

**BIOCHEMISTRY, MICROBIOLOGY AND IMMUNOLOGY
POSTER PRESENTATIONS
APRIL 11, 2002**



Université d'Ottawa
University of Ottawa

**BIOCHEMISTRY, MICROBIOLOGY AND IMMUNOLOGY
451 SMYTH RD., OTTAWA, ONTARIO, CANADA K1N 6N5
(613) 562-5424 or 562-5800 x 8164**

POSTER EVALUATION SCHEDULE

8:00 - 9:00 Set up time

9:00 - 10:15 Group I mans poster, Group II evaluates (you have 15 minutes for each evaluation)

10:15 - 10:30 Coffee Break and “Surprises “

10:15 - 11:15 Group II mans poster, Group I evaluates (you have 15 minutes for each evaluation)

11:15 - 11:45 Free discussion (go back and set up collaborations, get details, beg reagents, visit others, etc...)

12:30 Lunch in 3rd floor lounge - More “surprises”

Boards can stay up

Vas Mezl and Kathryn Wright
Department of Biochemistry, Microbiology and Immunology

**1. McKenna, Neil
GLYCOPROTEIN INTERACTIONS IN HUMAN
PARAINFLUENZA VIRUS TYPE 3**

By : McKenna, Neil, Ebata, Sharon, Murphy, Lise, Zolkiewska, Bogna and Dimock, Ken

The paramyxovirus human parainfluenza virus type 3 (HPIV3) is an important upper and lower respiratory tract pathogen in infants and children. HPIV3 virions contain two surface glycoproteins, the hemagglutinin-neuraminidase (HN) and fusion (F) proteins, which are responsible for virus entry into host cells. HN is involved in receptor binding and F mediates virus-cell membrane fusion. Although the mechanism of membrane fusion is unclear, it is believed that following receptor binding, conformational changes occur in both proteins that result in membrane fusion, which may include formation of a complex between heptad repeat (HR) structural elements of F. Previous studies on HN and F of paramyxoviruses have used immunoprecipitation and cross-linking in efforts to study HN/F interactions. We have developed an assay based on hexa-histidine tagging and nickel column chromatography to isolate native complexes of HN and F. The assay was used to screen F proteins with mutations in their membrane-proximal HR2 domain for their ability to form HN-F complexes. HN-F complexes include cleaved, active F proteins, uncleaved, inactive F proteins, and fusion-negative F protein HR2 mutants. GST-fusion peptides based on HR1 and HR2 sequences were constructed and screened for HR1-HR2 complex formation. Peptides based on fusion-competent F proteins were able to form HR1-HR2 complex; fusion-deficient F proteins were unable to form HR1-HR2 complexes.

**3. DAVIES, Christine
DETERMINING THE RATE OF INCIDENCE OF
MYOTONIC DYSTROPHY TYPE 2 (DM2),**

By : Davies, Christine, Carson, Nancy, Korneluk, G. Robert

Myotonic dystrophy (DM) is an autosomal dominant neuromuscular disorder with varying clinical symptoms including muscle weakness and wasting, myotonia, and cataracts. It is caused by either a CTG repeat expansion in the DM protein kinase (DMPK) gene (DM1), or a CCTG repeat expansion in the zinc finger 9 (Znf9) gene (DM2). It has also been suggested that a third disease locus exists. The prevalence of DM1 is approximately 1 in 8500. The incidence rate of DM2 is not known, but it has been suggested that it may be as high as that for DM1 (Meola, 2000; Liquori *et al.*, 2001). In an attempt to determine the incidence rate of DM2 in our sample population, derived primarily from eastern Ontario and western Quebec, we have begun screening a panel of 57 research samples from clinically diagnosed DM individuals who lack the CTG repeat expansion consistent with DM1. To determine if the DM2 expansion is present, we have begun screening these samples by capillary electrophoresis genotyping. Preliminary results show that 40/57 individuals do not have the DM2 repeat expansion by genotype analysis. These results suggest that DM2 may not be prominent in our sample population.

5. HARDER, J. Chris

**2. REGO, Dorothy
ALTERED IL-6 PRODUCTION IN SHP-1 DEFICIENT
BONE MARROW MACROPHAGES.**

By : Rego, Dorothy and Kozlowski, Maya

Cytokines are important mediators of immune responses. SHP-1 deficient *me/me* mice develop severe immune dysfunction and inflammation caused by the vast expansion of myeloid cell lineages resulting in alopecia, splenomegaly, pneumonitis and premature death. SHP-1 is known to negatively regulate signaling pathways activated by cytokine receptors. The morphological/cellular changes in *me/me* macrophages (m_s) suggest a deregulation of signaling cascades leading to the production of cytokines. To understand the mechanism(s) of inflammatory responses in *me/me* mice, we compared the expression/production of cytokines in *me/me* and normal bone marrow m_s (BMMs). RT-PCR analyses of mRNA for IL-1 β , IL-6, TNF α and IL-10 in GM-CSF and LPS stimulated *me/me* and normal m_s revealed decreased expression of IL-6 in *me/me*. This observation was further supported by ELISA. LPS mediates its action by the induction of cytokines such as IL-1 β and TNF α , known inducers of IL-6. Stimulation of normal and *me/me* BMMs by IL-1 β or TNF α resulted in an efficient production of IL-6 in normal but not in *me/me* m_s. The suppression of IL-6 production could not be rescued by either GM-CSF, LPS, IL-1 β or TNF α stimulation, suggesting a common element and a SHP-1 substrate activated by these distinct signaling pathways. As a first step to identify this putative protein critical for IL-6 expression, we examined the activation of MAPKs. Western analyses revealed hyper-activation of MAPKs in LPS stimulated *me/me* m_s.

**4. ALY, Mahmoud
GENETIC ANALYSIS TO HOST SUSCEPTIBILITY OF
CARDIOVIRULENT COXSACKIE B3 INFECTION**

By : Aly, Mahmoud M.A., Ladjamil, Zakia N., DE Bold, Mercedes K., Vidal, Silvia

Coxsackie virus B3 (CVB3) is responsible of 50% of the viral myocarditis on North America 1. Differences severity of to the disease caused by a given strain of CVB3 are thought to be largely related to host genetic factors 2. Little is known about the key genes that encode the determinants of myocarditis 3. To identify these genes and further understand the molecular mechanisms of the disease progression we are using inbred strains of mice as an experimental model. Inbred strains of mice recapitulate the heterotypic response observed in human. In this study we have characterized A/J and C57BL/6 mice for several CVB3 induced phenotypes as a pre-requisite to the genetic dissection of CVB3 induced pathogenesis. Viral load in target organs and heart histopathology statistical analysis indicated that these phenotypes would be useful for genetic mapping furthermore understanding of the differences in host response mechanism which can lead to early diagnosis and newly treatment for viral myocarditis.

INTRACELLULAR TRAFFICKING OF SR-BI

By : Harder, J. Chris & McPherson, Ruth

Selective uptake of HDL cholesterol involves uptake of free cholesterol and cholesteryl ester without concomitant degradation of the HDL particle, thus being distinct from the classic receptor-mediated pathway of lipoprotein particle endocytosis. Scavenger receptor (SR)-B1 is the only well established receptor for selective uptake. However, the cellular mechanisms for SR-B1 uptake of CE remain in dispute. Recent data suggest that the process of selective uptake may involve a recycling endosome. Using surface biotinylated SR-B1, we have demonstrated that SR-B1 undergoes retroendocytosis with a recycling time of 10 min. This short recycling time suggests that it is unlikely that SR-B1 follows the recycling itinerary characteristic of transferrin (TF) and its receptor. To further demonstrate that SR-B1 undergoes retroendocytosis, we have generated a construct with cyan fluorescent protein (CFP) attached to the N-terminus of SR-B1. Our preliminary experiments show that, in addition to the cell surface, a relatively large amount of SR-B1 is localized in the endosomal system. In the further characterization of this compartment, we demonstrate that SR-B1-CFP does not co-localize with Cy3-labeled transferrin in the early, sorting and recycling endosomes. In addition, CFP-SR-B1 also does not co-localize with Rab 7 and lysotrackerTM, marking late endosomal or lysosome directed compartments. In ongoing studies we are using live cell microscopy techniques to study SR-B1 trafficking in reference to the endosomal markers Rab 4, 5 and 11. These studies will also be carried out in a polarized hepatocyte line (WIF-B).

7. MURPHY, Lise

GROWTH AND CYTOPATHOGENICITY OF FUSION PROTEIN EXPRESSION MUTANTS OF HUMAN PARAINFLUENZA TYPE 3.

By : Murphy, Lise.L and Dimock, Ken

Human Parainfluenza Virus type 3 (HPIV3) is a member of the family Paramyxoviridae, a group of enveloped, non-segmented, negative-strand RNA viruses. Typically HPIV3 causes acute respiratory infection which can lead to bronchiolitis, and pneumonia, especially in young children. In order for HPIV3 to infect and replicate inside the target cell, membrane fusion with the cell must occur. The HPIV3 fusion gene encodes the fusion protein, F which carries out membrane fusion. Recent evidence suggests that the quantity of functional F protein in the target cell, may be critical to virus growth and cytopathogenicity. We hypothesize that the amount of functional F protein expressed in infected cells is regulated at the level of transcription, translation and F protein processing. Transcriptional regulation at the Matrix-Fusion gene junction will be examined using a reverse genetics approach. Translational regulation will be examined by comparing the ability of the 5' non-translated regions of each of the HPIV3 genes to direct the synthesis of a reporter gene product *in vitro* and following the transfection of cells in culture. The effect of enhanced post-translational cleavage of the F protein will be examined by fusion assay and virus rescue.

6. MOJIBIAN, Majid

HUMAN IMMUNE RESPONSES TO *LEISHMANIA* RECOMBINANT ANTIGENS

By : Mojibian, Majid and Webb, John

We are characterizing the human cellular immune responses to a panel of seven recombinant *Leishmania* antigens that were previously cloned on the basis of their immunoreactivity during *Leishmania* infection. Five donors were chosen from an area of Iran endemic for cutaneous leishmaniasis (CL) due to infection by *L. major*. In addition, we have characterized one donor who received a live parasite vaccine. PBMC from all donors exhibited strong responsiveness to parasite lysate as measured by *in vitro* proliferation and IFN- γ production. PBMC response to individual recombinant antigens was low and variable, however significant responses to α -tubulin (1 of 6 donors) and histone H2b (2 of 6 donors) were detected. *Leishmania*-specific T cell lines were generated from bulk PBMC of all donors. These cell lines exhibited much higher responsiveness to parasite lysate and individual recombinant parasite antigens than did PBMC. Of particular interest, T cell lines responded to α -tubulin (1 of 6 donors), histone H2b (5 of 6 donors), LmSTI1 (5 of 6 donors), β -tubulin (5 of 6 donors) and TSA (6 of 6 donors). Our results indicate that donors with self-resolved CL have T cells responsive to several of the recombinant antigens being considered as recombinant vaccine candidates. Furthermore our data indicate that the use of short-term T cell lines generated against total parasite lysate dramatically increases the ability to detect memory T cell responses specific for individual antigens.

