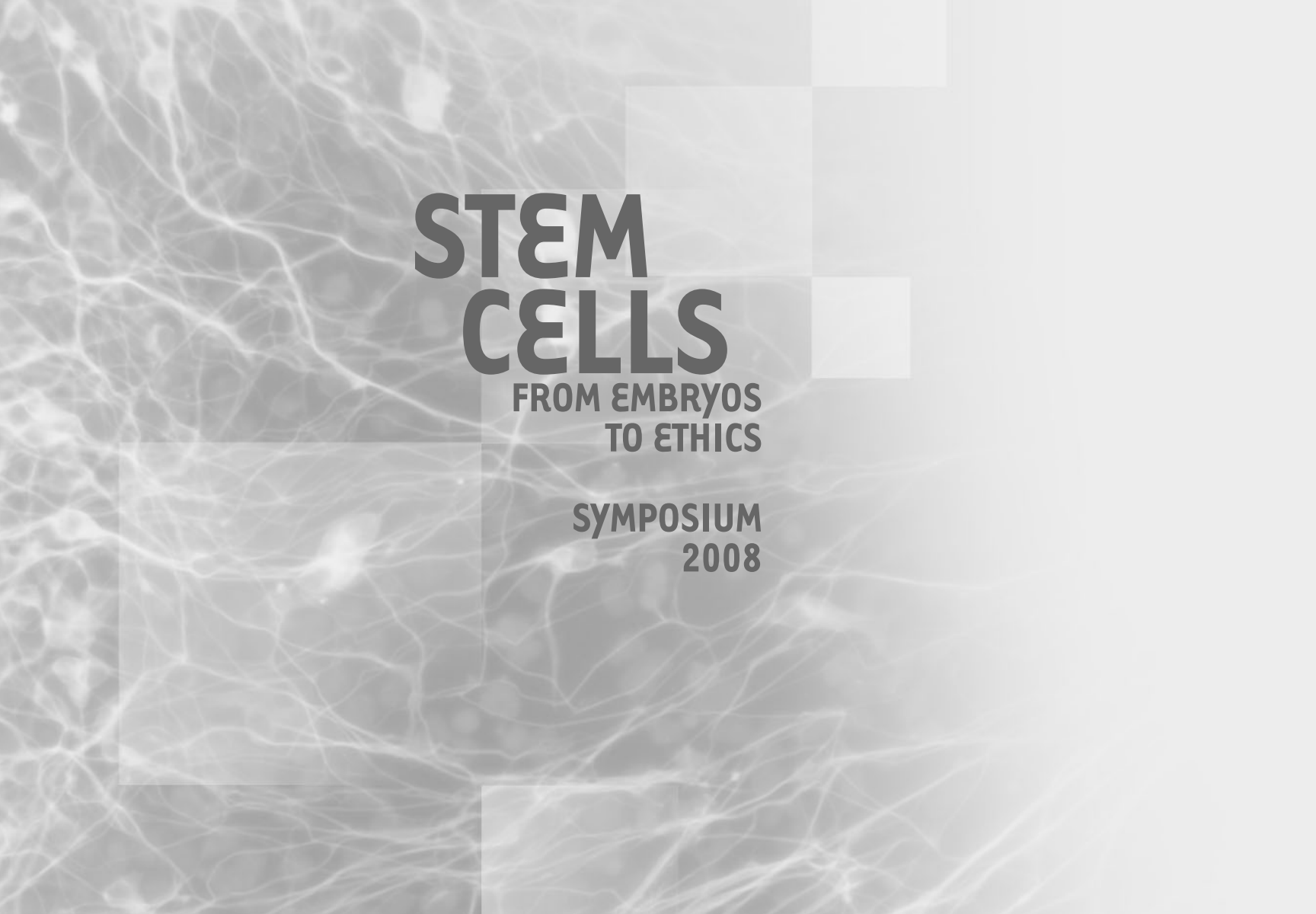


CANADIAN
STUDENT

Health Research Forum

STEM CELLS

FROM EMBRYOS
TO ETHICS
JUNE 3 – 5, 2008

The background of the image is a grayscale micrograph showing a complex network of interconnected, glowing fibers, likely representing a neural network or a similar biological structure. Overlaid on this is a grid of semi-transparent squares that tapers off towards the right side of the image. The text is centered in the upper-middle portion of the image.

STEM CELLS

**FROM EMBRYOS
TO ETHICS**

**SYMPOSIUM
2008**



Dean's Welcome

The Canadian Student Health Research Forum is an outstanding opportunity to learn science, but even more importantly to network, learn from peers, develop new friendships and collaborations and expand horizons.

The potential of stem cell research to truly be translated for new treatment as regenerative medicine is becoming a reality.

The work in this field is more exciting than ever before and, surprisingly, more relevant than expected to a variety of different human conditions. We have an outstanding group of experts who will serve as inspiration, mentors and provide context and stimulation. The topics will provide insight into the exciting, new developments in stem cell research and the current state of activity to translate this knowledge to decrease disability and death from many diseases and improve overall quality of life.

We expect a great contingent of visitors from across Canada and stiff competition in the CIHR National Health Research Poster Competition.

Winnipeg is a wonderful environment in which to live, learn and network. Along with your new ideas, enjoy your new experiences and your leisure time in Winnipeg.

J. Dean Sandham MD FRCPC FACP
Dean, Faculty of Medicine

PROGRAMME

**Thursday
June 5/08**

**Theatre 'A'
Basic Medical
Sciences Building
730 William Ave**

8:00am Registration & Refreshments (Concourse)

9:00am Welcome and Opening Remarks (Theatre A)

Dr. J. Dean Sandham

Dean, Faculty of Medicine, University of Manitoba

Dr. Joanne Kesselman

Vice-President (Research), University of Manitoba

Chair's Overview

Dr. Geoffrey Hicks

9:15am **Dr. Ron Worton**

Ottawa Health Research Institute and Stem Cell Network

'Stem Cells: Their Discovery, Healing Power and Social Impact'

Introduced by Dr. Patrick Choy

10:00am Coffee Break (Mezzanine)

10:30am **Dr. Geoffrey Hicks**

Canada Research Chair in Functional Genomics,

University of Manitoba

'Genetic Modelling of Disease in Embryonic Stem Cells'

11:15am **Dr. Lin He**
Assistant Professor of Cell and Developmental Biology,
Berkeley Stem Cell Centre, University of California at Berkeley
'miRNAs in Cancer Biology: Small RNAs with a Big Impact'
Introduced by Dr. Geoffrey Hicks

12:30pm Lunch and Poster Session (Brodie Atrium)

12:30pm Presentations of Recognition (Brodie Atrium)

- Graduate Students Assoc. Award for Distinction in Mentorship
- Heart and Stroke Foundation
- Dr. Kenneth Hughes Young Investigator Award
- Dr. Aubie Angel Young Investigator Award
- Faculty Recognition for Outstanding Contribution to Research

2:00pm Chair's Overview (Theatre A)
Dr. Grant Hatch

Dr. André Terzic
Professor of Medicine and Molecular Pharmacology and Experimental
Therapeutics, Mayo Medical School
'The Regenerative Medicine Paradigm in Heart Repair'
Introduced by Dr. Grant Pierce

3:15pm Coffee Break (Mezzanine)

3:15pm **Dr. Timothy Caulfield**
Professor, Public Health Sciences and Faculty of Law,
University of Alberta
'Stem Cell Policy: The Next Generation'
Introduced by Dr. Grant Hatch

4:00pm Round Table Discussion

4:30pm Awards Ceremony

5:00pm Reception (Concourse)

AWARDS

**Apotex Fermentation
Inc. Award**

– Molecular Biology

**Canadian Institutes of
Health Research**

– Poster Presentations

**Children's Hospital
Foundation Award**

– Child Health

Dean of Dentistry

– Poster Presentations

**Dean of Graduate
Studies**

– Poster Presentations

Dean of Medicine

– Poster Presentations

**E. L. Drewry Memorial
Award**

– Health Research

**Graduate Students
Association**

– Poster Presentation

**Health Sciences Centre
Foundation Inc. Award**

– Neurobiology

**Hoffman-La Roche
Ltd. Award**

– Population-based
Research

**Manitoba Health
Research Council**

– Postdoctoral Fellow
Poster Presentation

**Manitoba Medical
Service Foundation Inc.**

– Poster Presentations
– Awards for Excellence
in Research

**Merck Frosst Canada
Inc. Award**

– Cell Biology

**S. W. Prowse Memorial
Award**

– Clinical Research

**St. Boniface General
Hospital Research
Foundation Inc. Award**

– Cardiovascular Biology

AWARDS COMMITTEES

Advisory Committee

Dr. Patrick Choy
Dr. Michael Czubryt
Dr. Grant Hatch
Dr. Geoffrey Hicks
Dr. Thomas Klönisch
Mr. Alan McGreevy
Dr. Yvonne Myal
Ms. Ifeoma Okwor
Dr. Grant Pierce
Dr. Louise Simard
Dr. Donald Smyth
Dr. Karl Tibelius

Major Awards Committee

Dr. Yvonne Myal, Chair
Dr. Klaus Wrogemann
Dr. Robert Tate
Dr. Jeff Wigle
Dr. Kevin Coombs
Dr. Sam Kung
Dr. Elissavet Kardami
Dr. David Merz

Manitoba Poster Competition

Dr. Michael Czubryt,
Chair
Dr. Rakesh Arora
Dr. Rajinder Bhullar
Dr. Kevin Coombs
Dr. Hao Ding
Dr. Malcolm Doupe
Dr. Brenda Elias
Dr. Darren Freed
Dr. Sabine Hombach
Dr. Jiming Kong
Dr. Sam Kung
Dr. Tooru Mizuno
Dr. Mohammed
Moghadasian
Dr. James Nagy
Dr. Karmin O
Dr. Steven Pind
Dr. Julia Rempel
Dr. Barb Shay
Dr. Bob Tate
Dr. Carla Taylor
Dr. Jude Uzonna

CIHR National Poster Competition

Dr. Grant Hatch, Chair
Dr. Bernie Bressler
Dr. Anne-Cécile Desfaits
Dr. Janice Dodd
Dr. Mary-Lynn Duckworth
Dr. Thomas Klönisch
Dr. Lisa Lix
Dr. Brian MacNeil
Dr. Verena Menec
Dr. Karmin O
Dr. Jacquie Ripat
Dr. Louise Simard
Dr. Abdelilah Soussi
Gounni
Dr. Robert Tate
Dr. Karl Tibelius
Dr. Jeffrey Wigle
Dr. Elizabeth Worobec
Dr. Ronald Worton
Dr. Peter Zahradka
Dr. Teresa Zelinski

GUEST SPEAKERS

2008

Our outstanding speakers are recognized internationally for their contribution to the field and ability to communicate with a broad-spectrum audience.



**Timothy
Caulfield LLM**

Director, Health Law
Institute
– University of Alberta

Timothy Caulfield has been Research Director of the Health Law Institute at the University of Alberta, since 1993. In 2001 he received a Canada Research Chair in Health Law and Policy. He is also a Professor in the Faculty of Law and the School of Public Health. Over the past several years, he has been involved in a variety of interdisciplinary research endeavours that have allowed him to publish over one hundred and fifty articles and book chapters.

He is a Senior Health Scholar with the Alberta Heritage Foundation for Medical Research, the Principal Investigator for Genome Canada project on the regulation of genomic technologies, the theme leader in the Stem Cell Network and the Advanced Foods and Materials Network (National Centres of Excellence) and has several projects funded by the Canadian Institutes of Health Research. Professor Caulfield is and has been involved with a number of national policy and research ethics committees, including Canadian Biotechnology Advisory Committee, Genome Canada's Science Advisory Committee and the Royal Society of Canada's Expert Panel on the Future of Food Biotechnology (2001). He is a member of the Royal Society of Canada and the Canadian Academy of Health Sciences.

