavioral models in three strains of knockout mice lacking either mu, delta or kappa opioid receptors, and we have observed interesting cross-interactions between the two systems that may help to understand the mechanisms of action of cannabinoids. The functional interactions between the endogenous cannabinoid and opioid systems were also evaluated in pre-proenkephalin and prodynorphin deficient mice.

Antinociception induced in the tail-immersion test by acute THC administration was reduced in both lines of opioid peptide precursor mutant mice, whereas no differences between genotypes were observed in the effects induced on body temperature and locomotion. Cannabinoid withdrawal syndrome was significantly attenuated in preproenkephalin mutant mice.

These results indicate that the endogenous enkephalinergic and dynorphinergic systems are involved in the antinociceptive responses of THC. Endogenous enkephalins also participate in the expression of the cannabinoid abstinence.

The present study provides new data to clarify the involvement of the different components of the endogenous opioid system in the adaptive responses occuring during chronic THC exposure leading to the development of tolerance and dependence.

197P ASSOCIATIVE MECHANISMS IN ADDICTION: PROSPECTS FOR TREATMENT

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Environmental stimuli paired with self-administered drugs acquire motivational significance, induce drug craving and relapse to drug-taking habits in humans, and also control drug-seeking behaviour in humans and animals.

We have investigated in rats the neural basis of the associative processes by which environmental stimuli gain these motivational properties, and have shown the differential involvement of sub-regions of the amygdala and associated limbic cortical-ventral striatal structures, including the anterior cingulated cortex and nucleus accumbens core. These data indicate that the nucleus accumbens, with its cortical, glutamatergic and mesolimbic dopamine afferents, may form a locus where associative information about drug cues gains access to the mechanisms controlling drug-seeking behaviour. Using a second-order schedule of cocaine reinforcement to measure drug-seeking, we have shown the importance of the basolateral amygdala and the nucleus accumbens core, since selective lesions of these structures effectively prevent the acquisition of cocaine-seeking behaviour.

We hypothesize that drugs which interfere with glutamatergic and dopaminergic mechanisms in the nucleus accumbens or limbic cortical structures might thereby have therapeutic utility in diminishing cue-controlled drug-seeking and relapse. In testing this hypothesis, we have shown that a partial agonist at the D3 dopamine receptor decreases cocaine-seeking behaviour selectively. The neural sites mediating this effect are unknown, but may include the amygdala, nucleus accumbens and mesolimbic dopamine neurons, which are all enriched in dopamine receptors. In addition, an AMPA receptor antagonist, but not an NMDA receptor antagonist, infused directly into the nucleus accumbens core, but not shell, also decrease cocaine-seeking.

These results indicate that targeting such conditioned influences on drug-seeking may provide a novel approach to the treatment of addiction.

198P GENETICS AND AGE-RELATED CONTRIBUTIONS TO LONG-TERM CHANGES IN CNS STRUCTURE AND FUNCTION FOLLOWING BINGE DRINKING

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Binge drinking and parental history of alcoholism are two factors related to chronicity of alcohol dependence (Hasin et al,2001). CNS alterations associated with bingeing, were studied in an animal model of using Sprague-Dawley rats receiving multiple doses of ethanol for four days (Knapp & Crews, 1998). Multiple genes were induced by ethanol treatment including fos, an immediate early gene, and cyclooxygense 2, a prostaglandin synthetase that increases cellular oxidative stress.

Gene induction was most prominent in corticolimbic areas. Agyrophilic silver stain also indicated that neurodegeneration in corticolimbic areas occurred after 2 days of treatment and significantly increased after 4 days when neuronal loss was prominent in the granule cells of the dentate gyrus in the ventral hippocampus, in the perirhinal and entorhinal cortices and the olfactory bulb. TUNEL staining of these areas revealed few apoptotic cells, while H+E staining and electron microscopy revealed large numbers of necrotic cells. Areas of neurodegeneration also revealed increased phospho-MAPkinase levels.

Genetics and adolescent drinking are biological risk factors that contribute to the development of alcohol dependence. To determine if binge drinking induced CNS changes were related to age, gene expression and neurodegeneration were compared in adolescent and adult rats. Adolescent rats were found to have significantly more damage in frontal areas of brain. The anterior portions of the piriform and perirhinal cortices were only damaged in JVN rats. For example, in the anterior piriform cortex damage in binge treated adolescent and adult rats was 586 ± 162 vs 145 ± 39 ($mm^2 \times 10^3$ argyrophilic area; $p<0.05$), respectively.

To determine if binge drinking induced CNS changes were related to genetics, gene expression and neurodegeneration were compared in P and NP rats. The P-preferring rats strain has been bred to prefer alcohol and is a genetic model of alcohol dependence that most often is paired with genetic controls the non-preferring NP rat strain. P rats were found to have significantly greater brain damage in the posterior perirhinal and posterior entorhinal cortices, being 239%± 50% ($p< 0.02$) and 219% ± 46% ($p< 0.01$) of 'NP respectively. Phospho-MAPkinase levels were also found to differ between strains.

Thus, both genetic and adolescent risk factors for development of alcohol dependence result in differential gene induction and neurodegeneration following binge drinking. These differences in gene expression and neurodegeneration in response to binge drinking could be related to the progression to alcohol dependence. .

(Supported by NIAAA).

199P THE ROLE OF DOPAMINE D_3 RECEPTORS IN DRUG ABUSE

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The mesocorticolimbic dopaminergic system has been implicated in motivation, reinforcement and mediating some aspects of drug abuse. Dopamine D_3 receptor mRNA is found in the nerve terminal areas of the mesocorticolimbic system within the ventral striatum, nucleus accumbens, dentate gyrus and cortex of rat and human brain. Autoradiographic studies, with a variety of ligands, confirm this distribution. Since its discovery (Sokoloff *et al.,* 1990), the dopamine D_3 receptor has been implicated in addiction processes (Caine & Koob, 1993) but confirmation of this hypothesis has been hampered by a lack of selective pharmacological tools.

The recently identified D_3 receptor antagonist, SB-277011-A, has high affinity for cloned human dopamine D receptors (pKi=7.95) with 80 fold selectivity over hD_2 receptors (Reavill *et al.*, 2000) and studies have been conducted to explore the effects of this compound on dopaminergic functions. Repeated administration (uid/21 consecutive days) of SB-277011-A (1, 3 and 10 mg/kg p.o.) was studied for its effects on the activity of dopaminergic neurons using extracellular single unit recording. SB-277011-A significantly decreased the number of spontaneously active DA neurons in the ventral tegmental area, but not the substantia nigra, confirming a selective pharmacological action of the compound on the mesocorticolimbic system. In studies of brain stimulation reward (BSR) SB-277011-A has been found to attenuate the enhancing effect of cocaine on BSR thresholds, but by itself produced no elevations of response thresholds. In studies of cocaine induced conditioned place preference (CPP) acute treatment with SB-277011-A produced dose-dependent attenuation of both acquisition and expression of cocaine-induced CPP, without producing significant place preference or aversion. Sub-chronic treatment with SB-277011-A also attenuated expression of cocaine-induced CPP.

These effects appear specific to drug-seeking behaviour as SB-277011-A did not attenuate food-induced CPP. Finally, in rats trained to intravenously self-administer cocaine, acute treatment with SB-277011-A produced a dose-dependent attenuation of cocaine-triggered re-instatement of extinguished self-administration behaviour. These data support the hypotheses that dopamine D_3 receptors play a role in regulating the functions of mesocorticolimbic dopaminergic neurons and that they play a role in mediating at least some of the behavioural effects of cocaine which are thought to be predictive of its abuse liability.

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