8. GARRATT, Michelle

ANTI- and PRO-APOPTOTIC SIGNALING BY EGFRvIII

By : Garratt, Michelle M. and Lorimer, Ian

The epidermal growth factor receptor is activated in cancer by multiple mechanisms including mutation. The most common mutant is EGFRvIII which is ligand independent, constitutively active and present in approximately 50% of glioblastomas and cancers of the breast, lung, ovary, and prostate. We are currently studying EGFRvIII signaling in glioblastoma cell lines to better understand the pro- and anti-apoptotic pathways induced by EGFRvIII and the involvement of atypical PKCs in downstream signaling. Using a human glioblastoma cell line, U87MG, with or without the expression of EGFRvIII preliminary results have shown that EGFRvIII induces activation of the anti-apoptotic PI3K and ERK pathways and the downregulation of the pro-apoptotic p38 pathway. Adenoviral transduction of these cell lines with a kinase deficient aPKC causes a decrease in the levels of activated Akt showing that aPKC acts upstream of Akt. EGFRvIII is also known to confer chemotherapeutic resistance to common treatments like cisplatin. We have assessed the effects of Roscovitine, a novel, potent and specific cdk2 inhibitor, on U87MG cell lines overexpressing EGFRvIII. Results from MTS assays and Hoescht staining have revealed that U87MG Δ EGFR and U87MG show similar sensitivities to apoptosis and EGFRvIII is not conferring resistance to treatment with this drug.

9. LUM, Julian

VPR MUTATION R77Q IS ASSOCIATED WITH LONG TERM NON PROGRESSIVE HIV INFECTION AND AN IMPAIRED ABILITY TO INDUCE T CELL DEPLETION *IN VIVO*

By : Lum, Julian J., Cohen, Oren J., Yao, Xiaojian, Pilon, André A., Hawley-Foss, Nanci, Kim, John E., Chen, Zhaoxia, Montpetit, Michael, Sanchez-Dardon, Jaime, Garber, Gary, Cohen, Eric E. and Badley, Andrew D.

Background: 25-30% of long-term nonprogressors have mutations in CCR5/CCR2, yet reasons for 75% of LTNP remain undefined. We have evaluated vpr sequences in LTNPs and assessed its effects on apoptosis.

Results: 8/10 LTNP had an R77Q mutation in Vpr consistent with a search of the Los Alamos database (LTNP 109/146). *In vitro*, MT Vpr induces less apoptosis and less τ -Tm in Jurkat cells than WT Vpr. These effects were dose dependent and were inhibited by the caspase inhibitor Z-VAD. Immunoblot analyses revealed a reduction in caspase 8, 9, 3 activation and release of cytochrome c with MT Vpr compared to WT Vpr. Mice injected with WT Vpr had severe T cell depletion and tail vein vasculitis resulting in tail discoloration and petechia which was significantly attenuated by MT vpr.

Conclusions: 8/10 patients in our LTNP cohort have an R77Q Vpr, a mutation frequency that is similar to other cohorts of LTNP. *In vitro*, R77Q induced lower levels of apoptosis and caspase activation than with WT Vpr. Mice injected with R77Q vpr had significantly less T cell depletion and local tissue damage compared to mice injected with WT vpr. Therefore, R77Q in Vpr may represent an additional mechanism of LTNP.

11. HARDER M, Zdena

THE ROLE OF DYNAMIN-RELATED PROTEIN 1 AND INTERACTING PARTNERS IN MITOCHONDRIAL FISSION

By : Harder M, Zdena and Dr. Heidi McBride

Mitochondria are dynamic organelles constantly moving, transiently contacting each other, fusing with one another and dividing. The mechanisms underlying these dynamics are complex and not well understood. Mitochondrial fission, in particular, has only very recently been observed at steady state conditions and whose exact mechanism and function(s) in the cell are unknown. Dynamin related-protein 1 (DRP-1) has been implicated to be essential for fission. However, it does not perform this function alone. To better understand this phenomenon, I have used the yeast two-hybrid system to uncover DRP-1 interacting partners and have identified an interaction between DRP-1 and proteins of the SUMOylation pathway, among others. I am now characterizing these interactions and trying to determine their significance in the overall mechanism of mitochondrial fission.

13. WANG, Jing

10. WALLACE, Timothy

TH2-PROMOTING PROBIOTICS ON DIABETES INCIDENCE IN THE BB RAT

By : Wallace, Timothy D., & Scott, Fraser W.

Insulin-Dependent Diabetes Mellitus is an autoimmune disease characterized by insulin-producing beta cell destruction in the pancreas by autoreactive T cells. This autoimmune response arises because of poorly understood interactions between genetic and environmental factors, including pathogenic agents and diet. Beta cell destruction is characterized by a TH1 bias, resulting in inflammation and infiltration of the autoreactive Th cells into the pancreatic islets of Langerhans. Although it is unknown why such a response arises, a strong link exists between diet and diabetes outcome, suggesting that this response may be gut related. To test this hypothesis, BB rats were subjected to daily gavaging with 2×10^9 CFU of *Lactococcus lactis* pTRmIL-10 suspended in freeze medium (1:1 ratio of M17 broth/ glycerol). pTRmIL-10 is genetically engineered to secrete the TH2 derived cytokine Interleukin-10. Feeding was initiated at 26 days and terminated at 130 days or upon positive testing for diabetes, after which animals were sacrificed.

Preliminary results suggest that pTRmIL-10, pTR1NX (control plasmid), and p663 (control *L.lactis*) are not different in influencing diabetes outcome (52%, 52%, and 48% diabetic, respectively) while freeze medium alone may provide moderate protection (36%). Preliminary erythromycin selective plating results for pTRmIL-10 and pTR1NX show that little growth occurs in the stomach or upper small intestine, but that these levels rise in the colon from 1.0×10^5 - 1.0×10^6 CFU/g digesta dependent upon strain and time. Diabetic animals appear to have a further increase in colonic growth (1.0×10^8 CFU/g digesta), suggesting a link between intestinal microbial dysfunction and diabetes.

12. PHAM, Van Thong

IN VACUO CHEMICAL MODIFICATION OF PROTEINS AS AN APPROACH FOR THE CHARACTERIZATION AND IDENTIFICATION OF PROTEINS.

By : Pham, Van Thong; Altosaar, Illimar; and Kaplan, Harvey

Proteins often undergo post synthetic proteolytic processing in vivo. Thus, determination of the amino and carboxyl terminal regions is required in the characterization of proteins. Current methodologies are deficient in the ability to obtain sequence in a facile manner. The method fails when the protein has a blocked amino terminus, not pure or consists of multiple chains. The focus of this research is to develop a novel approach in sequence determination that would overcome the above deficiencies. The proteins are chemically modified and then the N-terminal and C-terminal peptides are isolated by high voltage paper electrophoresis for subsequent analysis by tandem mass spectrometry. A repeat pattern-matching algorithm elucidates peptide sequence from the MS spectrum.

G-PROTEIN COUPLED RECEPTOR KINASE IN FROG

OOCYTE MATURATION

By : Wang, Jing and Liu, X. John

Fully grown *Xenopus* oocytes physiologically arrest at the prophase of meiosis I. Resumption of meiosis or oocyte maturation is triggered by progesterone. We have demonstrated that G protein dimmer is responsible for maintaining oocyte meiosis arrest. We speculated that resumption of meiosis is induced by progesterone via a mechanism that suppresses the release of G_i. G protein coupled receptor kinases (GRKs) regulate G protein signalling through association with, and phosphorylation of, ligand-activated G protein coupled receptor (GpCR). Phosphorylated GpCR further binds another regulatory protein, β -arrestin, which in turn prevents G protein coupling to the GpCR and inhibits the release of G_i. Therefore, in the present study, GRKs are targets for further studying of oocyte maturation. In preliminary experiments, we found that rat GRK3 was most efficient, among several mammalian GRKs tested, in inducing oocyte maturation. Thus, GRK3(ARK2) has become a key molecular for further study. To see if kinase activity and C terminal region of GRK3 are required for inducing oocyte GVBD(Germinal Vesicle Break Down), we constructed two mutants GRK3. GRK3(K220R) lost ATP binding site and was kinase dead. GRK3 Δ C missed the C-terminal responsible for binding G_i. Either mutation affects the ability of GRK3 to induce GVBD. We further found that co-injection of GRK3 and β -arrestin2 was more efficient in inducing GVBD and maturation than injection of either alone. Our results suggest that GRK may mediate progesterone induced GpCR inhibition in *Xenopus* oocyte maturation.

15. YU, Weiling

THE GENE PRODUCTS OF *LISTERIA MONOCYTOGENES* INDUCED SPECIFICALLY DURING RABBIT INFECTION

By : Yu, Weiling and Lin, Min

The gram-positive, facultative intracellular bacterium *Listeria monocytogenes* causes the disease listeriosis in both humans and animals. For identification and functional characterization of the *L. monocytogenes* proteins specifically expressed *in vivo* during infection, rabbits were used as an animal model for experimental infections. The animals, which were either infected with viable bacteria or heat-killed cells, produced a high titer of antibody responses to the organism, as demonstrated by ELISA. The antisera from both groups of rabbits are useful probes for the investigation of regulated expression of the *L. monocytogenes* gene products due to infection by differential screening of a recombinant protein expression library. The genomic DNA has been isolated from *L. monocytogenes* and is being employed to construct an expression library using pSCREEN vector or λ SCREEN vector. Work is in progress to screen the library with *anti-L. monocytogenes* sera from both infected and immunized rabbits.

17. BONIN, Fanny

MOLECULAR MECHANISMS OF LIS-1 SIGNALING IN

14. BEVILACQUA, Lisa

THE EFFECT OF 2 WEEK AND 2 MONTH ENERGY RESTRICTION ON PROTON LEAK AND REACTIVE OXYGEN SPECIES PRODUCTION IN FBNF1 RATS.

By : Bevilacqua, Lisa and Harper, Mary Ellen

Energy Restriction (ER) without malnutrition is the only intervention that has consistently been shown to increase maximum life span in a wide variety of species. The mechanism for the effect of ER on aging is unknown, although ER may alter cellular oxygen consumption and the production of reactive oxygen species (ROS). We hypothesize that ER leads to quick and sustained decreases in proton leak and in turn results in decreased ROS production, followed by decreases in damage to cellular macromolecules. To test the above hypothesis, we have studied proton leak using top-down metabolic control analysis on skeletal muscle mitochondria of 6 month old FBNF1 rats following 2 week and 2 month 40% ER regimens. We found that State 4 (maximal leak dependent) oxygen consumption is 42% lower for the 2 month ER rats and 26% lower for the 2 week ER rats, and is highest in the ad libitum fed control groups. State 4 protonmotive force is highest in the two ad libitum fed control groups followed by the 2 week ER rats (6% lower) and the 2 month ER rats (13% lower). Production of ROS, determined using H₂O₂ fluorometric (PHPA) assay, demonstrated 50-63% lower ROS production (P>0.05) in the ER rats. These findings support the oxidative stress hypothesis of aging and suggest a role for proton leak in the mechanism of ER.