Stem Cell Policy: The Next Generation

Few areas of research have created as much social controversy as stem cell research. Most developed nations now have formal policies touching on the relevant ethics, with some countries banning a variety of research activities. Much of this law is a direct result of the concerns associated with the moral of the embryo. Over the past few years, a variety of new issues have emerged. The science is moving beyond the derivation of new embryonic stem cell lines and with this move comes a variety of challenges. This talk will highlight and critique a number of the most recent social controversies, including: the marketing of questionable stem cell therapies; the regulatory and social response to new methods for creating stem cells (e.g. 'cybrids and iPS cells'); and the commercialization and patenting of stem cell technologies.



Lin He PhD

Assistant Professor
of Cell and
Developmental Biology
– Berkeley Stem Cell
Centre, University of
California at Berkeley

Dr. He obtained her undergraduate degree at the Tsinghua University in China, and received her doctoral degree at the Stanford Medical School with Dr. Greg Barsh. Her PhD work was on the functional role of attractin, and its genetic and biochemical studies. The work has resulted in more than ten high quality publications, including first authorship papers in Nature, Nature Genetics, and Science.

After her PhD work, she went to do her postdoctoral fellowship with Dr. Greg Hannon at the Cold Spring Harbor Laboratories. Her studies on the microRNA polycistron as a potential human oncogene and as a component of the p53 tumour suppressor network also resulted in the publication of several ground-breaking papers in Nature, again with Dr. He as the first author on all these papers. Earlier this year, Dr. He was appointed Assistant Professor and Principal Investigator in the Department of Molecular and Cellular Biology, University of California at Berkeley. Her research program at UC Berkeley is to explore the roles of microRNAs in the oncogenic and tumor suppressor

pathways, and the work is supported by grants from the National Cancer Institute.

Dr. He is holding two US patents, the first one is on methods for cancer diagnosis and treatment, and the other one on TP53 function biomarker and miRNA-based therapeutics. In the last several years, Dr. He was invited to speak at many prestigious meetings, notably the East-West Alliance Meeting at Cambridge, UK, the American Association of Cancer Research (AACR) in San Diego, and the Keystone symposium on microRNAs and cancer in Keystone.

miRNAs in Cancer Biology: Small RNAs with a Big Impact

MicroRNAs are a family of small, non-coding RNAs that negatively regulate gene expression in a sequence specific manner. Recent studies suggest that microRNA may play an important role in the oncogenic and tumor suppressor pathways. We have previously identified a microRNA polycistron, mir17-92, as a potential oncogene for human B-cell lymphomas. To identify miRNA components in the tumor suppressor pathways, we compared miRNA expression profiles of wild-type and p53-deficient cells. This analysis revealed a family of miRNAs, mir-34, whose expression reflected p53 status. Precursors of all three miR-34 family miRNAs are direct transcriptional targets of p53, whose induction by DNA damage and oncogenic stress depends on p53 both in vitro and in vivo. Ectopic expression of miR-34 induces cell cycle arrest in both primary and tumor-derived cell lines, consistent with the observed ability of miR-34 to down-regulate a program of genes promoting cell cycle progression. The p53 network suppresses tumor formation through coordinated activation of multiple transcriptional targets, and miR-34 may act in concert with other effectors to inhibit inappropriate cell proliferation.



Geoffrey G. Hicks received his undergraduate degree in Biochemistry from the University of Manitoba. His PhD thesis focused on p53 as a tumor suppressor where he studied in the lab of Dr. Michael Mowat at the Manitoba Institute of Cell Biology. Pursuing his interest in cancer biology, Dr. Hicks held National Cancer Institute of Canada postdoctoral fellowships at the Massachusetts Institute of Technology and the Vanderbilt University School of Medicine.

Geoff Hicks PhD

Director, Mammalian
Functional Genomics
Centre
Director, Genetic
Modeling Centre
Senior Investigator,
Manitoba Institute of
Cell Biology

With Dr. H. Earl Ruley he developed a novel gene trapping technology as a genetic strategy to identify recessive gene in mammalian cells. This high-throughput sequence-based screen is currently being applied to develop a library of knock-out mutations in mouse embryonic stem cells, and is an international resource provided by Dr. Hicks' Mammalian Functional Genomics Centre. Dr. Hicks is a founding member of the International Mouse Mutagenesis Consortium, the International Gene Trap Consortium, the Federation of International Mouse Resources and the International Knock-out Mouse Project. Most recently, he has established the Canadian Mouse Consortium, which

serves to integrate all major Canadian transgenic mouse platforms and services. Dr. Hicks' research program focuses on the functional analysis of genetic determinants of cancer and leukemia. His research team is currently examining the related RNA binding proteins, TLS and EWS, to identify the transforming potential of these genes in acute myelogenous leukemia and Ewing sarcoma, respectively.

Dr. Geoff Hicks is a Canada Research Chair in Functional Genomics and is the Director of the Mammalian Functional Genomics Centre, a centre in the Manitoba Institute of Cell Biology which is a joint

institute between CancerCare Manitoba and the University of Manitoba and the Genetics Modeling Centre in the University of Manitoba. He is currently leading the North American Conditional Mouse Mutagenesis Project (NorCOMM), the Canadian component of The International Knockout Mouse Project. NorCOMM is supported by Genome Prairie with funding of \$13.5 million from Genome Canada and other partners. Dr. Hicks and his team are working with other scientists from around the world to create knockout mice lines for each of the approximately 20,000 mouse genes.

Mouse Embryonic Stem Cells: Functional Analysis of Genetic Determinants of Human Disease

NorCOMM (North American Conditional Mouse Mutagenesis project) is a large-scale research initiative to develop and distribute a resource of mouse embryonic stem (ES) cell lines carrying single conditional knockout mutations across the mouse genome. We are creating a publicly accessible library of ES cells suitable for drug discovery, target discovery and validation, and investigating mouse models of human diseases. Development of the knockout mouse resource itself is the major activity using a combination of high throughput random gene trap mutagenesis and sys-

tematic high-throughput gene targeting. NorCOMM vectors are designed to generate expression and functional information for the gene targeted in each mutant ES clone. To ensure the widest utility of the NorCOMM mouse knockout resource, we have developed a universal docking site strategy with a tool box of exchangeable cassettes. This design will ensure that each mutation can be used to create a conditional knockout allele and a variety of functional replacement alleles. Availability of the genetically altered ES cell resource to be generated in this project will significantly impact biomedical disease-focus research programs broadly and thereby increase the rate at which new medical discoveries are moved into health care delivery.



**André
Terzic MD, PhD**

Professor of Cardiovascular Diseases
– Mayo Medical School

Dr. Andre Terzic holds the Marriott Endowed Professorship of Cardiovascular Research at Mayo Clinic, where he is Professor of Medicine and Pharmacology, Medical Genetics, Director of the Marriott Heart Disease Research Program, and Co-Director of the Mayo Clinic Center for Individualized Medicine. Dr. Terzic also serves as the Mayo Clinic Associate Director for Research, Discovery Science and Experimental Medicine.

By integrating advanced technology with a focus on clinical problems addressed at a fundamental level, Dr. Terzic has pioneered pathogenomic research of maladaptation in heart disease, and the application of cardioprotective and cardioregenerative therapeutic modalities. He has authored over 200 scientific manuscripts.

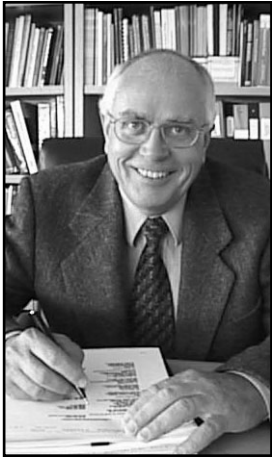
Dr. Terzic is the recipient of numerous national and international awards including the Medal of Merit from the International Society for Heart Research, the Leon I. Goldberg Award from the American Society for Clinical Pharmacology and Therapeutics, the Klaus Unna Award from the University of Illinois, the Established Investigatorship from the American Heart

Association, and the Excellence in Teaching Award, Outstanding Cardiovascular Research Mentor Award and Outstanding Investigator Award from the Mayo Clinic College of Medicine. He is the Past President of the American Society for Clinical Pharmacology and Therapeutics.

The Regenerative Medicine Paradigm in Heart Repair

Beyond the palliative reach of today, medical therapies of tomorrow aim to treat the root cause of chronic degenerative diseases. Therapeutic repair encompasses the converging triad of rejuvenation, replacement or regeneration strategies that rely on self-healing processes, stem cell regeneration and/or organ trans-

plantation. Natural healing or rejuvenation exemplify inherent, baseline repair secured by tissue self-renewal and de novo cell biogenesis, particularly effective in organs with a high endogenous reparative capacity. Transplant medicine exploits a replacement strategy as a valuable option to recycle used parts, and restore failing organ function by means of exogenous substitutes – it is however limited by donor shortage. Stem cell-based regeneration offers the next frontier of medical therapy through delivery of essentially unlimited pools of autologous or allogeneic, naive or modified, progenitor cells to achieve structural and functional repair. Translation into clinical applications requires the establishment of a regenerative medicine community of practice capable to bridge discovery with personalized treatment solutions. Indeed, this multidisciplinary specialized workforce will be capable to integrate the new science of embryology, immunology and stem cell biology into bioinformatics and network medicine platforms, ensuring implementation of therapeutic repair strategies into individualized disease management algorithms.



Dr. Worton is retired after a 37 year career as a medical research scientist and scientific leader. Following degrees in Physics at the University of Manitoba and a PhD in Medical Biophysics at the University of Toronto, Dr. Worton spent two years as a research fellow at Yale. In 1971, he moved to the Department of Genetics at the Hospital for Sick Children in Toronto, where he spent 25 years as a scientist, the last 11 years as Geneticist-in-Chief, and Professor in Molecular and Medical Genetics at the University of Toronto.

**Ronald G.
Worton PhD**

Past CEO and Scientific
Director
– Ottawa Health
Research Institute and
Stem Cell Network

In 1996, Dr. Worton moved to Ottawa as Scientific Director of a new Research Institute at the former Ottawa General Hospital. Following the merger of this Institute with the Loeb Health Research Institute to create the Ottawa Health Research Institute in 2001, he became its first CEO and Scientific Director, retiring in April, 2007. During that 6 year period he was also VP Research of The Ottawa Hospital, and Professor of Medicine at the University of Ottawa.

Dr. Worton's research has been on the genetics of human disease, including identification of the gene responsible for Duchenne muscular dystrophy and elucidation of the mechanism of mutation in patients.

His work has resulted in more than 120 publications in leading journals and books and over 150 invited presentations at international meetings.

Dr. Worton's national and international leadership role has included 12 years as Associate Director of the Canadian Genetic Diseases Network, six years as Head of the Canadian Genome Analysis and Technology Program and four years as Founding Scientific Director of Canada's Stem Cell Network.

He has also served on the Boards of the Ottawa Health Research Institute, the Ottawa Hospital Foundation, the Canadian College of Medical Geneticists, the Muscular Dystrophy Association of Canada and the

American Society of Human Genetics, his last year as President of the Society. He also served on the Interim Governing Council that created the Canadian Institutes of Health Research. Currently he is Chair of the Board of Research Canada and Vice Chair of the Board of the Ontario Research Fund

Dr. Worton is the recipient of several national and international awards including the prestigious Gairdner Foundation International Award. He holds honorary degrees from the University of Manitoba and Université Catholique de Louvain and is an honorary Fellow of the Royal College of Physicians and Surgeons of Canada. He is a Fellow of the Royal Society of Canada and a member of the Order of Canada.