16. WU, Kechun

PUROINDOLINES IN OAT AND WHEAT RELATIVES: IMMUNOLocalIZATION AND STRUCTURAL PREDICTION

By : Wu, Kechun and Altosaar, Illimar

Puroindoline a (PINa) and PINb are small, basic, cysteine-rich, seed proteins possessing a unique tryptophan-rich domain. PINa and PINb are major genetic factors influencing wheat endosperm hardness. We have identified a homologue, tryptophanin, in oat. Immunofluorescent labeling of plastic-embedded Hinoat seeds with a monoclonal anti-puroindoline antibody was restricted to the starch endosperm layer where it circumscribed solitary amyloplasts as well as the diagnostic compound starch granules. We expressed recombinant PINb in OrigamiTM, an *E. coli* strain with high expression level. Western blots showed rtPINb was recognized by a monoclonal anti-PIN antibody. The 3D structure determination of rtPINb with NMR is in process. Current data suggests that oat tryptophanin may contribute to oat endosperm softness. Future works involves application of anti-sense RNA technology to silence the oat tryptophanin expression to increase hardness of oat grain.

NEURONS

By : Bonin, Fanny, Franks, Doug, Bennett, Steffany

The LIS-1 gene product codes for the regulatory subunit of platelet activating factor acetylhydrolase 1b (PAFAH1b). Platelet activating factor (PAF: 1-*O*-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a biologically active phospholipid and potent neurotoxin that accumulates in brain following seizure, ischemia, HIV-infection, and in the developmental brain disorder Miller Dieker Syndrome (MDS). Mutations in the LIS-1 gene are the genetic determinant of MDS. The goal of this thesis is to identify molecular mechanisms of LIS-1 signaling responsible for neuropathology. We will determine whether PAF directly interacts with LIS-1 to initiate cell signaling or whether the role of LIS-1 is confined to its participation in the PAFAH1b complex. In our first series of experiments, we show that the kinetics of PAF-induced cell death are regulated by functional PAFAHs identified for the first time in PC12 cells. Using a commercial assay and thin layer chromatography, we demonstrate inactivation of PAF to lyso-PAF occurs within 15 min of ligand internalization. RT-PCR was performed using primers that recognize the three subunits of PAFAH 1b (1, 2, and LIS-1) and PAFAHII. PC12 cells express PAFAHII and all three subunits of PAFAH1b. To identify a cell model that expresses only PAFAH1b, RT-PCR was performed on rat cerebrum. PAFAH1b but not PAFAHII is expressed in rat hippocampus. Future studies will investigate the role of LIS-1 and mutant LIS-1 expression on PAFAH1b activity, PAF-mediated neurotoxicity, and PAF-mediated cytoskeletal alterations.

19. LANDRY, Roxanne C.
THE CRYSTALLIZATION OF PorB PORIN FROM
NEISSERIA MENINGITIDIS
By : Landry, Roxanne C. and Evans, Stephen V.

PorB porin is a homotrimeric anion-specific pore protein from the outer membrane of *N. meningitidis*. It has properties usually not seen with the porins of commensal Neisseriae and other unrelated bacterial pathogens: it translocates into target cell membranes and binds nucleoside triphosphates. These properties are thought to provide a crucial mechanism for the pathogen to control apoptotic signaling and accommodate into target cells. Structural investigations of PorB will provide insights into the basis of its ion selectivity, nucleoside triphosphate binding and overall gating mechanism. Such knowledge would greatly enhance our understanding of membrane channels as well as the process of infectivity of *N. meningitidis*. Membrane protein crystallization represents a great challenge for structural biologists. Crystals of PorB have been obtained and show diffraction to 25 Å. Current work is focused on improving the quality of the PorB crystals.

21. OKENWA, Chinonso
REGULATION OF INFLAMMATORY AND ANTI-

18. HILL, Danny
DISSECTING THE CHEMISTRY OF NICOTINIC
RECEPTOR-LIGAND INTERACTIONS WITH
INFRARED DIFFERENCE SPECTROSCOPY
By : Ryan, Stephen E., Hill, Danny G., and Baenziger, John E.

The physical interactions that occur between the nicotinic acetylcholine receptor from Torpedo and the agonists carbamylcholine and tetramethylamine have been studied using both conventional infrared difference spectroscopy and a novel double-ligand difference technique. The latter was developed to isolate vibrational bands from residues in a membrane receptor that interact with individual functional groups on a small molecule ligand. The binding of either agonist leads to an increase in vibrational intensity at frequencies centered near 1663, 1655, 1547, 1430, and 1059 cm⁻¹ indicating that both induce a conformational change from the resting to the desensitized state. Vibrational shifts near 1580, 1516, 1455, 1334, and between 1300 and 1400 cm⁻¹ are assigned to structural perturbations of carboxylate, tyrosine and possibly tryptophan residues upon the formation of receptor-quaternary amine interactions. Other vibrational bands suggest the involvement of additional side chains in agonist binding. Two side-chain vibrational shifts from 1668 and 1605 cm⁻¹ to 1690 and 1620 cm⁻¹, respectively, could reflect the formation of a hydrogen bond between the ester carbonyl of carbamylcholine and an arginine residue. The results demonstrate the potential of the double-ligand difference technique for dissecting the chemistry of membrane receptor-ligand interactions and provide new insight into the nature of nicotinic receptor-agonist interactions.

20. KAMEL, Chris
BIOLOGICAL AND BIOCHEMICAL ACTIVITIES OF
MAMMALIAN SIR2
By : Kamel, Chris and McBurney, Michael W.

Silent information regulator 2 (Sir2) proteins are a family of NAD⁺ dependent deacetylases conserved in organisms from bacteria to humans. These proteins catalyze a unique reaction, coupling deacetylation of protein substrates with conversion of NAD⁺ to O-acetyl-ADP-ribose. While they have been found in complexes associated with telomeric, mating type and rDNA silencing in yeast, little is known about the natural role of the Sir2 family in mammals. In order to study Sir2 function in a mammalian system, we looked at biological and biochemical differences between wild-type and Sir2 knock-out mouse embryonic stem cells generated in the lab. NAD⁺ levels and redox state of these cell lines was determined using an enzyme cycling assay. While it is predicted that loss of Sir2 function would lead to an increase in cellular NAD⁺ levels, preliminary results indicate that the reverse is true. Additionally, because Sir2 has been shown to deacetylate histones and p53 *in vitro*, we are looking to identify other biologically relevant deacetylation targets. In doing so, we hope to better understand the natural role of Sir2 proteins in the mammalian system.

INFLAMMATORY CYTOKINES BY SHP-1: MOTH-
EATEN MOUSE MODEL

By : Okenwa, Chinonso and Kozlowski, Maya

Loss of SHP-1 a cytosolic tyrosine phosphatase predominantly found in hematopoietic cells results in the moth-eaten disorder characterized by necrosis, inflammation, chronic infiltration of tissues by myeloid cells and neutrophils and consequently premature death. SHP-1 has been shown to down-regulate intracellular activation mechanisms by dephosphorylating cytosolic proteins at tyrosine residues. SHP-1 deficiency results in abnormal regulation of immune responses such as hyper-proliferation and hyper-activation. In an effort to explore mechanisms regulating hyper-activation of *me/me* macrophages (*m* s), we studied the cytokine production. In contrast to normal *m* s, SHP-1 deficient *m* s exhibited elevated levels of TNF- and reduced production of IL-10 in response to LPS stimulation. TNF- production is a subject of IL-10 regulation. Supplementing *me/me* *m* cultures with exogenous rIL-10 significantly reduced levels of TNF-. Preliminary data suggests that IL-10 signaling via IL-10R may be intact but its production may be a subject of SHP-1 regulation. This notion is supported by decreased production of chemokines by exogenous rIL-10. Elevated levels of TNF- with a concomitant lower production of IL-10 in LPS stimulated *me/me* splenic *m* s reveals a critical role for SHP-1 in regulating cytokine networks leading to the regulation of immune responses and chemokine production.

**23. INGREY, Keely
IDENTIFICATION OF AN ABC TRANSPORTER
INVOLVED IN HEME UPTAKE IN PATHOGENIC
NEISSERIAE**

By : Ingrey, Keely T. and Dr. Lee, Craig B.

Pathogenic *Neisseriae* are obligate human bacterial pathogens. *N. meningitidis* causes meningitis, and *N. gonorrhoeae* is the etiological agent of the sexually transmitted disease gonorrhea. Iron, essential to sustain bacterial growth, is obtained in the human host from transferrin, lactoferrin, and from heme and heme-containing compounds. The mechanisms for heme uptake are still not well understood. Heme iron acquisition proceeds via a receptor-mediated process. We hypothesize that a dedicated ABC transporter is an essential component of this uptake pathway. In collaboration with TIGR, we will construct, using transposon insertional inactivation, mutants of 24 putative ABC transporters identified in *N. meningitidis* by nucleotide sequence homology. We will characterize the ABC transporter mutants by heme binding assays in order to identify the putative heme ABC transporter. Subsequently, transcriptional and translational analysis, complementation studies, and LD₅₀ studies in mice will be conducted. Whole genome scanning of the gonococcal chromosome using BLAST will be applied to identify the homologous ABC transporter in *N. gonorrhoeae*.

25. ARBOUR, Nicole

**22. ENG, Nelson
IMPROVING GENETIC TOOLS FOR STUDYING
EXPRESSION OF CELL DIVISION GENES IN
NEISSERIA GONORRHOEAE**

By : Eng, F., Nelson and Dillon, R., Jo-Anne

The selection of proper bacterial cell division sites is mediated, in part, by MinC, MinD, and MinE proteins. The role of these proteins in cocci has only recently been investigated in our laboratory using the gram-negative coccus *Neisseria gonorrhoeae* (Ng) as a model organism. *N. gonorrhoeae* divides along two perpendicular planes, resulting in a tetrad of daughter cells. We constructed a shuttle vector, pFP20, and have used it to co-express MinC_{Ng} and MinD_{Ng} in gonococcal backgrounds. We found that these proteins inhibit cell division as evidenced by enlarged cells, whereas in *Escherichia coli*, inhibition is shown by filamentation. However, expression from our shuttle vector was solely from the cloned promoter region of *min* genes. I improved pFP20 by introducing an inducible promoter, P_{lac}, creating pNE2. Then, I cloned *minCDE*_{Ng}, *minC*_{Ng}, *minD*_{Ng}, *minE*_{Ng}, *minCD*_{Ng}, and *minDE*_{Ng} to generate pNE10 to pNE15, respectively. Morphological analysis and Western blots confirmed MinC_{Ng} overexpression when pNE11 was transformed into *E. coli* PB103. I am currently transforming pNE10 to pNE15 into Ng backgrounds. These constructs provide new tools required to control expression of the Min_{Ng} proteins in its native organism and thus allow us to better characterize Min function which was previously not possible to perform.