Stem Cells: Their Discovery, Healing Power and Social Impact

Stem cells have been known in the scientific literature for over 40 years, but only in the last 10 years have they burst into the public consciousness. The first stem cells were discovered in mouse bone marrow in the early 1960s and found to be primitive unspecialized cells that were capable of giving rise to the many different types of specialized cells found in blood. Later, stem cells were discovered in other tissues such as brain and muscle, and found to give rise to special-

ized cells in those tissues. In 1980 mouse embryos at the blastula stage were found to contain stem cells that were capable of giving rise to all the cells of the adult mouse. It was not until 1998 when human embryonic stem cells were reported with similar properties that scientists and the public began to think seriously about the use of such cells for repair or regeneration of tissues destroyed by trauma or disease. Stem cell research came of age, and with it came the controversy over the use of stem cells from human embryos. Some of the social concern is very real, and touches on both ethical principles and religious beliefs, but much of the debate is also due to ignorance of the facts, or on distortion of the facts to serve a particular purpose. The recent discovery that ordinary skin cells can be re-programmed to behave like stem cells may prove to be the best route to the new field of Regenerative Medicine based on stem cell therapeutics, and at the same time remove any concern about the use of embryonic stem cells for therapeutic applications.



**POSTER
PRESENTATIONS**

2008

Stress Echocardiography: Utility of Tissue Doppler Imaging for Early Detection of Cardiac Allograft Vasculopathy

Roien Ahmadi¹, Thang Nguyen², Tielan Fang¹, Matthew Lytwyn¹, Ivan Barac³, Farrukh Hussain³, Shelley Zieroth³ and Davinder S. Jassal^{1,3,4}

¹Institute of Cardiovascular Sciences, St. Boniface Research Centre, University of Manitoba,

²Department of Internal Medicine, St. Boniface General Hospital, ³Section of Cardiology, Department of Cardiac Sciences, University of Manitoba,

⁴Department of Radiology, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction: The most significant predictor of long-term survival in heart transplant patients is the development of accelerated cardiac allograft vasculopathy (CAV). Several studies have demonstrated the usefulness of dobutamine stress echocardiography (DSE) for screening CAV, by detecting regional wall motion abnormalities. Tissue Doppler imaging (TDI) derived indices during DSE allows for the early detection of ischemic heart disease (IHD), prior to a reduction in regional or global systolic function. These indices include a reduction in annular systolic velocity (S'), a decrease in early diastolic annular velocity (E') and prolongation of time to E' . In cardiac transplant patients, the application of these TDI abnormalities as an early noninvasive marker of CAV remains unknown.

Objective: To evaluate if Tissue Doppler imaging (TDI)-derived indices of systolic and diastolic function during dobutamine stress echocardiography (DSE) can predict cardiac allograft vasculopathy (CAV) in cardiac transplant patients.

Methods: A retrospective evaluation of 30 patients (mean age 54 ± 11 years) who had both DSE and coronary angiography was performed. The control group consisted of 15 patients referred to rule out coronary artery disease while the study group consisted of 15 cardiac transplant patients referred for routine annual follow-up. During each stage of DSE, tissue Doppler measurements of systolic (S'), early (E'), and late (A') diastolic velocities of the lateral annulus were taken.

Results: All 30 patients had normal DSE based on systolic regional function and normal coronary angiograms with no stenosis $>50\%$. There was no difference in hemodynamic parameters during the DSE at baseline and with stress. Despite normal coronaries, cardiac transplant patients demonstrated lower S' , E' , and A' velocities at peak stress compared to the control patients.

Conclusion: Dobutamine-induced augmentation of TDI velocities of the lateral annulus, normally observed in the absence of ischemia in non-transplanted adults, is reduced in cardiac transplant recipients.

4-Hydroxy-2-Nonenal Modifies Neurofilament Protein, Impairs Axon Outgrowth, Causes Accumulation of Mitochondria in Aberrant Axonal Structures and Mimics the Effect of Diabetes in Cultured Adult Sensory Neurons

Eli Akude, Elena Zhrebetskaya and Paul Fernyhough

Div. of
Neurodegenerative
Disorders, St. Boniface
Hospital Research
Centre, Dept. of
Pharmacology &
Therapeutics,
University of
Manitoba, Winnipeg,
Canada

Introduction: Protein adducts of the lipid peroxidation product 4-hydroxy-2-nonenal (4-HNE) have been associated with disease states involving oxidative stress. Modification of proteins by 4-HNE in peripheral nerve tissue occurs in diabetic sensory neuropathy, however, the target proteins and impact on nerve function are unknown. We, therefore, tested the ability of 4-HNE to induce amino acid adduct formation on axonal cytoskeletal proteins and determined if such modifications were associated with aberrant axon morphology, mitochondrial accumulation, and suboptimal axon outgrowth in cultured adult sensory neurons. The impact of type 1 diabetes on these processes was also investigated.

Methods: Adult rat dorsal root ganglion (DRG) sensory neurons were cultured in defined F12-media supplemented with neurotrophic factors (NTFs; 1 nM insulin, 1 ng/ml NGF, 10 ng/ml GDNF and 10 ng/ml NT-3) and treated with 4-HNE concentrations ranging from 1.0 μ M to 10 μ M. Cell survival, axonal morphology and level of axonal regeneration were assessed at 24 hours in culture. Western blot and immunofluorescent staining were utilized to detect protein adduct formation by 4-HNE (anti-4-HNE, Alexis Biochemicals) and phosphorylation levels of neurofilament H protein (antibody SMI31, Covance). Also, a mitochondrial specific dye (1.0 μ M Mitotracker red CMXRos; Invitrogen) was utilized to detect the presence of accumulation of actively respiring in the 4-HNE-induced aberrant morphological structures along the axons.

Results: 4-HNE induced formation of amino acid adducts on neurofilament H protein and impaired axon regeneration by approximately 50% (ED₅₀ 3 μ M) whilst having no effect on neuronal survival. 4-HNE initiated formation of aberrant axonal structures and caused the accumulation of mitochondria in these structures, which mimicked those seen in axons of neurons under diabetic conditions (in animal models and humans). The formation of protein adducts also led to diminished levels of phosphorylation of neurofilament H protein. Sensory neurons from 3 month streptozotocin-diabetic rats showed abnormal axonal swellings which were filled with 4-HNE protein adducts and exhibited impaired levels of axon outgrowth; control neurons exhibited negative staining for 4-HNE in axons.

Conclusion: This study demonstrates that 4-HNE induces amino acid adduct formation on neurofilament H protein, causes mitochondria to accumulate, and this modification is associated with impairment of axonal regeneration. The results show that 4-HNE might be an important link between oxidative stress-triggered lipid peroxidation and subsequent modification of key neuronal cytoskeletal and mitochondrial proteins in diabetic sensory neuropathy. It is proposed that 4-HNE mediates abnormalities in neurofilament and mitochondrial function, and possibly other cytoskeletal proteins, and may cause distal axon degeneration through suboptimal mitochondrial motility and localization.

A Large-scale Chemical-genetics Approach to Identify Novel Genes Involved in Protein Synthesis in Yeast

Md. Alamgir, Veronika Eroukova, Matthew Jessulat and Ashkan Golshani

Department of
Biology, Carleton
University, Ottawa,
Ontario

Chemical-genetics approaches have been recently employed in a variety of biological investigations such as functional genomics or identifying mechanism of drug action and target pathways. Here we employ a systematic large-scale chemical-genetic approach to identify novel genes involved in translation in the yeast, *Saccharomyces cerevisiae*.

In our experiment, we screened the entire non-essential yeast gene deletion array (yGDA) (~4700 strains) for sensitivity to different aminoglycoside antibiotics such as paromomycin along with the genetic interactions analysis to identify genes involved in translation. Paromomycin is known to affect translation by binding to the small ribosomal subunit of eukaryotic cells. With this approach, we have identified a previously uncharacterized ORF, *YBR261C*, along with numerous yeast genes such as *YEL009C*, *YER050C* and *YBL027W* which are already known to be involved in translation. It was found that *YBR261CΔ* was also sensitive to other translation-inhibitory drugs such as 3-amino-1,2,4-triazole (3-AT) and cycloheximide. The translation fidelity and efficiency of the yeast deletion strain for *YBR261CΔ* was examined using specialized β -galactosidase expression constructs. It was observed that when *YBR261C* was deleted both the efficiency and fidelity of translation were reduced. In addition, ribosome profile analyses demonstrated that the deletion of *YBR261C* altered the ribosomal 60S:40S subunit ratio in the mutant cells. We found that *YBR261C* genetically interact with 16 ribosomal and 7 amino acid or protein biosynthetic genes, resulting in reduced-growth phenotype. Further, overexpression of *YBR261C* compensated the hypersensitivity of 28 yeast gene deletion strains for known translation genes to different aminoglycosides. These observations suggest that *YBR261C* may have a role in yeast translation. On the whole, these results confirmed that integration of chemical and genetic interaction analysis can be useful to identify other novel genes involved in translation and the opportunity to develop new and improved anti-microbial drugs.

The Role of Cellular Sialidase in Ligand-Induced Toll-Like Receptor Activation and Signaling

S. Ray Amith¹, Preethi Jayanth¹, Rudi Beyaert² and Myron R. Szewczuk¹

¹Queen's University,
Kingston; ²VIB-Ghent
University, Belgium

Toll-like receptors (TLRs) play a vital role in the pathophysiology of infectious and inflammatory diseases, and possibly autoimmune diseases. Thus, understanding the structural integrity of TLRs, their ligand interactions and signaling components, are essential for subsequent immunological protection and provide important opportunities for disease alteration through TLR manipulation. The ectodomain of TLRs is highly glycosylated, and it is known that the glycosylation of secreted and cell membrane bound receptors may be an important requirement for their transport and function. However, to date a direct link between TLR glycosylation and receptor activation following natural ligand binding has not been reported. To investigate the role of glycosylation in TLR activation, and based on our previous work on the role of cellular sialidase in TrkA tyrosine kinase receptor activation, we hypothesize that cellular sialidase(s) may also be involved in the activation of TLRs. To test this, we utilized an assay to detect the sialidase activity of TLR-expressing cells in culture as assessed by fluorescence of the cleaved product of the sialidase-specific substrate 4-MUNANA (2'-(4-Methylumbelliferyl)- α -DN-acetyl-neuraminic acid). We discovered that a cellular sialidase(s) is induced when specific ligands bind to their respective TLRs, and that this sialidase enzyme(s) specifically targets and hydrolyzes α -2,3-linked β -galactosyl sialic acid (SA) residues, as shown by lectin colocalization and inhibition experiments using *Maackia amurensis* agglutinin (MAL-2, specifically binds α -2,3-SA) and *Sambucus nigra* agglutinin (SNA, specific for α -2,6-SA). When LPS binds to TLR4, cellular sialidase(s) desialylates the receptor and facilitates MyD88/TLR4 interaction and NF κ B activation. Sialidase-specific inhibitor Tamiflu (oseltamivir phosphate) blocks not only sialidase activity but also inhibits LPS-induced NF κ B activation as evidenced by ICC, EMSA and supershift assays. In addition, MyD88 co-immunoprecipitates with TLR4 in lysates from LPS-treated HEK-TLR4 cells, but this is significantly inhibited in cells pretreated with either Tamiflu or MAL-2 lectin. Our findings indicate for the first time that ligand induced TLR activation and signaling are dependent on cellular sialidase(s) and that the hydrolysis of α -2,3-sialyl residues is a critical step in LPS-induced TLR4 activation and subsequent downstream signaling.

Overwhelming HIV-1 Vif's Blockage and Inhibiting Viral Infection by a R88-Apobec3G Fusion Protein

Zhujun Ao, Zhe Yu, Lina Wang, Yingfeng Zheng, and XiaoJian Yao

Laboratory of
Molecular Human
Retrovirology,
Department of
Medical Microbiology,
Faculty of Medicine,
University of Manitoba,
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Background: APOBEC3G (A3G), a deoxycytidine deaminase, is a potent host antiviral factor that can restrict HIV-1 infection. During Vif-negative HIV-1 replication, A3G is incorporated into HIV-1 particles, induces mutations in reverse transcribed viral DNA and inhibits reverse transcription. However, HIV-1 Vif counteracts A3G's activities by inducing its degradation and by blocking its incorporation into HIV-1 particles. Thus, it is interesting to elucidate a mechanism that would allow A3G to escape the effects of Vif in order to rescue its potent antiviral activity and to provide a possible novel therapeutic strategy for treating HIV-1 infection.

Methods and Results: In this study, we generated an R88-A3G fusion protein by fusing A3G to a virion-targeting polypeptide (R14-88) derived from HIV-1 Vpr protein and compared its antiviral effects relative to those of HA-tagged native A3G (HA-A3G). Our study showed that transient expression of the R88-A3G fusion protein in both Vif- and Vif+ HIV-1 producing cells drastically inhibited viral infection in HeLa-CD4-CCR5-cells, CD4+ C8166 T cells and human primary PBMCs. Moreover, we established CD4+ C8166 T cell lines that stably express either R88-A3G or HA-A3G by transduction with VSV-G-pseudotyped lentiviral vector that harbor expression cassettes for R88-A3G or HA-A3G, respectively, and tested their susceptibility to Vif+ HIV-1 infection. Our results clearly reveal that expression of R88-A3G in transduced CD4+ C8166 cells significantly blocked Vif+ HIV-1 infection. In an attempt to understand the mechanism underlying the antiviral activity of R88-A3G, we demonstrated that R88-A3G was efficiently incorporated into viral particles in the presence of Vif. Moreover, PCR analysis revealed that R88-A3G significantly inhibited viral cDNA synthesis during the early stage of Vif+ virus infection.

Conclusion: Our results clearly indicate that R88 delivers A3G into Vif+ HIV-1 particles and inhibits infectivity and spread of the virions among CD4+ T cells. This study provides evidence for an effective strategy to modify a host protein with innate anti-HIV-1 activity and rescue its potent anti-HIV potential in the presence of Vif. Further characterization and optimization of this system may lead to the development of an effective therapeutic approach against HIV-1 infection.

cAMP Accelerates Retinal Ganglion Cell Growth by During Development

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During development, retinal ganglion cells (RGCs) extend their axons toward their specific targets in the thalamus and the superior colliculus. RGC axon navigation is largely directed by guidance cues present in their environment. cAMP has been proposed as an important second messenger during axon guidance and brain wiring in the developing central nervous system. Furthermore, it was reported that intracellular cAMP levels decrease significantly following birth in purified dorsal root ganglion cells and RGCs. In the present study, we tested if an early postnatal modulation of the cAMP/Protein Kinase A (PKA) pathway would affect RGC axon navigation. We observed a significant decline in retinal cAMP levels during early postnatal development. To investigate the effect of an increase in cAMP on retinal projection development, at postnatal day 1, hamsters received a unilateral injection of either 0.9% saline, 12mM of the membrane-permeable cAMP analogue dibutyryl cAMP, or 10 μ M of PKA inhibitor KT5720. Compared with the control group, intraocular elevation of cAMP significantly accelerated RGC growth whereas inhibition of PKA activity delayed axonal growth. When highly purified RGC cultures were treated with several pharmacological agents activating the cAMP/PKA pathway, neurite length, growth cone (GC) surface area and GC filopodia number were increased significantly. These effects were prevented by PKA inhibitors. Taken together, these results suggest that cAMP levels promote projection growth in part by directly modulating the PKA pathway in RGCs. Intraocular elevation of cAMP could potentially be an interesting route to promote retinal projection growth in pathological conditions particularly during early development.

Characterization of the Molecular Basis of Bowen-Conradi Syndrome

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Introduction: Bowen-Conradi Syndrome (BCS) is a lethal autosomal recessive disorder found in 1 in 355 live births among the Hutterites of the Canadian Prairies. A genome-wide scan approach to map the gene responsible for BCS led eventually to the identification of an A to G mutation in *EMG1* (Greenberg, Triggs-Raine, Wrogemann, Zelinski, unpublished data). This results in an Asp to Gly substitution which is the probable cause of BCS. The specific function of the EMG1 protein is not yet understood in humans; however its homologue in yeast plays a role in ribosome biogenesis. We hypothesize that the Asp to Gly substitution in the EMG1 protein disrupts its structure and function, interfering with normal ribosome biogenesis in BCS patients.

Methods: Vectors expressing HA-tagged wild type and mutant EMG1 were constructed and western blots using anti-HA antibodies were then performed on cell lysates from transiently transfected BHK cells to determine the levels of EMG1 expression. Metabolic labeling with ^{35}S -Cys/Met was then performed to establish if the mutant EMG1 was degraded at a different rate than the wild type protein. To compare the sub-cellular localization of mutant and wild-type EMG1, fluorescence microscopy was performed.

Results: During transient expression experiments, very little soluble mutant EMG1 was detected when compared to the wild type control. Metabolic labeling revealed that the levels of soluble wild type and mutant EMG1 were initially the same, but that soon after synthesis, the mutant protein became preferentially associated with a detergent-insoluble fraction of the cell while the wild type protein was divided between the soluble and insoluble fractions. Fluorescence microscopy did not show a difference in sub-cellular localization.

Conclusions: Metabolic labeling results suggest that the mutated EMG1 protein is sequestered, either by electrostatic interactions with itself or with proteins in a different cell compartment. However, fluorescence microscopy did not differentiate the sub-cellular localization of mutant and wild type EMG1.

Youth Engagement as a Protective Factor in Rural and Urban Suicidality

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Suicide embodies a considerable but preventable loss of life. As the second leading cause of death for young people in Canada between the ages of 15 and 24, nearly one-quarter of all annual deaths for this age group are accounted for by those who have died by suicide. Greater still is the prevalence of suicidal behaviour. Annually, suicidality is estimated to cost Canadians \$2.2 billion. Given the high personal costs to victims, survivors, and taxpayers, it seems crucial that prevention strategies be implemented to reduce the high rate of suicide and suicidal behaviours, particularly in youth. Primary suicide prevention is especially relevant for youth living in rural areas. Fewer mental health resources are available in rural communities than in urban centres. Moreover, youth living in rural, low population density areas often do not believe that their anonymity and right to privacy would be protected if they were to seek mental health services. Thus, the present research examines the promotion of positive mental health and suicide risk prevention in rural and urban youth through engagement in extracurricular activities, such as sports, music, drama, or community groups. A survey was conducted with over 800 (421 females; 401 males) secondary school youth in rural and urban regions of Eastern Ontario. It was found that rural youth report significantly more suicidal thoughts, depressive symptoms, lack of satisfaction with social support, and fewer supportive persons in their lives than urban youth ($p < .01$). Furthermore, self-reported depressive symptoms, risk behaviours, low self-esteem, and low social support were also found to be important predictors of suicidal thoughts for both rural and urban youth ($p < .05$). Regressions involving interaction analyses were carried out with youth engagement as a moderator for depressive symptoms, risk behaviours, self-esteem, and social support in the prediction of suicidality. Through simple slope analyses, it was found that youth engagement significantly reduces the relationships between suicidality and depressive symptoms, risk behaviours, low self-esteem, and low social support ($p < .001$). Therefore, engagement in extracurricular activities may promote positive mental health and act as a protective factor in rural and urban youth suicidality. In particular, youth engagement may be a relevant prevention strategy for rural youth, given the higher suicide risk, more significant mental health concerns, and barriers to mental health service access in rural communities.

Characterizing Hypoxia-Induced Autophagy: The Role of BNIP3 and Reactive Oxygen Species

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Introduction: Autophagy is a regulated degradation pathway that functions in both cell survival and cell death. The role of autophagy in cancer is controversial: depending on cell type and conditions, it may be protective or destructive to tumor cells. Hypoxia (low oxygen) is often associated with solid tumors, correlating with poor prognosis. Reactive oxygen species (ROS) are required for starvation-induced autophagy, and are important signaling molecules in hypoxia. This study investigates autophagic cell death in cancer cells under hypoxia and examines the role of ROS and the pro-death Bcl-2 family member, BNIP3.

Methods: We analyzed one embryonic and four cancer cell lines (U87, U373, MDA-MB-231, ZR75) for response to etoposide and hypoxia (<1% O₂). Cell death was measured by membrane permeability assay. Apoptosis was quantified by caspase activity, nuclear condensation and phosphorylation of histone H2A.X. Autophagy was assayed by LC3-GFP distribution, electron microscopy, staining of acidic vacuoles, and siRNA knock-down of autophagy genes. Inhibitors of autophagy (3-methyladenine, 3MA) and apoptosis (zVAD-fmk) were employed to differentiate the two types of cell death. BNIP3 was overexpressed by transient transfection or inhibited using siRNA or a dominant-negative mutant (BNIP3 Δ TM). ROS were measured using fluorescent redox-sensitive dyes, and manipulated using the ROS scavengers Tiron and catalase.

Results: Etoposide induced apoptotic cell death in all five cell lines whereas hypoxia induced autophagy and cell death but failed to induce apoptosis. Hypoxia-induced cell death was reduced by 3-MA or knock-down of autophagy genes, but not by zVAD-fmk. Expression of BNIP3 siRNA or BNIP3 Δ TM reduced hypoxia-induced autophagy. Hypoxia also induced ROS and further experimentation will determine how ROS and BNIP3 may cooperate to regulate hypoxia-induced autophagy.

Conclusion: Taken together, these results indicate that prolonged hypoxia can induce autophagic cell death in apoptosis-competent cancer cells, through a mechanism involving BNIP3. The precise mechanism is unknown, but we hypothesize that BNIP3 mediates hypoxia-induced autophagic cell death by inducing mitochondrial ROS production.

Using X-Ray Crystallography to Design Selective Inhibitors to Target a Family 3 Glycoside Hydrolase Involved in *ampC* Beta-Lactamase Induction

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Introduction: NagZ is a family 3 β -glucosaminidase involved in remodeling of the bacterial cell wall. It removes terminal N-acetylglucosamine residues from internalized cell wall degradation intermediates that are subsequently recycled back into the cell wall during biosynthesis. In the presence of beta-lactam antibiotics however, the NagZ product 1,6-anhydroMurNAc-oligopeptide accumulates in the bacterial cytosol to levels sufficient to induce the expression of the *ampC* beta-lactamase gene through direct activation of the AmpR transcriptional activator.

Methods and Results: Previously we determined the crystal structure of *Vibrio cholerae* NagZ in complex with PUGNAc, a potent and fairly selective inhibitor was determined to 1.7Å. This structure revealed a large open pocket beneath the 2-acetamido methyl group of the inhibitor. This is in contrast with the architecture of the human family 20 hexosaminidases and family 84 O-GlcNAcases. Family 20 and 84 enzymes form a tight envelope around the 2-acetamido group, holding it in position to participate as a nucleophile during catalysis. This structural difference suggests that extensions off the 2-acetamido group of PUGNAc would confer high inhibitor selectivity toward NagZ. Subsequent synthesis of derivatives two N-butylPUGNAc and Valeryl-PUGNAc possessing modifications to this region have been chosen for crystallographic analysis. These derivatives are potent and selective for NagZ.

Conclusion: We present a detailed structural comparison of the family 3 NagZ:N-butylPUGNAc complex (to 2.5Å) with human family 20 and 84 glycosidase crystal structures and discuss further design strategies for highly selective family 3 glycoside hydrolase inhibitors.

Modulation of Monosynaptic Reflex by Extrasynaptic GABA_A Receptors on Turtle Spinal Cord

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Since tonic inhibition by extrasynaptic GABA_A receptors was first described a decade ago, increasing evidence of the presence of this type of inhibition in the brain, cerebellum and recently on spinal cord substantia gelatinosa neurones has now emerged. Tonic inhibition results from the permanent activation of high affinity extrasynaptic GABA_A receptors. Modulation of neuronal excitability is important for maintaining the firing rate within an operational range over a wide range of excitatory drive (Mitchell and Silver 2003). This modulation is efficient enough in terms of energy that allows controlling the excitability of a neuronal network without getting interneurons firing constantly, simply taking the advantage of the GABA ambient concentration. This is crucial to maintain focused a target activity within a dynamic network exposed to several sources of excitability, the spinal reflex activity is a very good example of this because it is always present even during the most complex behaviours. The aim of the present study was to determine by electrophysiological and pharmacological methods either if the synaptic or extrasynaptic GABA_A receptors participate in the monosynaptic reflex modulation on turtle spinal cord, for that purpose we tested the effect of synaptic and extrasynaptic GABA_A antagonists picrotoxin and furosemide on monosynaptic reflex response where we observed a potentiation of the monosynaptic reflex. In order to discriminate between presynaptic or postsynaptic modulation, DRP and evoked presynaptic inhibition was induced. Additionally presynaptic inhibition was observed for the first time in the turtle lumbar spinal cord, our results strongly suggest that extrasynaptic GABA_A receptors are at the motoneurons, therefore this is the first evidence illustrating tonic inhibition of monosynaptic reflex mediated by extrasynaptic GABA_A receptors on turtle spinal cord.