**24. ROBERTS, Danielle
IN VITRO AND IN VIVO GENETIC STABILITY OF
RECOMBINANT ADENOVIRUS WILDLIFE RABIES
VACCINES**

By : Roberts, Danielle R., Nadin-Davis, Susan, Wandeler, Alexander

The development of new effective and safe vaccines for wildlife vaccination against rabies continues to be important in regards to the health of humans and domestic animals. A variety of recombinant vaccines have been developed, using canarypox, vaccinia, and most recently adenovirus as viral vectors. One particular human adenovirus recombinant, AdRG1.3, contains a rabies G-glycoprotein insert within the E3 region. The focus of this research centers on the genetic stability of this recombinant adenovirus both *in vitro* and *in vivo*, since so far relatively little is known. *In vitro* stability studies planned include nucleotide analysis of the insert following multiple passaging of AdRG1.3 on HEK 293 cells, to observe any mutations that may be acquired. The possibility of re-acquisition of deleted functions from the helper cell line or from recombination with wild type adenoviruses will also be investigated. *In vivo* stability studies will make use of a cotton rat animal model in order to detect the possible emergence of mutants in a live animal host.

P53 UP-REGULATES NOXA, A BH3 ONLY MEMBER

OF THE BCL-2 FAMILY, WHICH PROMOTES NEURONAL CELL DEATH IN A P53 AND BAX DEPENDANT FASHION

By : Arbour A. N., Cregan¹, S. P., MacLaurin, J. G., Callaghan¹, S. M., MacKenzie, A. E., Park, D. S. and Slack, R. S.

p53 is a transcriptional activator that has been identified as a critical factor in the regulation of neuronal cell death. We have previously demonstrated that Bax, a pro-apoptotic member of the Bcl-2 family, plays an important role in the regulation of p53-mediated neuronal cell death. Despite its importance in this pathway Bax is not up regulated in p53-mediated neuronal cell death. Using DNA microarray analysis of total RNA isolated from neurons undergoing p53-induced apoptosis we detected a 5-7-fold increase in the expression of the BH3 only Bcl-2 family member, Noxa. We show that under conditions of neuronal cell death induced by camptothecin, a DNA-damaging agent that functions through a p53-dependant mechanism, Noxa is up regulated in p53 positive, but not in p53-deficient neurons. Furthermore, Noxa protein levels were increased, in both *in vitro* and *in vivo* models of p53-induced neuronal cell death. Finally, we show that enforced expression of Noxa using a recombinant adenoviral vector was sufficient to induce cell death in wild type, but not in Bax deficient neurons. We propose that p53 up regulates Noxa, which binds to and inhibits anti-apoptotic Bcl-2 family members and thereby facilitates Bax-mediated cell death processes. This work was supported by Canadian Institutes of Health Research (CIHR) grant to RSS.

**27. BEAUDOIN, Gregory
FUNCTIONAL STUDY OF APOPTOSIS RESISTANCE IN MACROPHAGES**

By : Beaudoin, G. and Badley, A.

Apoptosis is a form of programmed cell death that is critical to such processes as development and immunity. Macrophages are long-lived, resilient cells that are generally resistant to apoptotic stimuli. In the differentiation process from blood monocyte to macrophage, these cells up regulate anti-apoptotic proteins such as cFLIP, XIAP and Bcl-2 family members, and down regulate proapoptotic proteins such as caspases 3 and 8. Functional involvement in resistance to apoptosis has been shown for cFLIP and XIAP, but not others. This study aims to characterize functional differences in the various apoptotic pathways, between monocyte-derived macrophages (MDM) and other cell types known to have fully functional apoptosis capabilities. Preliminary results in a cell-free system indicate that MDM are not susceptible to caspase 8-mediated activation of caspase 3, whereas Jurkat T cells are. Further experiments will determine which points in the various apoptosis pathways remain functional in macrophages, and which points are inhibited.

**29. ALLI, Zaman
A PLANT-DERIVED HEPATITIS-B CORE PARTICLE**

**26. CHAKIR, Habiba
GENETICALLY RESISTANT MICE LACKING THE IL-12R β 2 GENE ARE SUSCEPTIBLE TO *LEISHMANIA MAJOR* INFECTION AND DEVELOP A SPECIFIC TH2 IMMUNE RESPONSE**

By : Chakir, Habiba, Campos-Neto, Antonio and Webb, John R

IL-12 is critical for Th1 immune cell differentiation and resistance to *Leishmania major* infection. To determine the *in vivo* role of IL-12 receptor in the development of protective immunity, genetically resistant mice lacking the IL-12R β 2 subunit were infected with *L. major* and compared to wild type mice of susceptible and resistant backgrounds. After infection with *L. major* promastigotes, IL-12R β 2 KO mice developed footpad swelling characteristic of a susceptible Th2 phenotype. *In vitro* stimulation of popliteal lymph node (LN) cells from *L. major*-infected IL-12R β 2 mice revealed a typical Th2 response (high IL-4, high IL-5, low TNF α and low IFN γ). In addition, RPA analysis revealed the presence of IL-10 and IL-13 mRNAs in IL-12R β 2 KO LN cells in response to parasite antigen stimulation. IL-12R β 2 KO mice and susceptible BALB/c mice produced similar levels of parasite-specific IgE after *L. major* infection; however, unlike BALB/c mice, IL-12R β 2 KO mice did not produce any detectable parasite-specific IgG2a, consistent with a defect in IL-12 inducible IFN γ production. We conclude that IL-12R β 2 subunit is critical for the development of the Th1 response necessary for protective immunity against *L. major*.

**28. CONTE, Damiano
HIAP1 IS ESSENTIAL FOR THYMOCYTE SURVIVAL DURING EARLY STAGE T CELL DEVELOPMENT**

By : Conte, Damiano, Korneluck, R.G., Wright, Kathryn E.

T cell and thymocyte-triggered programmed cell death as well as T cell function/activation are partially dependent on the activation of caspases through their proteolytic processing. IAP family members inhibit apoptosis by directly interacting with key initiator and effector caspases.

Over-expression of HIAP1 inhibits apoptosis in tissue culture cells challenged with a wide variety of apoptotic triggers. Interestingly, human thymocytes and peripheral lymph nodes contain high levels of human *hiap1* mRNA. More importantly, it has also been shown that the key genetic lesion involved in approximately 50% of mucosa-associated lymphoid tissue lymphomas includes a rearrangement of *hiap1* gene, and that there is an upregulation of HIAP1 and XIAP in activated T lymphocytes from patients with multiple sclerosis.

Here, by generating a *miap1* gene-knockout mouse, we demonstrate that HIAP1 is essential for thymocyte survival during early stage T cell development. Furthermore, thymocytes derived from *hiap1* KO mice are more susceptible to T cell-specific apoptotic triggers, and display a retarded proliferative response to a variety of proliferative agents relative to wild-type littermates.

VACCINE SHOWED IDENTICAL IMMUNOSTIMULATORY ACTIVITY AS THE *E. COLI*

DERIVED PARTICLE WHEN ADMINISTERED TO MICE

By : Alli, Zaman and Altosaar, Illimar

The spread of viral diseases such as hepatitis and AIDS is alarming. Scientists are endeavouring to develop new and better vaccines. The focus here is the development of edible and low-cost vaccines for worldwide protection. Plants can process and correctly assemble heterologous proteins in specialized tissues or organellar compartments. Further, plants are relatively free of human pathogens and have edible tissues making them ideally suitable for oral vaccines. To produce an edible hepatitis B core particle (HBCP) vaccine, the HBCP expression construct was used to transform carrots, rice, squash, tobacco, and wheat tissues. The first batch of vaccinogen plants to mature is tobacco plants and these produced correctly assembled viral-like particles of 25-30 nm in diameter as determined by electron microscopy. Therefore, the purified HBCP from transgenic tobacco leaves was used to immunize mice. The *E. coli* derived HBCP was also tested. Upon analysis of the immunized mice, equal immune responses were obtained for both groups. The selection and regeneration of transgenic plants are continuing for the remaining plants. The next phase is to determine the ability of the plant produced HBCP for stimulation of oral immunity. Plant produced vaccines may lead to global, low-cost, edible vaccinations.

**31. CHRISTOU, Marie-Grace
TARGETING HEPATITIS C INFECTED CELLS USING
VESICULAR STOMATITIS VIRUS**

By : Christou, M. Grace, Lichty, Brian D. and Bell, John C.

Hepatitis C virus downregulates interferon response through its NS5A/5B protein, making infected cells immune to normal cellular anti-viral responses. Vesicular Stomatitis Virus has been previously reported to be exquisitely sensitive to interferon and a potent killer of cells lacking an interferon response. We hypothesize that Hepatitis C infected cells can be preferentially targeted for killing by VSV.

To confer additional specificity to VSV, viral gene expression can be placed under the regulation of Hepatitis C components. Hepatitis C encodes a trans-acting protease complex NS3/4A that recognizes cleavage sites in the 3' end of the Hepatitis C polyprotein and is vital for generating a functional RNA replicase. We hypothesize that expression of NS3/4A protease in infected cells can be exploited for therapeutic purposes by engineering a recombinant VSV whose replication is dependent on cleavage by NS3/4A.

**30. LEFRANÇOIS, Brice
MECHANISMS UNDERLYING ABNORMAL
REGULATION OF THYMIDYLATE SYNTHASE IN
COLON CANCER**

By : Le François, Brice, and Birnboim H. Chaim

Thymidylate synthase (TS) plays an essential role in cellular metabolism by catalyzing the last step of the *de novo* synthesis pathway of thymidine. TS is a cell-cycle dependent enzyme undergoing an upregulation in cells entering S-phase. Furthermore the TS protein is reported to have a mRNA binding activity, possibly suggesting a much more complex role than previously understood. Many drugs used in chemotherapy, such as FUDR or Tomudex, target this enzyme and kill cells by interfering with DNA repair and replication. To date, several mechanisms of resistance to these compounds have been described, like point mutations decreasing the protein affinity for these drugs. In our study, we screened (i) tumor bank samples, (ii) colon cancer cell lines and (iii) HeLa cell lines resistant to FUDR for spontaneous point mutations. Using a PCR-based strategy, we amplified and sequenced two exons of the TS gene corresponding to residues involved in substrate binding. Only one of the resistant HeLa cell lines showed a heterozygous point mutation within the coding region of the gene. Currently, we are testing whether this mutation can confer resistance when introduced into a sensitive cell line. We are also investigating upstream and downstream regulation of TS by trying to develop a TS double knock-out cell line and characterizing the role of two transcription factors, E2F and LSF, involved in the regulation of the gene.

**32. WEAGANT, Brodie
THE MECHANISM FOR INHIBITION OF ANGIOGENESIS
BY ANGIOSTATIC MOLECULES**

By : Weagant, Brodie, Addison, Christina L., McBurney, Michael

Angiogenesis, the formation of new blood vessels from pre-existing ones, is important in wound healing, pregnancy, tumour growth and metastases. This process involves the proliferation and migration of endothelial cells as new capillaries are formed. Endothelial cells rely on signals from surrounding cells and the extra-cellular matrix (ECM). These signals are mediated in part by integrins, which are cell/ECM attachment proteins. Integrins are capable of inside:out and outside:in signaling and influence many functions such as survival, migration, signal transduction and gene expression. Angiostatic molecules may directly affect the ability of integrins to transduce the necessary information via changes in signaling molecules such as focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI3-K) and integrin-linked kinase (ILK). We have found that the angiostatic molecule, endostatin, decreased FAK phosphorylation, which may be important for the deactivation of angiogenic signaling cascades. We are currently examining these issues in the laboratory.

33. DUHAIME, Suleena

DIFFERENTIAL REGULATION OF P-GLYCOPROTEIN GENE EXPRESSION IN TUMOR CELLS BY CYCLIC AMP-DEPENDENT PROTEIN KINASE

By : Duhaime, Suleena K and Parissenti, Amadeo M

Several studies have demonstrated that the over-expression of the drug transporter, P-glycoprotein (P-gp), leads to multi-drug resistance (MDR) in tumour cells. A number of studies in rodent systems suggest that cyclic AMP-dependent protein kinase (PKA) stimulates P-gp expression. Dominant inhibition of PKA in these cells resulted in lower expression levels of the P-gp gene and protein. In contrast, Parissenti *et al.* (1999) found that dominant inhibition of PKA in adriamycin resistant human breast cancer cells (MCF7ADR) had no effect on P-gp levels. Together, these findings suggest differential regulation of P-gp expression amongst cell lines. In our present line of work we are examining whether the ability of PKA to modulate P-gp expression is cell-type specific or is lost as a consequence of selection for doxorubicin resistance. To assess this, additional wild-type and drug-resistant human and rodent cell lines have been transfected with PKA dominant inhibitory mutations. Interestingly, our findings to date suggest that PKA inhibition can block P-gp expression in doxorubicin-resistant human uterine sarcoma cells, suggesting cell type-specific differences. Findings in our laboratory also indicate that the human promoter for the P-gp gene is unaffected by dominant inhibition of PKA, suggesting regulation at the level of mRNA stability. Future studies will specifically address this hypothesis.

35. NAAS, Turaya

HEPATITIS C STRUCTURAL PROTEINS (CORE AND ENVELOPE) ARE EXPRESSED IN THE LIVER AND SALIVARY GLANDS IN A TRANSGENIC MOUSE MODEL

By : Naas, Turaya, Ghorbani, Masoud, Alvarez-Maya, Ikuri, Diaz-Mitoma, Francisco

Hepatitis C virus (HCV) is the major cause of non-A, non-B hepatitis. More than 200 million people worldwide are infected with HCV, including 4 million individuals within the United States. Very little is known about the role of HCV structural proteins in the virus-cell interaction and the pathogenesis of HCV infection. To study the pathogenesis of hepatitis C infection, we established a transgenic mouse model expressing the encoded HCV structural proteins (core, E1, E2) under the control of the CMV promoter. These transgenic mice showed expression of core, E1, E2 in the liver and salivary glands as detected by immunofluorescent staining. We are performing histological analysis to detect pathological changes that may appear in the liver and other tissues of this HCV transgenic mouse model. Preliminary analysis demonstrated hepatocyte steatosis in transgenic mice. This animal model will be used to analyze the effect of structural HCV proteins in the pathogenesis of chronic hepatitis C.

34. BROWN, Robert

IDENTIFYING THE BIOLOGICAL ROLE OF HEPATIC LIPASE CELL SURFACE ASSOCIATION.

By : Brown, Robert J. and Yao, Zemin

Human hepatic lipase (hHL) exists mainly bound to cell surface heparan sulfate proteoglycans (HSPG) *in vivo*. In contrast, mouse HL (mHL) is found circulating in the bloodstream. Using a catalytically active hHL mutant (hHL₄₇₁) in which the C-terminal 5 predominantly basic amino acid residues (KRRIR) were deleted, we demonstrated that these residues account for 40% of the hHL cell surface affinity by densitometry and pulse-chase analyses. Additional determinants of HSPG binding were identified within the C-terminal 73 amino acid residues of hHL. Using a catalytically active chimera (hHLmt) in which the C-terminal region of hHL (73 residues) was replaced with an equivalent segment derived from mHL, we demonstrated that the region accounts for 68% of the hHL cell surface affinity. To understand the role of hHL binding to the cell surface, CHO cells expressing hHL and hHLmt were used to study high density lipoprotein (HDL) cholesteryl-ester (CE) association. The CE association by control and hHL cells did not differ, demonstrating that hHL is not involved in CE association. However, hHLmt reduced CE association by half. This may suggest that HDL is being hydrolyzed in the media, thus generating HDL that poorly donates CE to cells. To study this effect further, adenoviruses expressing hHL and hHLmt have been generated for *in vivo* expression using *HL*^{-/-} mice.

36. FALLAHI, Firouzeh

STUDY OF THE EFFECT OF PENCICLOVIR AND ACYCLOVIR IN LYMPHOCYTE PROLIFERATION

By : Fallahi, Firouzeh and Diaz-Mitoma, Francisco

Several guanosine analogues, i.e. acyclovir (and its oral prodrug valaciclovir), penciclovir (in its oral prodrug form, famciclovir), are widely used for the treatment of herpes viruses [i.e. herpes simplex virus type 1 (HSV-1), and type 2 (HSV-2), varicella-zoster virus (VZV) and/or human cytomegalo virus (HCMV)] infections. Penciclovir and acyclovir possess similar potent antiviral activities and mechanism of action *in vivo*. The antiviral activity of these drugs depends on their phosphorylation by the viral thymidine kinase (TK). We investigated the possibility of a concomitant induction of proliferation of peripheral blood monocytes (PBMCs) by the guanosine analogues, as it has been observed in previous studies in our laboratory. In this study we examined the ability of penciclovir and acyclovir to induce the proliferation in PBMCs in the presence of an antigenic proliferative response in PBMCs, using influenza virus.

37. SLATER, Kathryn

CHARACTERIZATION OF REOVIRUS $\mu 2$ EXPRESSION AND INTERACTIONS WITH VIRAL PROTEINS AND THEIR ROLE IN INCLUSION BODY FORMATION

By : Slater, Kathryn and Brown, Earl

The site of virion synthesis in reovirus-infected cells is characterized by inclusion body formation. Previous work has shown that the rate of inclusion body formation differs between two reovirus prototype strains, Type 1 Lang (T1L) and Type 3 Dearing (T3D). The genetic basis for this difference has been attributed primarily to the M1 gene, encoding the $\mu 2$ protein, of reovirus with the NS encoded by the S3 gene being a contributing factor. Further investigation into the protein-protein interactions between $\mu 2$ and other reoviral proteins in order to determine their involvement in inclusion body formation, will aid in the characterization of the sequential pathway that leads to inclusion body formation and viral replication. Copies of all ten genes of both T1L and T3D reovirus have been produced. These genes will be cloned into an expression vector containing the M1 gene in order to express both genes from the same mRNA. Protein interactions will be examined by dual fluorescence labeling and immunoprecipitation. Since it is difficult to express detectable amounts of $\mu 2$ protein *in vitro*, we also expect that one or other of the remaining viral proteins will enhance $\mu 2$ protein expression or stability. These studies will provide a basis for the central role of $\mu 2$ protein in reovirus replication and morphogenesis.

39. MCKENZIE, Andrea

CAVEOLIN-1 INVOLVEMENT IN CHOLESTEROL EFFLUX AND CHOLESTEROL TRANSPORT (METABOLISM ?)

By : McKenzie, Andrea G. and Marcel, Yves L.

Caveolin-1 is a 21kDa protein involved in movement of cholesterol between the endoplasmic reticulum (ER) and the plasma membrane (PM), from where it can be transferred by efflux. Previous work in our lab showed that over expression of caveolin-1 in McA-RH7777 increased cholesterol transport from ER to PM, and increased cholesterol efflux to HDL upon prolonged incubations. In contrast, short term over expression of caveolin-1 by adenovirus vector infection of HepG2 cells did not increase efflux of ^3H -cholesterol to apoA-I or HDL, but increased the synthesis of cholesterol from ^3H -acetate. Because the method of labeling varied pools of cellular cholesterol affects transport to efflux sites, we are now comparing cholesterol transport and efflux as a function of labeling PM or endocytic compartment and ER. Labeling the PM with ^3H -cholesterol pre-equilibrated with HDL increases with caveolin-1 expression in McA-RH7777 without change in the labeling of Triton X-100 resistant membranes. Future studies will examine the transfer and efflux of endogenously synthesized cholesterol.

41. PARADIS, Madeleine

ESTROGEN RECEPTOR EXPRESSION, ACTIVATION AND

38. GULZAR, Naveed

T-CELL WARS, EPISODE CD8+: ATTACK OF THE HIV

By : Gulzar, Naveed & Copeland, F. T., Karen

It has been over 20 years since acquired immune deficiency syndrome (AIDS) was first recognized as a novel disease. In that time, it was discovered that the causative agent of AIDS is the human immunodeficiency virus (HIV). HIV is able to infect a number of cell types, which includes T-lymphocytes. CD8+ T-cells, a subset of T-lymphocytes, plays an important role in host cell defense in the control of viral replication by either direct lysis of infected cells and/or by the secretion of soluble anti-viral factors. With the development of viral progression and clinical symptoms, the anti-HIV response normally attributed to CD8+ T-cells is diminished or lost. This phenomenon could be attributed to either a loss in CD8+ T-cell function or the loss of a CD8+ T-cell subset. We hypothesize that CD8+ T-cells are lost in HIV infection through an apoptotic mechanism. We have demonstrated that HIV-IIIB infected CD8+ T-cells undergo DNA fragmentation, a feature of apoptosis. In addition, infected CD8+ T-cells underwent phosphatidylserine exposure, an early apoptotic event, as demonstrated by Annexin V staining. Western blots will be performed on cellular lysates of HIV infected CD8+ T-cells with antibodies to caspases 8,9 and 3, the effectors of the apoptotic pathway. By examining the mechanism of cell death of CD8+ T-cells in response to HIV infection, therapeutic interventions can be researched to prevent the loss of these cells in viral pathogenesis.

40. RUOSO, Patrizia

THE MATRIX PROTEIN OF VESICULAR STOMATITIS VIRUS AND VIRUS BUDDING

By : Ruoso, Patrizia and Bell, John C.