A Novel Role for PROX1 as a Transcriptional Co-Activator

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Introduction: The homeobox transcription factor PROX1 is necessary for the development of the lymphatic vascular network. Expression of PROX1 in a subset of blood endothelial cells in the cardinal vein switches their fate to a lymphatic phenotype. PROX1 has been shown to upregulate expression of CYCLIN E1 in both *Drosophila melanogaster* and mammalian cells. CYCLIN E1 is involved in the transition from the G₁ to S phase of the cell cycle. Expression of CYCLIN E1 is controlled by a bipartite E2F-Sp1 binding site and an E2F binding site located on either side of the transcriptional start site. We have shown in our laboratory that PROX1 is able to activate a 1 kb *Cyclin E1* promoter via a DNA-binding independent mechanism. We hypothesize that PROX1 binds to repressive complexes located at the two E2F sites during G₁ phase and either removes them from the *Cyclin E1* promoter or inhibits their function thus allowing for gene transcription.

Methods: We have used immunocytochemistry, western blotting and luciferase assays to determine the subcellular localization, expression levels and activity of various mutant PROX1 versions transiently transfected into Human Embryonic Kidney (HEK) 293 cells. We have generated two truncated versions of the *Cyclin E1* promoter as well as versions in which the E2F-Sp1 and the E2F sites are mutated.

Results: We have determined that PROX1 is able to activate the 1 kb, 557 bp and 206 bp *Cyclin E1* promoters in HEK 293 cells. We observed a significant decrease in PROX1's ability to activate a 206 bp *Cyclin E1* promoter in which we had mutated the E2F-Sp1 site. In contrast, we did not detect a significant difference in PROX1's ability to activate a 206 bp *Cyclin E1* promoter in which the downstream E2F site had been mutated. We then measured the ability of PROX1 to activate either the 1 kb or the 206 bp *Cyclin E1* promoters in presence of Sp1 and observed a synergistic effect.

Conclusion: We have established that the bipartite E2F-Sp1 site is involved in PROX1 mediated transcriptional activation of *Cyclin E1* promoter. We have also observed a potential role for Sp1 in PROX1 mediated activation of *Cyclin E1*. Our studies represent a novel role for PROX1 as a transcriptional co-activator.

Correlation between Structurally Abnormal Y Chromosomes, Abnormal Sexual Development and Gonadal Tumors

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Infrastructural rearrangements of the Y chromosome are often seen in both males and females. These structurally abnormal Y are unstable and can be implicated in infertility and azoospermia in men, and in gonadal dysgenesis, sex reversal, and increased risk of developing gonadal tumors when found in women. Such tumors might arise from germ cells that abnormally proliferate in undifferentiated gonadal tissue (UGT) of dysgenetic gonads under the influence of *TSPY* (Yp11.2). Currently, these patients usually undergo systematic prophylactic gonadectomy because we are unable to predict their risk of developing a gonadal tumor. Furthermore, molecular characterization of the rearranged Y chromosomes by fluorescence *in situ* hybridization (FISH) is greatly limited due to the lack of probes specific to this chromosome. Thus, in the first phase of our project, we are mapping Bacterial Artificial Chromosomes (BACs) of the Y chromosome by FISH in order to establish the cytogenetic position and order of each BAC on the normal Y. Then, we use these BACs to determine the molecular breakpoints in patients having an abnormal Y chromosome to better correlate the phenotype of these patients with their genotype. In parallel, we also study by FISH, histology and immunohistochemistry the gonads of patients who underwent gonadectomy to understand their abnormal sexual development and identify risk factors that lead to the development of gonadal tumors. The results obtained so far indicate that UGT is indeed a high risk factor for gonadal tumors, and that there can be a great difference in the proportion of cells which retained the Y chromosome between the blood lymphocytes and the gonads, explaining the female phenotype of many patients carrying an abnormal Y chromosome.

Genome-wide RNAi Screen to Identify Stromal Factors Regulating Mammary Progenitor Cell Growth and Differentiation

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Stromal-epithelial interactions have been implicated in all stages of mammary gland development and malignancy. Although the stromal composition is dynamic over time, fibroblasts account for a large proportion of the cellular compartment. The relationship between the fibroblasts and the mammary epithelial cells hitherto remains ill defined. A striking example of the relationship between the two cell types lies in the obligatory requirement for fibroblasts in the Colony Forming Assay (CFC assay). When plated with irradiated fibroblasts, mammary epithelial cells obtained from dissociated normal tissues are able to form colonies containing either all myoepithelial cells, all luminal cells or both. Mixed colonies represent derivation from bipotent progenitor cells, the most primitive detectable cell within the breast lineage hierarchy.

The fibroblast dependency exhibited by primitive cells within the CFC assay is mimicked by the heterogeneous 184-hTERT mammary epithelial cell line. A series of clonal cell lines were derived from an earliest available passage of 184-hTERT cells. Interestingly, all of these clonal lines have the propensity to selectively gain chromosome 20 during passaging. Copy number gain of chromosome 20 is associated with poor clinical prognosis in breast cancer and is thought to play a role in oncogenic progression.

A siRNA screen has been completed targeting the 184-hTERT cells in a modified CFC assay to identify genes that are involved in fibroblast dependant growth. The screen was done in parallel using an early passage diploid 184-hTERT clonal line and a later passage (48,XX,+20,+20) clonal line. Dharmacon's human siGENOME library was used to individually interrogate approximately 22,000 genes. Effects on growth were established through cell counts derived from images collected on GE's InCell Analyzer and segmented using Cell Profiler. Genes were sorted based on their relative effect on the cells lines; a greater number of genes show an effect on the karyotypically normal cell line. Secondary screening using a combination of RNAi methods is currently underway to validate genes with the greatest effect. This systematic screen will provide a solid understanding of cellular stromal regulation of mammary epithelial cells.

The Peripheral-Central Nervous System Interaction: A Novel Technique in Adult Bone and Nervous Tissue Cryosectioning

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Objective: To perfect a decalcification technique allowing cryosectioning of the spinal column to maintain the integrity of spinal cord attachments to the dorsal root ganglia (DRG) via the peripheral nerve roots.

Introduction: Studies looking at factors crossing the blood-brain-barrier at the dorsal horn of the spinal cord, encounter obstacles associated with obtaining a cross section of the entire spinal column, including peripheral roots, DRG and spinal cord. Many studies have attempted to compensate by obtaining cryosections of DRG and spinal cord separately, and then matching the sections to provide a single cross-section. Techniques that allow the sectioning of the entire vertebral column are time-consuming and require expensive specialized equipment, as sectioning bone, particularly adult bone, is technically difficult. An alternative approach is to decalcify the vertebral column prior to cryosectioning. However, if the study includes analysis of myelin the decalcification process can disrupt or even destroy the myelin structure. We have devised a novel protocol to decalcify adult rat vertebral column allowing cryosectioning at 10µm, which maintains the structure of the DRG and the spinal cord connections, without disrupting myelin structure.

Materials and Methods: We compared commercially available decalcification reagents RDO Gold (less than 10% HCl) and Krajian solution (20%-30% formic acid), and a commonly used decalcification solution EDTA-Glycerol, with 6% TCA. The entire spinal column was removed from perfusion-fixed adult rats. Vertebral segments, less than 1cm in length, were placed into decalcification solution for the required period, after which the segments were cryopreserved in 30% sucrose, and embedded in OCT for cryosectioning. Incubation times for the commercial reagents were as per manufacturers instructions, 6% TCA solution and EDTA-G solutions were assessed at several time points using insertion of a sharp needle into the bone to determine the end-point of decalcification. Serial sections, 10µm thickness, were stained for myelin basic protein (MBP) immunoreactivity. Images were processed in Image Pro (7.0) via an Olympus IX51 microscope, coupled to a monochrome digital camera. Confocal images were collected via an Olympus FV500 Confocal Microscope.

Results: MBP IHC of *RDO GOLD* and *Krajian solution* treated tissue resulted in indistinct myelin structure, with no ring structures visible. MBP IHC showed more distinct myelin structure in *EDTA-G treated tissue*, how-

ever, the rings are not clear. MBP IHC of TCA treated tissue showed clear structure of myelin, with very distinct MBP rings. Further, using confocal microscopy, we are able to visualise the nodes of Ranvier present within the grey matter of the spinal cord.

Conclusion: We conclude that decalcification using 6 % TCA solution results in the maintenance of the myelin structure, allowing accurate visualization of specific structures such as the nodes of Ranvier. Using this decalcification method we can successfully section the entire spinal column, maintaining the integrity of the peripheral root/DRG connections linking the PNS to the CNS.

Isolation of Novel Adeno-Associated Viruses from Porcine Tissues

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Introduction: Gene transfer vectors derived from adeno-associated viruses (AAVs) are highly efficient and can deliver genes in a variety of tissues *in vivo*. Recent observations have also promoted the evaluation of AAVs as potential genetic vaccine vector due to their long-term expression profile which can stimulate robust antibody responses. Currently, 12 serotypes of AAVs have been characterized; the majority isolated from human tissues. Novel serotypes of AAVs have recently been isolated including those from nonhuman primates and are currently being evaluated also regarding the safety associated to the administration of monkey-derived viruses into human patients. The goal of the present study was to detect, isolate and characterise AAVs from porcine tissues. We speculated that the high antigenic compatibility and low toxicity associated with xenograft transplantation of porcine tissues in immunodeficient human recipients would also correlate with high compatibility of porcine AAVs to humans.

Methods and Results: Porcine tissues including gut, lung, liver, heart, and spleen were screened for the presence of AAV using universal primers designed from an alignment of known AAV sequences. In total, porcine AAVs were detected in 9 out of 21 farmed pigs. Several 252bp and 255bp fragments (signature regions) were isolated from various tissues, cloned, sequenced, and BLAST analysis confirmed high to low homology with known AAV sequences. A majority of AAVs were detected from the spleen, with the liver and lung testing second highest for the presence of AAV sequences. The remaining sequences of porcine AAVs were identified for some isolates and extracted by PCR using specific primers based on the signature region and degenerate primers based on conserved regions. Sequence analysis confirmed the isolation of at least three novel porcine AAV isolates which we named AAV Po1, Po2, and Po3. The AAV Po1 *cap* gene demonstrated the highest homology to human AAV 5. AAV Po2 was found to be closely related to human AAV 2 whereas the isolated sequence of AAV Po3 is highly divergent from all AAV isolates previously described. Interestingly, AAV sequences with 100% identity to human AAV 2 were also isolated from several porcine tissues suggesting co-evolution and/or zoonotic transmission of AAV 2 between pigs and humans. Pseudotyping of AAV 2 *rep* with AAV Po1 *cap* has been performed and hybrid vector particles were successfully produced. The novel AAV 2/Po1 particles were administered in the lung, liver and muscle of BALB/c or C57BL/6 mice to determine tissue tropism in parallel to AAV 2/5 as a positive control.

Conclusion: AAV 2/Po1 transduced muscle cells with efficiencies comparable to that observed with AAV 2/5. AAV 2/Po1 was subjected to anti-sera produced against AAV 2/5, and it was determined that AAV 2/Po1 appears to be a unique serotype, or at least unique from AAV 2/5. New sequences, phylogenetic analysis, tissue tropism and biodistribution will be presented. Novel AAVs derived from porcine tissues may significantly contribute to the generation of new preventive or curative clinical modalities acceptable for human use.