Our lab has recently discovered an oncolytic virus, vesicular stomatitis virus (VSV), that has the ability to kill a number of different cancer cells both *in vitro* and *in vivo*. The replication process behind this virus, however, has yet to be completely elucidated. The matrix protein of VSV is the major viral protein thought to be involved with viral replication. Various host factors are also thought to be utilized by the virus to aid in budding from the host cell membrane. Recent studies published on various viral replication proteins of different viruses (i.e. Gag protein of HIV and Rous Sarcoma Virus) have shown that TSG101, a recently discovered tumour suppressor protein, and Nedd4, an E3 ligase, bind to specific domains contained in the proteins involved in viral replication. VSV has also been found to contain homologous binding domains to those found in other viruses. To determine these protein interactions, we are using co-immunoprecipitations and immunofluorescence to determine if these host factors are necessary in viral replication of VSV. By determining the replication pathway of VSV, we can exploit viral functioning for cancer therapies that are resistant to current forms of treatment as well as developing anti-viral agents.

CONTROL OF PROTEOLYTIC ENZYMES, IN HUMAN AORTIC VASCULAR SMOOTH MUSCLE CELLS

By : Paradis, Madeleine A. and O'Brien, Edward R.

Acute events in the progression of atherosclerosis are thought to be primarily a result of plaque rupture, a mechanical event dependent on the structural integrity of a given lesion. The mechanism by which estrogen confers putative vascular benefits may be related to expression or activation of degradative enzymes influencing plaque stability. The estrogen signal is transmitted by estrogen receptors in the vascular wall. In this study, human aortic VSMCs were characterized for ER expression by means of Western blot and immunocytochemistry. ER alpha was found to be primarily in the nucleus, while ER beta was cytoplasmic. Cells were transiently transfected with a reporter vector designed to contain an ERE enhancer upstream of a promoter controlling transcription of EGFP. Transcriptional activity was monitored in the presence of various ER ligands and antisense, at 6h and 24h post dose. Cells were assessed for relative gelatinase activity and urokinase activity. All ligands caused a minor increase in relative gelatinase activity as compared to baseline, but were not significantly different from each other. Upregulation of urokinase activity was found to be elevated in the presence of the pure antagonist faslodex, and the partial antagonist 4-hydroxytamoxifen. This suggests an overall increase in proteolytic activity in the absence of ER activation.

**43. SWAN, Katherine
THE POTENTIATION OF C/EBP -MEDIATED
TRANSCRIPTION BY THE GLUCOCORTICOID
RECEPTOR AND STEROID RECEPTOR COACTIVATOR
1**

By : Swan, Katherine M. & Haché, Robert J.G.

The glucocorticoid receptor (GR) has been shown to functionally interact with C/EBP, a key regulator of early adipogenesis. Previous research revealed that the GR ligand binding domain can enhance transcription mediated by C/EBP independent of DNA binding or direct interaction with C/EBP. Furthermore, the GR LBD has been shown to stimulate the differentiation of 3T3 L1 preadipocytes into mature adipocytes. Nuclear receptors, such as GR, are known to recruit coregulators through their C-terminal activation domain in order to regulate transcription. The p160 family of coactivators, including steroid receptor coactivator 1a (SRC1a), has been shown to interact with nuclear receptors in a ligand-dependent manner. It was postulated that a p160 coactivator may be the bridge between the GR LBD and C/EBP. These results indicate that C/EBP associates with SRC1a and that this interaction potentiates C/EBP-mediated transcription. The characterization of the interaction between SRC1a and C/EBP will provide valuable insight into their role as regulators of adipogenesis.

**45. THOMSON, Errol
INDUCTION OF ENDOTHELIN EXPRESSION BY**

**42. ROMERO, Julia
ROLES OF Cdc7 AND Dbf4 IN THE ACTIVATION OF DNA
REPLICATION IN MAMMALIAN CELLS**

By : Romero, Julia I., Lee, Hoyun, and Dillon, Jo-Anne R.

It is thought that, like in yeast, the association of origin recognition complex (ORC) onto an origin of replication (*ori*) is the first step in activating replication initiation in mammalian cells. Subsequently, a Mcm protein complex binds to the *ori*-ORC to form a pre-replication complex (pre-RC), by which the chromatin becomes replication-competent or "licensed." A licensed *ori* is replication-competent but it still cannot initiate DNA replication. The S-phase-promoting protein kinases (S-Cdks) and the Cdc7-Dbf4 complex are further required for the *ori* activation. In particular, Cdc7-Dbf4 kinase is involved in the final two steps in *ori* activation (i.e., the activation of Mcm and Cdc45 proteins), suggesting that it is a critical molecular switch for replication initiation. Although Cdc7 binds chromatin throughout the cell cycle, it is only activated in S phase by associating with Dbf4. Since only a small number of many potential *oris* are activated in mammalian cells, this observation raises two possibilities: (1) Cdc7 binds to many *oris* but only some of them are activated by Dbf4; or (2) Cdc7 binds only to those *oris* that will subsequently be activated by Dbf4. Using the well-known hamster DHFR replicon model and the chromatin immunoprecipitation (ChIP) assay, we want to discern between these possibilities.

**44. HOSSEINI, Mona
GENETIC MODULATION OF THE ONCOLYTIC
PROPERTIES OF REOVIRUS**

**By : Hosseini, Mona ; Marius, Ricardo M. ; Bell, John C. ;
Brown, Earl G.**

Cancer is currently one of the major causes of death in the world. Cancerous cells circumvent normal growth and differentiation and become parasitic within the host organism. Cancer therapies are designed to exploit these differences between cancer and normal cells to selectively destroy tumor cells in the body. One of the strategies makes use of oncolytic viruses that can preferentially replicate in tumor cells relative to normal cells. Reovirus is one of the viruses that fall in to this category. This dsRNA virus is naturally oncolytic lysing some transformed cells, but not normal cells. Different serotypes of reovirus show different oncolytic properties on different transformed cell lines. The segmented nature of reoviral genome also makes it possible to reassort the genetic material between different isolates in order to increase the oncolytic ability. This project seeks to produce new reassortants of reovirus with increased oncolytic abilities to destroy cancers that can not currently be destroyed. In this process we also hope to gain an understanding of the genetic basis for tumor specific replication of reovirus.

AMBIENT PARTICULATE MATTER

By : Thomson, LM, Errol, Vincent, Renaud, and Aubin, Remy

Air pollution has been recognized for many years to have a detrimental effect on human health. Epidemiological studies have found an association between fluctuations in air pollution levels and hospital admissions for cardiovascular and respiratory problems, suggesting a causal relationship. However, uncovering a biologically plausible mechanism to explain the apparent association of health problems with low levels of pollutants has proved elusive. Recently, the effect of airborne particulate matter on the circulating levels of endothelins has been proposed as a possible mechanism for the adverse effects of air pollution on human health. Endothelins are potent vasoconstrictors, and have been implicated in many cardiovascular and pulmonary disorders. Studies involving rats and humans have shown that plasma levels of endothelin-1 (ET-1) and endothelin-3 (ET-3) rise soon after exposure to airborne particles. The mechanism of particle induction of endothelins is not known; however, protein kinase C has been shown to mediate ET-1 induction caused by cigarette smoke extract. This study will use cell cultures to examine endothelin responses to particle exposure. The hypothesis that protein kinase C mediates induction of endothelins by airborne particulate matter will be addressed.

47. MRAD, Rim
HOST GENE EXPRESSION DURING COXSACKIEVIRUS B3 INFECTION IN MICE

By : Mrad, Lejmi Rim; Nait-Ladjemil, Zakia; Mercedes, de Bold Kurosky and Vidal, Silvia

Enterovirus such as coxsackievirus of group B serotype 3 (CVB3) induce myocarditis in human and mice. Human CVB3 infections can be mimicked in mice. Inbred strains of mice present a large spectrum of CVB3-induced pathology. For example C57BL/6 mice are highly resistant. In contrast, A/J mice are the most susceptible presenting severe myocardial inflammation at 7 days post-infection. Several genes have been shown to be differentially expressed during the disease process, however it is not clear whether they have a direct impact in the pathology. To initiate a functional dissection of CVB3-induced pathology in A/J mice, we have characterized the expression of genes known to be overexpressed during infection. We have studied three gene categories including 1) genes coding for the viral receptors, 2) innate immunity genes, and 3) genes expressed during the fetal program. Semi-quantitative PCR analysis indicate a differential expression of interferon-g (*Ifn-g*), tumor necrosis factor- α (*Tnf-a*) and brain natriuretic peptide (*Bnp*). To determine a causative effect of these differentially expressed genes in CVB3-induced disease, their expression will be characterized in informative panels of recombinant inbred strains between A/J and C57BL/6 strains.

49. KELETA, Liya
IDENTIFICATION OF MUTATIONS INVOLVED IN

46. MÉLANSON-DRAPEAU, Lysanne
FUNCTIONAL CHARACTERIZATION OF AN *IN VIVO*
MODEL OF NEURAL PROGENITOR ENRICHMENT
By : Melanson-Drapeau, Lysanne and Bennett, Steffany

Previous work in the Bennett laboratory has demonstrated that the connexin32 knockout (Cx32 KO) mice retain more cells with immunogenic characteristics of neural progenitors in the subgranular zone (SGZ) of the dentate gyrus. These data lead us to hypothesize that alterations in gap junctional coupling influences the fate of neural progenitors. The present study was undertaken to functionally characterize the ability of KO progenitor population to activate and regenerate damaged brain tissue *in vivo*. To determine the proliferative potential of KO and WT progenitors, cells were labeled *in vivo* with bromodeoxyuridine (BrdU). Adult KO mice exhibit a marked increase in the number of actively dividing cells in the SGZ of uninjured animals. To activate progenitors, mice underwent kainic acid-induced epileptiform seizure. Preliminary data indicate that Cx32KO mice exhibit reduced neuropathology relative to WT. To assess functional indices of learning and memory, we are testing Cx32KO and WT mice in the radial arm maze - a behavioural test of spatial learning. Pilot data suggest that uninjured KO mice have a diminished spatial memory capacity relative to WT mice. The effect of progenitor activation on behavioural recovery following seizures is currently under investigation.

48. THÉRIAULT, Steven
ACUTE HYPERTENSIVE RESPONSE TO CENTRAL
SODIUM IN MICE: MEDIATION BY AN ENDOGENOUS
BRAIN OUABAIN-LIKE SUBSTANCE.

By : Theriault, S., Hou, X. and Van Huysse, J.

Intracerebroventricular (i.c.v.) administration of sodium is thought to mimic the effects of a high salt diet in salt-sensitive individuals, increasing brain sodium levels, causing sympathetic nervous system hyperactivity and hypertension. In rats, high brain Na levels upregulate an endogenous brain ouabain-like substance (OLS), which in turn inhibits brain Na, K-ATPases, leading to heightened sympathetic nervous activity and hypertension. To test the hypothesis that high brain sodium, ouabain and OLS exert similar effects in mice, the following studies were carried out. Artificial cerebrospinal fluid (aCSF) with normal or high concentrations of Na was injected i.c.v. In separate mice, aCSF with or without ouabain was also injected i.c.v. Both the aCSF-high Na and aCSF-ouabain injected mice showed increased mean arterial pressure and heart rates, whereas aCSF showed no change. Co-injections of antibodies with a high affinity for ouabain produced a progressive reduction in the pressor response to aCSF-high Na in mice. There are three Na, K-ATPase isoforms in the brain (1, 2 and 3). The above experiments will be extended to 1 and 2 isoform knockout mice, in order to determine if the 1 or 2 isoform mediates the hypertensive effects of brain sodium, OLS or ouabain.

VIRULENCE OF MOUSE-ADAPTED HONG KONG
STRAINS OF INFLUENZA VIRUS

By : Keleta, Liya and Brown, Earl

In influenza viruses the genetic basis for virulence is unknown. The goal of this project was to study the mutational basis for increased virulence. It has been shown that an increase in virulence is associated with a change in the pH of fusion. However, it is still unclear if there is an increase or a decrease in pH. The hemagglutinin (HA) gene of influenza is involved in fusion and receptor binding activities. Mouse-adapted Hong Kong (A/HK/1/68) strains were used to perform this study. The approach we used was to take the mouse-adapted strains sequence them and then perform fusion assays to determine their pH of fusion. It is expected that the pH of each of the isolates will correlate with their virulence. This in turn will help us identify the mutations that are important for virulence. Based on our results we have identified interesting mutations that we think play a part in increasing virulence. Another experiment that is under way deals with introducing a single amino acid change in the cytoplasmic tail of the HA gene by in vitro site directed mutagenesis. We ensured that there were two nucleotide changes accounting for a single amino acid change in order to decrease the chances of a back mutation to compensate for the existing mutation. It is expected that this will increase the chances of suppression which deals with the action of a new mutation on another site on the HA or on the M1 protein that provides wild type function. It has been proven that the M1 (matrix) protein interacts with the cytoplasmic tail of HA. However it is not known what portion of M1 interacts with HA. It is our goal that by serial passaging our mutants and sequencing our samples we will be able to identify residues of M1 that interact with the cytoplasmic tail of HA.

**51. KIELCZEWSKA, Agnieszka
CONSTRUCTION OF A IGG FC-LY49H FUSION PROTEIN
AS A TOOL FOR THE STUDY OF LY49H LIGAND.**

By : Kielczewska, Agnieszka and Vidal, Silvia M.

The gene underlying host resistance/susceptibility mouse cytomegalovirus infection recently been identified in our laboratory. It encodes an activating natural killer cell receptor, belonging to the C-type lectin family, Ly49H. While NK cells have been implicated in innate resistance to viral infection, Ly49H is the first NK cell receptor shown to control it specifically. The identification of a ligand binding Ly49H receptor would provide a deeper understanding of the mechanism of host resistance to cytomegalovirus, as well as open opportunities for design of possible therapeutic agents. Since other members of the Ly49 receptor family expressed on NK cells have been shown to bind MHC class I, it is likely that the Ly49H ligand is a MHC class I homologue, either as a cellular protein induced by MCMV infection or as a virally encoded protein. In order to initiate this study, we have constructed a soluble fusion protein between the Fc part of human IgG1 and the extracellular domain of the Ly49H receptor. The fusion protein FcEC has been cloned and expressed in 293T cells. FcEC provides a powerful tool for further experiments, including FACS screening of mouse splenocytes and MCMV infected cell lines, as well as phage display techniques.

**50. HUNTER, Allison
PEPTIDE INHIBITORS OF THE IAPS**

By : Hunter, Allison M., Ho, Jenny Mei-Yen., and Liston, Peter and Korneluk, Robert G.

Apoptosis signal pathways converge on the caspases, a family of proteases that form a self-amplifying cascade, the activation of which generates the characteristic morphological and biochemical features of apoptotic cell death. The Inhibitors of Apoptosis (IAPs) are a family of proteins that bind and inactivate caspases at both the initiation and terminal, effector stage of this cascade. The recent structure determinations of XIAP in conjunction with caspases and Smac/Diablo lead to the identification of a critical pocket and groove on the surface of each BIR domain (Baculovirus IAP Repeat). Within XIAP BIR3, the binding pocket interacts with caspase 9, and can be disrupted by the presence of Smac protein, which competes for the same site. In this respect, the interaction surface of the BIR motif resembles an enzyme catalytic site, and is therefore amenable to drug development strategies comparable to more traditional drug discovery targets, such as kinases, receptors, or proteases. We have taken a random peptide, phage display approach to identify novel, high affinity peptide ligands that bind to individual BIR motifs of XIAP and the other IAPs. These studies were initiated with the most highly characterized of the IAP BIR domains, XIAP BIR3. Phage encoding peptides identical to the amino terminus of Smac or the caspase 9 small subunit cleavage site were obtained. However, the majority of the selected phage encoded peptides that differed from both Smac and Caspase 9 in the second and fourth position of the tetrapeptide recognition sequence. We are employing a fluorescent polarization binding assay to identify and characterize the peptide sequence that binds to BIR3 with the highest affinity. The results of XIAP BIR1 and BIR2 screening will be presented.

**52. BOUCHER, Jonathan
HDL REGULATES LIPOPROTEIN LIPASE AND
MACROPHAGE METABOLISM OF OXIDIZED
LIPOPROTEINS.**

By : Boucher, Jonathan and Sparks, Daniel L.

Lipoprotein Lipase (LPL) plays two important roles in human lipid metabolism. The enzyme acts to hydrolyse acylglycerides in chylomicrons and VLDL, and thereby generates fatty acids for energy production or storage. LPL also acts as a cell surface ligand to enhance the binding and uptake of circulating lipoproteins into cells. Macrophage-associated LPL plays an important pro-atherogenic role in the uptake and deposition of oxidized LDL into the arterial wall. HDL has been described to play an anti-atherogenic role in lipid metabolism and previous studies have shown that HDL can inhibit the uptake of OxLDL by macrophages and prevent their conversion to lipid engorged foam cells. HDL may prevent OxLDL uptake by promoting the displacement of LPL from the macrophage cell surface. Studies have shown that HDL and apoA-I can displace LPL from pure heparan sulfate proteoglycans. HDL also appears to modulate the lipolytic functions of LPL. This investigation will explore the effect of HDL on the lipolytic and ligand functions of LPL and determine if the anti-atherogenic nature of this lipoprotein is related to LPL regulation.

53. GEE, Katrina

DIFFERENTIAL REGULATION OF CD44 EXPRESSION BY LIPOPOLYSACCHARIDE (LPS) AND TUMOR NECROSIS FACTOR- IN HUMAN MONOCYTIC CELLS: DISTINCT INVOLVEMENT OF C-JUN-N-TERMINAL KINASE IN LPS-INDUCED CD44 EXPRESSION

By : Gee, Katrina, Lim, Wilfred, Ma, Wei, Nandan, Devki, Diaz-Mitoma, Francisco, Kozlowski, Maya, and Kumar, Ashok

LPS, a bacterial cell wall component, regulates CD44 expression and modulates CD44-mediated biological effects in monocytic cells during inflammation and immune responses. We show that in normal human monocytes, LPS and LPS-induced cytokines IL-10 and TNF- enhance CD44 expression. To delineate the mechanism underlying LPS-induced CD44 expression, we investigated the role of mitogen-activated protein kinases (MAPK), p38, p42/44 extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) by employing their specific inhibitors. We demonstrate the partial involvement of p38 in TNF- induced CD44 expression in both monocytes and THP-1 cells. Neither p38 nor p42/44 were involved in IL-10-induced CD44 expression in monocytes. To further dissect the TNF-, and LPS-induced signaling pathways regulating CD44 expression independent of IL-10-mediated effects, we used IL-10-refractory THP-1 cells as a model system. We show that CD44 expression induced by the LPS-mediated pathway predominantly involved JNK activation through results derived from transfection of THP-1 cells with a dominant-negative mutant of stress-activated protein/Erk kinase 1 (SEK1), and by exposure of cells to dexamethasone, an inhibitor of JNK activity. Both treatments prevented CD44 induction in LPS-stimulated, but not in TNF- stimulated, THP-1 cells. Furthermore, we show that CD44 induction may involve JNK-dependent Egr-1 activation in LPS-stimulated monocytic cells. Taken together, these results suggest a predominant role of JNK in LPS-induced CD44 expression in monocytic cells.

55. DINNES, Donna M.

EFFECT OF MATERIAL SURFACE CHEMISTRY ON THE PHOSPHOLIPASE A₂ PATHWAY IN HUMAN MACROPHAGES

By : Dinnes, Donna M. and Labow, Rosalind S.

During the inflammatory response to implanted medical devices, the phospholipase A₂ (PLA₂) pathway may be activated resulting in the release of arachidonic acid (AA). In this study, U937 cells, previously validated as a model for MDM, were differentiated, resuspended and reseeded onto ¹⁴C-labelled polycarbonate-based polyurethane (PCNU) coated glass slips. PLA₂ inhibitors were added to media 1 h post-cell adherence and incubated for 48 h. Biodegradation was assessed by measuring the radiolabel release into the cell supernatant. Bromophenacyl bromide, arsitocholic acid, palmityl trifluoromethyl ketone (PMK) and quinacrine were assessed with PMK yielding the greatest inhibition of radiolabel release (>50%) with negligible effect on viability. AA release was measured by adding ³H-AA to differentiating U937s. The cells were resuspended and reseeded on polystyrene (PS) and a PCNU not labelled with ¹⁴C. The cells adhered to the two surfaces and supernatants were assessed for the rate of radiolabel release. Significantly more ³H-AA was released from the PCNU-adherent U937s than the control surface PS at 10 min. These results suggest that surface chemistry affects the activation of the PLA₂ pathway in adherent U937s which may lead to biodegradation.

54. POON, Stephanie

IDENTIFICATION AND DETERMINATION OF THE EXPRESSION OF NOVEL LMNA MUTATIONS IN DILATED CARDIOMYOPATHY

By : Poon, Stephanie, Bolongo, Pierrette, Frédérique Tesson

Dilated cardiomyopathy (DCM) is a cardiac muscle disorder characterized by ventricular dilatation and impaired systolic function leading to congestive heart failure and sudden death. LMNA, one of nine autosomal genes implicated in this disease, encodes for two alternatively spliced nuclear intermediate filament proteins, lamins A and C. In order to identify novel mutations in the LMNA gene that may predispose individuals to DCM, blood was obtained from DCM patients and their first-degree relatives with written and informed consent. DNA was extracted from whole blood and the LMNA gene was analyzed by polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP), denaturing high performance liquid chromatography (DHPLC) and DNA sequencing.

Two novel mutations in highly conserved regions of the LMNA gene were identified, resulting in Asp192Gly and Arg541Ser substitutions. Analysis of wild type and mutant lamin A/C expression levels in affected heart tissue samples by quantitative RT-PCR using the LightCycler system may aid in providing more insight into the pathogenesis of dilated cardiomyopathy.