Quantitative Proteomic Analysis of Reovirus-Infected Cells

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Introduction: Lacking independent metabolism, viruses require a host cell to provide necessary components for replication. Although transcriptional alterations within host cells have been determined for many virus/cell interactions by micro-array techniques, little is known about how global cellular effector molecules (proteins) are affected. We are using SILAC, a quantitative mass spectrometric-based method, to study the differences in a host cell's proteome before and after infection by mammalian reovirus.

Methods: The SILAC method allows for a more complete determination of the cell's proteome. 293 cells were adapted into different SILAC media and infected with the T1L strain of reovirus at MOI of 7 PFU/cell. Infected cells were harvested at various times post-infection (6hpi to examine early effects; and 24hpi to examine late effects) and host proteins resolved, both by SDS-PAGE/LC/MS and by 2-D LC/MS.

Results: The 2-D LC/MS method identified 3001 and 3079 proteins, respectively, from the 6 and 24hpi T1L-infected samples – approximately 3-fold more than were identified by the SDS-PAGE method. 356 proteins were found to be differentially regulated (either significantly up-regulated or down-regulated) at 6hpi and 387 proteins were differentially regulated at 24hpi. Differentially regulated proteins included those involved in host cell immunity, cell structure (ex. laminin, inositol polyphosphate 5-phosphatase, and mutant beta-actin), metabolism, protein function (ex. HSP70-2, ubiquitin-like 5 protein) and transcription (ex. RPL14 protein, small nuclear ribonucleoprotein, chromodomain helicase-DNA-binding protein 4). Quantitative validation of selected proteins was done by western blot.

Conclusion: SILAC is an effective way of generating an overview of the host cell and viral interactions, which can lead to particular host cell pathways that are specifically affected by viral infection.

Discovery of Vinyl-ether Lipid Changes During Cell Cycle Progression and Differentiation

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A common feature in many different types of human cancers is the loss of cell cycle control and cellular differentiation. It has been hypothesized that disruptions in the cell cycle may be the cause for the loss of differentiation, or that differentiation mechanisms are affected as secondary effects of carcinogenesis. Surprisingly, few studies have been carried out to investigate the metabolism associated with these processes. Using a non-targeted metabolomics strategy, we investigated global metabolite changes associated with differentiation and cell cycle progression by profiling the differentiation of U937 cells and synchronous progression of MRC5 fibroblast cells through the cell cycle. Non-targeted metabolomic analysis of differentiating U937 cells using high-resolution Fourier Transform ion Cyclotron Resonance Mass Spectrometry revealed significant and dramatic increases in a group of phospholipids harbouring vinyl-ether bonds at the SN1 position, otherwise known as plasmalogens. In a similar approach to characterize cell cycle-related metabolites, significant increases in triacylglycerols as well as a non-phosphate group of vinyl-ether lipids, otherwise known as 2-Acyl-1-(1-alkenyl)-sn-glycerols, were detected as the cells progressed into the G1 stage of the cell cycle. Vinyl-ether lipids are an interesting and often overlooked group of molecules possessing diverse functions from cell signalling to protection against oxidative stress. Their presence can also dramatically change the architecture and fluidity of cell membranes. We have recently begun to address the underlying causes for these molecular changes by investigating the role of peroxisomal enzymes involved in ether lipid synthesis. Our preliminary results using quantitative rt-PCR, interestingly, indicate no significant change in peroxisomal enzymes during differentiation, suggesting that transcriptional activation is not the key driving event. We have also been investigating the potential role of Phospholipase C (PLC) in cell cycle progression. Preliminary results show increased expression of particular PLC isoforms at specific stages in the cell cycle. Current studies are focused on investigating the activity of these and other relevant enzymes using RT-PCR, si-RNA and C13 stable isotope tracer studies.

Toll-like Receptor 5 as a Novel Anti-inflammatory Target for Cystic Fibrosis Lung Disease

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Novel therapies to target lung inflammation are predicted to improve the lives of people with cystic fibrosis (CF) but specific anti-inflammatory targets have not been identified. The goal of our study was to establish whether Toll-like receptor 5 (TLR5) signalling is the key molecular pathway mediating lung inflammation in CF, and to determine whether strategies to inhibit TLR5 can reduce the damaging inflammatory response. The innate immune responses were analyzed in both airway epithelial cells and primary peripheral blood mononuclear cells from CF patients and matched controls. In addition, 151 clinical isolates of *P. aeruginosa* from CF patients were assessed for motility and capacity to activate TLR5. Blood and airway cells from CF patients produced significantly more proinflammatory cytokine than control cells following exposure to the CF pathogens, *P. aeruginosa* and *B. cepacia* complex ($P < 0.001$). Stimulation with pure TLR ligands clearly demonstrated that TLR signalling appears to mediate the excessive cytokine production occurring in CF. Using complementary approaches involving both neutralizing antibody targeting TLR5 and flagellin-deficient bacteria, we established that specific inhibition of TLR5 abolished the damaging inflammatory response generated by CF airway cells following exposure to *P. aeruginosa* ($P < 0.01$). The potential therapeutic value of TLR5 inhibition was further supported by our demonstration that 75% of clinical isolates of *P. aeruginosa* retained TLR5 activating capacity during chronic CF lung infection. These studies identify the innate immune receptor—TLR5—as a novel anti-inflammatory target for reducing the damaging lung inflammation in cystic fibrosis.

Development and Characterization of a Neutralizing Murine/Human Chimeric IgG1/k Monoclonal Antibody to the Protective Antigen Toxin of *Bacillus anthracis*

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Introduction: There is a need for therapeutic reagents to biothreat agents such as *Bacillus anthracis*. A major virulence component of *B. anthracis* is a secreted tri-partite toxin including protective antigen (PA), lethal factor (LF) and edema factor (EF). The individual proteins are non-toxic, however combinations of PA and LF (lethal toxin, LeTx) or PA and EF (edema toxin) produce toxins capable of inducing toxic shock or edema, respectively. Neutralization of PA toxin has largely been attributed to antibody responses engaged to domain 4 of PA, a region key to binding to the host cell receptor. However, a LeTx neutralizing murine mAb, F20G75, has been generated in our lab and has been shown to bind via a defined epitope within the 2 β 2-2 β 3 loop region in domain 2 of PA (Gubbins *et al.*, 2006).

Method: Herein, we have developed a novel intronless two-plasmid human IgG1/k mammalian cell antibody expression system for the development of murine-human chimeric mAbs. Within the system, PCR-amplified heavy and light antigen-specific variable (binding) domains of murine origin were genetically fused to human constant (effector) domains. Full-length, functional chimeric antibody, huG1-F20G75, was generated by co-transfection of these heavy and light chain plasmids into HEK 293 cells for expression.

Results: Characterization of expressed huG1-F20G75 via ELISA, Western immunoblotting and pepscan analysis shows binding to the identical defined epitope on domain 2 of PA. Affinity analysis via surface plasmon resonance indicates the chimeric mAb retained the original binding affinity. Finally, *in vitro* testing indicates the chimeric antibody maintains the LeTx neutralizing activity of the parental mAb, while *in vivo* testing is ongoing.

Conclusion: This antibody expression system has broad use in the development of recombinant mAbs with high specificity to emerging pathogens and reduced immunogenicity as therapeutics.

Nurses' Health: Conceptual and Measurement Approaches

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Introduction: Nurses are one of the least healthy occupational groups. Many aspects of the work environment are negatively associated with nurse health, including: physical work, workload, nature of the work environment, managerial support and relationships with other health care professionals. Nurses have the highest prevalence of work-related back injuries and nurses' work-related injuries are more costly than those of high-risk occupations such as firefighters, police officers and transportation workers.¹ Nurses' rates of absenteeism and disability claims have a significant impact on the cost of providing health care services in Canada. With the global shortage of nurses that is already being felt in Canada, it is imperative that every effort be undertaken to retain a competent and healthy nursing workforce. As the average age of Canada's nursing workforce continues to rise, and many nurses look forward to retiring, it is critical that health care leaders, educators, researchers and occupational health professionals understand the impact of nurses' health on organizational, nurse, and patient outcomes.

Method: A critical appraisal of the nursing, occupational health, business and psychology literature (1995 to 2008) was conducted to examine the conceptual approaches to and measures of nurses' health. Research studies were included to illustrate the current dimensions, limitations and assumptions regarding nurses' health as a concept. Gaps in the existing literature along with areas for future research pertaining to nurses' health are identified.

Results: Results of this conceptual analysis will contribute to further understanding and measurement of nurses' health emphasize implications for nurse leaders, educators, policy makers and researchers in the development and evaluation of workplace interventions aimed at improving nurse health.

Conclusions: An adequate supply of healthy nurses is essential to the delivery of health care services in Canada. Recent research demonstrates relationships between nursing work environments and nurses' health. We risk losing nurses to poor health and other professions. Ultimately patients, the recipients of health care services, may suffer.

Regulation Of Mcl-1 and its Contribution to Cell Survival and Drug Resistance in Chronic Lymphocytic Leukemia (CLL) and Breast Tumours

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Introduction: Mcl-1 is a highly regulated member of the Bcl-2 family that plays an important role in regulating the balance between life and death in a variety of cell types. Depending upon the cellular context, Mcl-1 can act to either strongly repress or conversely activate programmed cell death. We aim to evaluate the contribution of Mcl-1 in cell survival and drug resistance in two distinct cancer types, CLL, a blood malignancy as well as solid breast tumours. Preliminary data indicates that Mcl-1 may be a key regulator of lysophosphatidic acid initiated pro-survival signalling in hematopoietic malignancies, such as CLL. Furthermore, *in silico* promoter analysis indicates that expression of Mcl-1 can be regulated through a variety of transcriptional activation pathways including Estrogen receptor mediated signalling as well as through the transcription factor NFkB. This project aims to identify if Mcl-1 plays a critical role in enhancing survival and resistance in these cell types. Knowledge gained will indicate whether specific inhibition of Mcl-1 activity may have a potential therapeutic benefit in these types of cancer.

Methods: Mcl-1 expression analysis was performed by Western blot following stimulation of cell lines with specific growth factors. Subcellular localization and changes in protein interaction were monitored by performing confocal microscopy, sub-cellular fractionation, and co-immunoprecipitation experiments. Transcriptional regulation of Mcl-1 was studied through cloning of the full-length Mcl-1 promoter and preparation of specific deletion mutants followed by luciferase assays. The requirement of Mcl-1 for growth factor induced cell survival signalling was assessed by inhibition of Mcl-1 activity through the specific inhibitor GX15-070 as well as shRNA mediated protein knockdown.

Results: Growth factor treatment led to increased resistance to chemotherapy induced apoptosis and coincided with elevated Mcl-1 protein levels. Following growth factor stimulation, Mcl-1 interaction with proapoptotic Bcl-2 family members was increased in both CLL and breast cancer cell lines indicating a repression of apoptotic signalling. Knock-down of Mcl-1 by shRNA or inhibition of its action through small molecule inhibitors resulted in a significant increase in apoptosis.

Conclusion: Mcl-1 expression is rapidly increased following growth factor stimulation in a variety of cancer cells. Mcl-1 plays a critical role in protecting these cells from apoptotic stimuli such as chemotherapeutic drugs. Blockage of Mcl-1 activity diminishes the protective effect of these survival factors. Mcl-1 plays a critical role in promoting cell survival and resistance to apoptosis, thus making it a favourable target for therapeutic intervention.

Fetal and Neonatal Nicotine Exposure in Wistar Rats Causes a Progression of Pancreatic Mitochondrial Alterations and Leads to Beta Cell Dysfunction.

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In Canada, nicotine replacement therapy (NRT) is recommended as a safe smoking cessation aid for pregnant women. However, there have been concerns regarding the safety of NRT use during pregnancy. In rats, fetal and neonatal nicotine exposure causes adult onset dysglycemia, which we hypothesize is related to progressive mitochondrial dysfunction in the pancreas. Therefore in this study we examined the effect of fetal and neonatal exposure to nicotine on pancreatic mitochondrial structure and function during postnatal development. Female Wistar rats were given saline (control) or nicotine bitartrate (1mg/kg/d) via subcutaneous injection for 2 weeks prior to mating until weaning. Male offspring were sacrificed at 3 (weaning) and 26 (adult) weeks of age for pancreas collection. Nicotine exposure resulted in increased islet reactive oxygen species (ROS) production at both weaning and 26 weeks of age ($p < 0.05$). At weaning there was no significant effect of nicotine exposure on mitochondrial DNA (mtDNA) deletions or mitochondrial enzyme activity in the pancreas ($p > 0.05$). However, by adulthood nicotine-exposed offspring had elevated mtDNA deletions and impaired enzyme activity relative to control animals ($p < 0.05$). Beta cells from nicotine-exposed animals had mitochondrial structural abnormalities which were observable by electron microscopy at weaning and progressively worsened with age. In addition, glucose-stimulated insulin secretion, an indicator of mitochondrial function in beta cells, was impaired at 26 weeks in the nicotine-exposed relative to saline-exposed offspring ($p < 0.05$). Taken together, these data suggest that maternal nicotine use during pregnancy results in postnatal mitochondrial dysfunction that may explain, in part, the dysglycemia observed in the offspring from this animal model. We propose that nicotine, a pro-oxidant, causes increased ROS production which in turn leads to the subsequent mitochondrial dysfunction. These results clearly indicate that further investigation into the safety of NRT use during pregnancy is warranted.

Characterization of the Immune Response to a Recombinant Vesicular Stomatitis Virus-Based Vaccine Against Lassa Fever

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Introduction: Lassa virus is an arenavirus that causes a hemorrhagic fever endemic in West Africa. Conservatively, over 200,000 infections are estimated to occur each year, resulting in 3000 to 5000 deaths. Our laboratory has previously demonstrated that an attenuated recombinant Vesicular Stomatitis Virus expressing Lassa virus glycoprotein (VSVdG/LASVGPC) is protective in a lethal non-human primate model of Lassa fever. The objective of the present study was to characterize the immune response to this vaccine in mice.

Methods: We have shown in a preliminary study that the antibody titre specific for the Lassa glycoprotein was weak in mice immunized with the VSVdG/LASVGPC vaccine. In the present study, different mouse groups were immunized with increasing doses of the vaccine and boosted on day 28 and day 70. The kinetics of the antibody response were studied by ELISA using serum samples taken every two weeks. In addition, the cellular immune response was measured by a T cell proliferation assay and ELISPOT (to assess production of IL-2, IL-4, IL-10, IL-12p70, IFN- γ and TNF- α cytokines) using leukocytes isolated from the spleen 10 days following the last boosting immunization.

Results: Preliminary analysis shows that the antibody titre does increase in a dose- and time-dependent manner but no or little T cell proliferation occurred following *in vitro* stimulation with Lassa glycoprotein peptides. The production of IFN- γ and TNF- α by splenocytes was significantly augmented with increasing doses of the vaccine.

Conclusions: These preliminary results suggest that the VSVdG/LASVGPC vaccine is able to stimulate an adaptive immune response in the mouse model. Further analysis will allow us to determine whether the humoral or cellular immune response is most important.

Decreased Levels of Immune Activation in Resistance to HIV-1 Infection are Associated with an Elevated Frequency of Regulatory T Cells

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Rationale: HIV-resistant commercial sex workers (CSW) provide a unique opportunity to study correlates of protection against HIV infection. Emerging data from studies on these individuals and other HIV-exposed uninfected individuals suggest that low levels of immune activation may play a role in protection against infection, yet the mechanisms controlling this differential activation state are poorly understood. The present study aims to characterize the role that regulatory T cells (Tregs) play in HIV-resistance. Tregs are a subset of circulating CD4+ T cells that have been implicated as mediators of the immune response to pathogens such as HIV. They exert a suppressive function on T cell responses and have been shown to maintain a low level of immune activation.

Methods: In this study, peripheral blood mononuclear cells (PBMC) were isolated from fresh blood obtained from HIV-infected (HIV+; n = 35), HIV-resistant (HIV-R; n = 25) and newly-enrolled HIV-uninfected control (HIV-N; n = 38) participants from the Pumwani CSW Cohort. PBMCs were immunophenotyped using multi-colour flow cytometry to determine levels of T cell activation (CD3, CD4, CD8, CD69) and Treg frequency (CD3, CD4, CD25, FOXP3).

Results: HIV-R CSW were shown to have fewer activated CD4+ and CD8+ T cells and elevated Treg frequencies when compared to HIV-N CSW. Consistent with previous data, Treg frequencies were shown to be depleted in HIV+ individuals.

Conclusions: HIV has previously been shown to preferentially infect activated T cells. The results of this study suggest a role for Tregs in maintaining low levels of T cell activation in HIV-resistant women, thereby minimizing the pool of cells susceptible to infection by the HIV virus.

A C-terminal TonB Derivative of *Escherichia coli* Binds the Periplasmic Binding Protein FhuD

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Gram-negative bacteria rely upon TonB-dependent transporters for acquisition of scarce and essential nutrients such as iron and vitamin B12. The Ferric Hydroxamate Uptake (Fhu) system of *Escherichia coli* participates in the uptake of ferric-hydroxamate siderophores. The system is comprised of an outer membrane receptor FhuA, a periplasmic binding protein FhuD and a cytoplasmic membrane (CM)-associated permease FhuB/C. Unidirectional siderophore transport requires energy input from the CM-anchored TonBnExbBnExbD multiprotein complex. We identified novel interactions between TonB and FhuD and proposed that in addition to its role as energy transducer, TonB acts as a scaffold to direct protein-protein interactions that culminate in iron uptake. We now extend our analyses of TonBnFhuD interactions by delineating regions within TonB that are essential for its interaction with FhuD. To obtain a form of TonB amenable to structural studies, we generated a soluble mutant of TonB containing the C-terminal residues 103-239 and analyzed its capability to interact with FhuD. By surface plasmon resonance, we demonstrated that TonB(103-239) forms a complex with FhuD in a dose-dependent manner; binding is independent of siderophore. To model the TonBnFhuD complex, we adopted the program AUTODOCK. An internal TonB oligopeptide derived from the most complete crystal structure (PDB 1U07) and containing the predicted FhuD-binding residues 151-162 was used as a flexible ligand which we docked to the crystal structure of FhuD (PDB 1EFD). A low-energy ensemble of docking solutions positioned the TonB oligopeptide against a region on FhuD that we previously predicted would serve as a TonB-binding surface. Our data indicate that the C-terminal region of TonB is sufficient to form a complex with FhuD. Given these outcomes, we propose that our C-terminal derivative of TonB will facilitate continued efforts towards understanding structural biology of iron transport in *E. coli*.

Peptides from the Novel Calmodulin-Binding Protein, cmbB, Increase Cell Motility and Chemotaxis in *Dictyostelium*

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Calmodulin is known to be involved in cell motility and development in *Dictyostelium* exerting its effects via downstream calmodulin-binding proteins. A novel calmodulin-binding protein, cmbB, has recently been partially characterized in this model organism, although its function is not yet known. This protein consists almost entirely of repeating units of an IP22 motif. The biological significance of this repeat is not yet known. In this study cells were treated with various peptides equivalent to conserved domains within cmbB and their effects on cell motility, chemotaxis, cell differentiation, and development were examined. The four peptides used were CaMBD (equivalent to the calmodulin-binding domain), N-Term (a non-conserved sequence equivalent to the N-terminal), and IP22-1 and -2 (representative of two general IP22 motifs not found in cmbB, but constructed based on the amino acid usage in IP22 motifs from several other proteins). While observing the process of development it was found that cells treated with either the CaMBD, N-Term, or IP22-1 peptide started to form aggregations 30 minutes before negative control cells. Subsequent experiments showed that starved cells treated with 50 μ M of CaMBD showed a 6-fold increase in the rate of cell motility. Similarly, 50 μ M of IP22-2 showed a 2-fold increase in the rate of cell motility. Equivalent rate increases were also observed when using N-Term or IP22-1 peptides, however the concentration needed to produce a maximal effect was 500 μ M. Similar results were observed during chemotaxis to cyclic AMP, with the CaMBD resulting in a 7-fold increase and the IP22-2 resulting in a 3-fold increase in chemotactic rate. Again, only high (500 μ M) concentrations of N-Term and IP22-1 produced similar results. There was no observable effect of any of these peptides on cell differentiation or fruiting body morphology. These peptides appear to affect only processes involving cell movement events which may have positive biomedical implications in wound healing or the immune response. Possible mechanisms, future studies, and implications are discussed.

The Molecular Epidemiology of Acute Infectious Diarrhea in the Pediatric cases attending the Winnipeg Children's Hospital Emergency Room

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Acute infectious diarrhea (AID) poses a considerable health burden on the population of developed countries, and even a higher burden in the developing world. The last study done in Winnipeg was in 1970 and that study showed 3% -5% of paediatric emergency room visits were due to AID, with about 50% being rotavirus infections. However a cause for the disease remained undiagnosed in almost 30% of cases. The distribution and frequency of pathogens associated with AID changes over time. Both astroviruses and caliciviruses have been identified as major causes of AID. The data for the Winnipeg study are not current, and with the evolving knowledge of the microbiology of newer pathogens, must now be considered inadequate. We are conducting a prospective, case-control study to elucidate the prevalence of various infectious causes of AID in pediatric patients presenting to the Winnipeg Children's Hospital Emergency Room (ER). This study of pediatric AID will help assess the current need and target population for rota virus vaccines and other possible therapeutic interventions. We will identify the spectrum of pathogens and the distribution and frequency of their occurrence. Finally, we will conduct molecular epidemiology, using genome sequence information, to further establish the range, distribution and frequency of pathogen subtypes, and to determine the relatedness of cases infected with the same specific type of pathogens.

Stool samples are being collected from pediatric cases with AID at the ER, and asymptomatic controls are being solicited from non- diarrheal admissions to the Winnipeg Children's Hospital. Bacterial and parasitic identification is being done by conventional bacterial culture and microscopic examination of stained smears. Stools are being tested for Human adeno, astro, calici (noro and sapo), entero, Hepatitis A, polio, reo and rotaviruses both by electron microscopy and molecular viral techniques (PCR and RT-PCR). The resulting information will make it possible to identify the proportion of cases attributable to bacterial, parasitic, and viral pathogens and may reduce the proportion of cases with AID of unknown etiology. This knowledge of the pathogens present will facilitate design of effective methods for prevention, treatment and intervention in the spread of these pathogens.

Navigational Skills in Early Blindness

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Navigation is a complex behaviour involving a multitude of sensory modalities. The hippocampus has been shown to be involved in visual spatial memory in primates and humans. Blind individuals from birth are not impaired on a spatial competence level or in the formation of novel spatial maps of the environment besides reduced right posterior hippocampus. The question therefore arises on how blind people form these spatial maps? We first tested the ability of blind subjects to navigate in an obstacle course. Wearing a camera mounted on glasses and the TDU grid on the tongue, they were requested to point to the obstacle (detection), move towards it and negotiate a path around it (avoidance). We showed that blind subjects had no difficulty to perform the task. In a virtual maze task where subjects used their tongue to move along a path blind subjects were able to find a path as well as blindfolded controls. While in a 3.0T fMRI scanner performing a virtual route recall task, they activated several brain regions involving posterior parietal cortex, anterior hippocampus and para-hippocampal area. These results demonstrate that early blind people can actually form maps and to do so call upon structures involved in route forming. These findings also confirm the brain reorganization of the blind's brain as demonstrated by voxel based morphometry and tensor diffusion imaging.