56. LESAGE, Jacinthe

IDENTIFICATION OF GENES ON CHROMOSOME 3p RESPONSIBLE FOR BICUSPID AORTIC VALVE

By : Le Sage, Jacinthe and Bulman, Dennis

In the human disease bicuspid aortic valve (BAV), the normally tricuspid aortic heart valve has only two leaflets. BAV can result from either a defect in valvulogenesis in the embryo, or from a fusion of two cusps in a normal adult valve. BAV shows familial clustering in about one third of cases. Our hypothesis is that congenital BAV is a genetic developmental disorder inherited as an autosomal dominant trait with variable penetrance. Our objective is to examine a region on chromosome 3p on which DNA marker D3S1259 has been found to possibly be linked to the disease in 8 families. In addition, a gene deletion syndrome in the same region is associated with congenital heart disease. Our genetic linkage study between microsatellite DNA markers and the disease's locus with genomic DNA from 14 unrelated families aims to find one or more candidate genes containing mutations. 2-point linkage analysis was performed with the MLINK program using PCR amplified alleles. Preliminary linkage results have shown that 7 families have attained their maximum LOD scores for at least one DNA marker within the 3p candidate region. Our results also favor genetic heterogeneity for BAV. We are currently recruiting additional families as well as performing high density screening and multi-point analysis to further refine our locus.

57. AMIN, Shahreen

THE ROLE OF PROTEIN TYROSINE PHOSPHATASE SHP-1 IN CELL TRANSFORMATION

By : Amin, Shahreen, Graziani-Bowering, Gina and Dr. Maya Kozlowski

The SH2-domain containing tyrosine phosphatase, SHP-1 has been implicated in the negative regulation of mitogenic cascades activated by the engagement of cytokine/antigen/growth factor receptors. The chromosomal region to which SHP-1 has been mapped frequently undergoes rearrangements in pediatric acute lymphoblastic leukemia (ALL), suggesting that SHP-1 is involved in the pathogenesis of at least some types of leukemia. The role of SHP-1 in the suppression of cell growth is also demonstrated by enhanced development of adenocarcinomas and lymphomas in mice heterozygous for SHP-1 gene mutations. To determine whether a deregulation of SHP-1 occurs in, and contributes to, cell transformation, the expression of SHP-1 gene in a panel of normal and tumor samples from humans have been analyzed. The results of these preliminary experiments indicate diminished expression of SHP-1 protein in leukemia cells. I will expand these studies by examining the expression of SHP-1 transcripts by RT-PCR and Northern analyses. To understand the aberrant expression of SHP-1 in leukemic cells, I will investigate the regulation of the SHP-1 gene. For this, I will examine the hematopoietic cell-specific promoter P2 and its deletion mutants as well as the non-hematopoietic cell-specific P1 promoter along with its deletion mutants, for their ability to drive the expression of the luciferase reporter gene in Jurkat T cells and MCF7 breast carcinoma cells, respectively. I will identify critical regions in both SHP-1 promoters required for the expression of the SHP-1 gene in normal cells and correlate the abnormal expression with their tumor counterparts. I will also identify transcription factors and signaling proteins involved in SHP-1 gene regulation in both hematopoietic and non-hematopoietic cells. Anti-oncogenic properties of SHP-1 will be further supported by the analyses of the signalosome and F-actin cytoskeletal organization in normal and hyper proliferating SHP-1-deficient *me/me* cells by confocal microscopy imaging.

59. VEERESWARAN, Vasanthi

HDL STRUCTURE AND INTRACELLULAR METABOLISM

By : Veereswaran, Vasanthi and Sparks, Daniel L.

High density lipoproteins (HDL) mediate the transport of cholesterol and other lipid soluble molecules between different tissues in the body. Cells take up these lipoproteins through a process called endocytosis, in which localized regions of the plasma membrane invaginate and pinch off to form endocytic vesicles. Some of the endocytosed molecules are transported to lysosomes, where they are degraded, and others are transported between the apical and basolateral surfaces of the cell, through a process called transcytosis. Epithelial cells generally endocytose at their apical surface and secrete the transcytosed molecule from the basolateral membrane. In the present project, we have evaluated the effect of HDL structure on the intracellular metabolism of native HDL, apoA-I and reconstituted HDL (rHDL) in polarized human proximal tubule epithelial cells (HK). Apical cell association of native HDL with HK cells was considerably less than that observed with apoA-I or rHDL. Both the transcytosis and degradation of native HDL was almost 2-fold greater than that observed for apoA-I and rHDLs. ApoA-I was degraded to a lesser extent than all other ligands. Addition of HDL lipids or pure lipids directly impaired transcytosis and stimulated the degradation of apoA-I. The results show that HDL composition and structure affect the intracellular metabolism of this lipoprotein.

58. GAUTHIER, André

SURFACE-ANCHORED CHOLESTEROL ESTER TRANSFER PROTEIN (CETP) FUNCTIONS AS AN HDL RECEPTOR AND MEDIATES SELECTIVE UPTAKE OF CHOLESTERYL ESTERS FROM HDL

By : André Gauthier, Xiaohui Zha, Ross Milne & Ruth McPherson

Cholesteryl ester transfer protein (CETP) is an intravascular neutral lipid transfer protein, predominantly expressed in liver and adipose tissue, and has an established role in human lipoprotein metabolism.

We show that a significant fraction of CETP is anchored to the plasma membrane surface, where it functions as a novel HDL receptor. CETP expression in Chinese hamster ovary cells results in specific HDL binding to the cell surface and markedly enhances selective uptake of HDL derived cholesteryl esters. Scavenger receptor B-I is not required for CETP mediated selective uptake. Studies with an inactive mutant CETP also demonstrate that CETP-mediated selective uptake occurs independently of neutral lipid transfer activity (CETP_{KD}). Furthermore, liver-specific expression of CETP_{KD} in C57BL6 mice results in decreased plasma cholesterol levels and a shift in the HDL subpopulation from the cholesteryl ester rich HDL₁ to the cholesteryl ester poor HDL₃. These data unequivocally support a novel role for CETP in the selective uptake of HDL-derived cholesteryl ester in the liver and adipose tissue.

60. LIM, Wilfred

DISTINCT ROLE OF P38 AND C-JUN-N-TERMINAL KINASES IN IL-10-DEPENDENT AND IL-10-INDEPENDENT REGULATION OF THE COSTIMULATORY MOLECULE B7.2 IN LIPOPOLYSACCHARIDE-STIMULATED HUMAN MONOCYTIC CELLS

By : Lim, Wilfred, Ma, Wei, Gee, Katrina, Aucoin, Susan, Nandan, Devki, Diaz-Mitoma, Francisco, Kozlowski, Maya and Kumar, Ashok (supervisor)

The costimulatory molecule B7.2 (CD86) plays a vital role in immune activation and development of T helper responses. The molecular mechanisms by which B7.2 expression is regulated are not understood. We investigated the role of mitogen-activated protein (MAP) kinases in the regulation of B7.2 expression in lipopolysaccharide (LPS) stimulated human monocytic cells. LPS stimulation of human monocytes resulted in the down-regulation of B7.2 expression that could be abrogated by anti-IL-10 antibodies. Furthermore, SB202190, a specific inhibitor of p38 MAP kinases, inhibited LPS-induced IL-10 production and reversed B7.2 down-regulation, suggesting that LPS-induced B7.2 down-regulation may be mediated, at least in part, via regulation of IL-10 production by p38 MAP kinase. In contrast to human monocytes, THP-1 cells which are refractory to the inhibitory effects of IL-10, LPS stimulation enhanced B7.2 expression. This IL-10 independent B7.2 induction was not influenced by specific inhibitors of either p38 or p42/44 MAP kinases. To ascertain the role of the JNK MAP kinases, dexamethasone, an inhibitor of JNK activation, was used which inhibited LPS-induced B7.2 expression. Transfection of THP-1 cells with a plasmid expressing a dominant-negative SEK kinase significantly reduced LPS-induced B7.2 expression, thus confirming the involvement of JNK. To study the signaling events downstream of JNK activation, we show that dexamethasone did not inhibit LPS-induced NFκB activation in THP-1 cells, suggesting that JNK may not be involved in NFκB activation leading to B7.2 expression. Taken together, our results reveal the distinct involvement of p38 in IL-10 dependent, and JNK in IL-10-independent regulation of B7.2 expression in LPS-stimulated monocytic cells.

62. CUI, Bo

Not available at time of printing

61. RAHIMI, Rahim

THE ROLE OF P42/44 EXTRACELLULAR SIGNAL-REGULATED PROTEIN KINASE (ERK) IN INTERLEUKIN-10-MEDIATED CD14 INDUCTION IN NORMAL HUMAN MONOCYTES

by Rahimi, Ali A. R., Lim, Wilfred, Gee, Katrina, and Kumar, Ashok (supervisor)

Keywords: MAPKs; IL-10; p42/44-ERK MAPK; HL-60; normal human monocytes

Abstract: IL-10, an immunoregulatory cytokine with inhibitory effects on inflammatory and cell-mediated immune responses, has enormous potential for the treatment of inflammatory and autoimmune disorders. Although IL-10 generally exhibits inhibitory effects, it has also been shown to facilitate the induction of Th2 cell types and exert stimulatory effects on B cell growth and differentiation. In this study, we show that IL-10 also enhances the expression of CD14 on normal human monocytes. The molecular mechanism by which IL-10 mediates its biological effects are not well understood. Therefore, we have investigated the role of intracellular signaling molecules especially that of the mitogen-activated protein kinases (MAPKs) in IL-10-induced CD14 upregulation in normal human monocytes. MAPKs play a crucial role in signal transduction that are activated by phosphorylation in response to a variety of mitogenic signals. However, the role of MAPKs in IL-10-mediated biological effects is not known. We provide an evidence that IL-10 can selectively induce the phosphorylation of p42/44 extracellular signal-regulated protein kinase (ERK) MAPK in highly purified normal human monocytes as well as in human monocytic cell line HL-60. IL-10-induced phosphorylation of p42/44 ERKs increased in intensity with time with a maximum increase in phosphorylation at 60 minutes post-stimulation in HL-60 cells. In contrast, IL-10 did not induce the phosphorylation of either p38 mitogen-activated protein kinase (p38) or c-Jun NH2-terminal kinase (JNK) in normal human monocytes and HL-60 at any concentration even up to 4 hrs of stimulation. PD98059, a specific inhibitor of ERK MAPK, inhibited the IL-10-induced phosphorylation in a dose dependent manner in both normal human monocytes as well as in HL-60 cells. In addition, PD98059 inhibited IL-10-mediated induction of CD14 expression on normal human monocytes in a dose dependent manner. These results suggest that IL-10 selectively induces the phosphorylation of p42/44 ERK MAPK which plays a critical role in IL-10-mediated CD14 induction in normal human monocytes.