The Steroid Receptor RNA Activator Protein (SRAP) Acts as a Transcriptional Repressor

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The bi-faceted products of the Steroid receptor RNA activator gene (SRA1) consist in a functional RNA, which acts as a transcriptional co-activator, and a protein (SRAP), conserved in all chordates and which function remains elusive. To investigate the functional role of SRAP independently of its RNA counterpart, we first identified co-immunoprecipitated proteins by Mass spectrometry. Several of these proteins were known transcriptional regulators. We further showed that SRAP directly interacts with various transcription factors and associates with chromatin. To investigate what possible effect this association of SRAP with molecules involved in regulating transcription might have, we artificially recruited SRAP in the vicinity of the herpes simplex virus regulatory protein VP16, a protein known to strongly activate transcription. This was performed either by fusing SRAP with VP16 or by targeting it in close proximity to the VP16 binding sites. In both systems, full length SRAP, as well as its two highly phylogenetically conserved N- and C-terminal domains, harbored a strong transcriptional repressive activity, sensitive to the HDAC inhibitor, trichostatin A. We further confirmed the presence of HDAC activity in SRAP co-immunoprecipitated complexes and showed that HDAC2 specifically interacts with SRAP. To investigate the possible effects of SRAP on the estrogen receptor signaling pathway we established MCF-7 cell lines stably expressing SRA RNA and protein. These cells have a significant decreased sensitivity to estradiol but no additional sensitivity to Tamoxifen as measured by a vitellogenin estrogen receptor element driven luciferase reporter assay. However, transient transcription experiments using Hela cells showed that SRAP differentially upregulates unbound and agonist/antagonist bound estrogen receptor alpha activity in an ERE dependent manner. Our results thus suggest that similarly to SRA, SRAP is also involved in modulating estrogen receptor alpha's and possibly other transcription factors activities in a cell and promoter context dependant manner.

Combination of Fgf and Wnt Signaling Pathways Mediate Positional Information During Cell Migration

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Cell migration requires spatial and temporal regulation; this is especially critical for developmental processes such as organogenesis. Understanding the interactions leading up to the appropriate cell guidance is important since tumor progression and developmental disorders stem from these errors in the regulation of cell migration behavior.

In *C.elegans*, the distal tip cells (DTCs) lead the extensions of the gonad arms. The pattern of DTC migration along the dorsoventral and anteroposterior axes of the animal give a characteristic mirror-image C-shape to the gonad arms. UNC-6/Netrin ligand is expressed from the ventral nerve cord of the animal and its receptors, UNC-5 and UNC-40 are expressed in the DTCs to cause chemorepulsion during the ventral to dorsal second migration phase. Genetic screens can be used to identify genes that interact with the *unc-5*, *unc-6* or *unc-40* to increase or decrease the frequency of DTC migration defects. We have previously shown that up-regulation of *unc-5* expression in the DTC is one of the key events that initiate the second migration phase. EGL-17/Fgf and EGL-20/Wnt regulate numerous cell migrations along the anteroposterior axis of *C. elegans*. EGL-20 is expressed by cells situated in the tail and EGL-17 by cells situated at the ventral mid-body of the worm. Both EGL-17 and EGL-20 are responsible for the proper anteroposterior migration of specific cell types (sex myoblasts and Q neuroblasts respectively). *egl-20* mutants display very few DTC migration defects whereas *egl-17* mutants have none. However, *egl-20; egl-17* double mutants display frequent defects in DTC migration patterns. In *egl-20; egl-17* double mutants, the posterior DTC frequently fails to reflex back toward the mid-body in the third migration phase. In addition, the second, V-D migration phase begins at a more anterior position than normal, suggesting that this phase is initiated precociously. We compared the timing of posterior DTC turning to that of the anterior DTC and also compared the timing of DTC turning relative to stages in vulval development in wild-type and double mutant animals. We found that the more anterior position at which the posterior DTC initiates the V-D migration phase in *egl-20; egl-17* results not from altered timing of the initiation of the second migration phase, but from a slower rate of migration during the first phase. In addition, expression of *unc-5* reporter constructs in the DTCs begins at the normal time in devel-

opment regardless of the position of the DTC. This suggests that EGL-17 and EGL-20 may normally act as guidance cues facilitating DTC migration along the anteroposterior axis.

We are currently testing different genes belonging to both EGL-20 and EGL-17 signaling pathways in order to narrow down the interactions that are necessary for proper longitudinal DTC migration. We hope to be able to reproduce the phenotypes first observed in *egl-20;egl-17* mutant strain by making double mutants incorporating the components of EGL-20 pathway with *egl-17* and vice versa. We propose that EGL-20 and EGL-17 gradients collectively mediate positional information to the DTC during its longitudinal 1st and 3rd migration phases.

Evaluation of Varied Repair Methods Applied to 3 Types of CAD/CAM Blocks

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Introduction: Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) technology is used in dentistry in order to produce restorations such as crowns, inlays and bridges in shorter time period. Occasionally the restoration will need adjustment or repair.

Objectives: The study aim was to determine the best repair method for three types of CAD/CAM blocks.

Materials and Methods: 3 types of CAD/CAM materials, two ceramic (Vitablocs Esthetic Line, Vident and ProCAD, Ivoclar Vivadent) and one composite (Paradigm MZ100, 3M ESPE), were sectioned into 48 specimens (5X2X3mm), then embedded and randomly divided into 12 groups (n=12). Specimen surfaces were either roughened by a fine diamond bur (Brasseler, USA) or air abraded by 30 μ m aluminum oxide particles (CoJet System, 3M ESPE). Clearfil Repair (Kuraray America) was applied and polymerized according to manufacturers' instructions. A cylindrical mould (2.3798X4mm) was used to fabricate composite cylinders from either flowable (Esthet X Flow, Dentsply) or hybrid (Filtek Z250, 3M ESPE) composite resin. Specimens were stored in water at 37°C for 24 hours and sheared using the Ultradent method with Zwick Z010 Compression Tester set to move at 1mm/minute (Figure-1). Data were analyzed using ANOVA single factor ($\alpha=0.05$). Scanning Electron Microscope (SEM) was used to assess the modes of fracture.

Results: Figure-2 and Table-1 summarizes the results. ProCAD and Paradigm blocks yielded statistically significant higher shear bond strengths as compared to Vitablocs ($p<0.05$). There was no statistically significant difference between bond strengths for bur and CoJet abrasion ($p>0.05$) or between hybrid and flowable resins ($p>0.05$). Failed specimens showed mostly cohesive fractures within the blocks

Conclusions: Within the study's limitation, the significant difference found in the reparability of CAD-CAM blocks may be attributed to block composition; however, all block/surface preparation/resin combinations yielded clinically acceptable shear bond strengths when bonded by Clearfil Repair. Both fine diamond bur abrasion and CoJet abrasion may be used to roughen CAD/CAM restorations after which either flowable or conventional hybrid composite resins can be bonded to repair the material.

Resting-state Functional Connections Indicate Language Lateralization in the Human Brain

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This study aimed to use resting-state connections and functional magnetic resonance imaging (fMRI) to characterize language lateralization in the human brain. In general, language is lateralized to the inferior frontal gyrus (IFG) of the left hemisphere; however, individual differences in language lateralization may be associated with individual differences in resting-state connectivity. In fact, greater intra-hemispheric connectivity between language related areas was found to be associated with greater lateralization of language function, but inter-hemispheric connectivity was not investigated. In addition, clinical research has shown that patients recruit additional areas to perform the same tasks as healthy controls, while pathology results in a disruption of functional resting-state connectivity, further suggesting a relationship between resting-state connectivity and language lateralization.

Methods: Subjects underwent two scans each of resting-state and language fMRI. Resting-state scans required the subject to relax and fixate on a cross. Language fMRI involved a noun being presented on a screen and subjects were asked to covertly generate as many verbs as possible associated with that noun. Language activation maps were created to illustrate those regions of the brain exhibiting significant activation in response to task performance. Connectivity maps were created to illustrate those areas of the brain that exhibited coherent activity with the IFG from the left hemisphere.

Results: Language related activation was consistent with previous findings, showing significant activation in the IFG (i.e., Broca's area). Resting-state connectivity maps exhibited inter-hemispheric connections between homologous language-related areas for both hemispheres. There was a linear relationship between resting-state connectivity and language lateralization measures.

Discussion: Subjects exhibiting greater inter-hemispheric communication during rest recruit language areas of the right hemisphere to a lesser degree. One explanation for the observed relationship is that those areas connected to Broca's area in the left hemisphere act silently during performance of a language task; whereas, when language areas are not strongly connected between the hemispheres, language-related areas in the right hemisphere are activated to help perform language tasks.

Significance: Connectivity may be a potential alternative for understanding language dysfunction in patients who have difficulty performing language tasks.

Functional Comparison of NCX1.4 and NCX2.1 Using Chimaeric Exchangers

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Introduction: The sodium-calcium exchanger (NCX), an ion counter-transporter essential for trans-sarcolemmal Ca^{2+} removal and homeostasis, is expressed as two isoforms (NCX1.4 and NCX2.1) in the brain. Located on different chromosomes, these NCX isoforms are differentially expressed in neurons and glial cells, and despite high primary structural similarity, they have distinct ionic regulatory profiles. NCX1.4 exhibits a pronounced Na^+ -dependent inactivation that is relieved by high intracellular Ca^{2+} levels, whereas NCX2.1 has intermediate inactivation which is stabilized by calcium. The regulatory regions in the intracellular loop involved in ionic regulation are present in all NCX isoforms but apparently confer different phenotypes. The XIP region, believed to regulate Na^+ -dependent inactivation, displays 70% identity between NCX1 and NCX2. The region corresponding to the mutually-exclusive exon of the alternative splicing region, located in the calcium binding domain, has little sequence similarity, and therefore may have an impact on regulation. To investigate the functional differences attributable to these regulatory regions of NCX1.4 and NCX2.1, chimaeras were constructed which interchanged these regions amongst the two parent exchangers.

Methods: Chimaeric constructs (NCX1.4 background with either the exon corresponding region and/or the XIP region from NCX2.1 and *vice versa*) were expressed in *Xenopus* oocytes and outward Na^+ - Ca^{2+} exchange activity was assessed using the giant, excised patch technique.

Results: Replacement of either the mutually exclusive exon or the XIP region in NCX1.4 with the corresponding region from NCX2 disrupts the native ionic properties of NCX1.4 which are re-established when both regions are exchanged simultaneously. However, NCX2.1 maintains its regulatory phenotype when either region is substituted with the analogous sequence from NCX1.4. Interestingly, the NCX1.4-like current profile is exhibited when both exon A and XIP 1 are expressed in an NCX2.1 background.

Conclusion: The mutually-exclusive exon and the XIP regions are critical determinants of ionic regulation and current expression for NCX1.4, but not for NCX2.1. The presence of both regions is fundamental, as synergy between these two areas is key in modulating the regulatory profile. NCX2 regulation does not seem as stringent as NCX1.4, as replacement of individual regulatory regions has little effect on the native phenotype. Our study highlights the long range interactions between these important regulatory domains of NCX1.4 and NCX2 and provides insight into their cell-specific cellular function

***Chlamydia Pneumoniae* Infection Impairs Contractility and Endothelium-Dependent Relaxation in Porcine Coronary Arteries**

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Introduction: *Chlamydomphila pneumoniae*(Cpn) infection has been associated with progression of atherosclerosis by inducing arterial thickening through stimulation of vascular smooth muscle cell proliferation. However, it remains unclear whether Cpn infection in the absence of the immune system can induce vascular dysfunction. We hypothesize that Cpn infection will lead to impaired contractile and relaxation responses in isolated porcine coronary arteries.

Methods: Left anterior descending coronary arteries isolated from porcine hearts were cut and placed in culture medium for 72 hours prior to administering 1 of 3 treatments: live Cpn, heat-inactivated (HI) Cpn, no treatment (Ctrl). Contractile response to 47mM KCl and 0.3nM-300nM U46619 and relaxation response to 1×10^{-10} - 1×10^{-6} M bradykinin (Bk) and 1×10^{-7} - 10^{-4} M sodium nitroprusside (SNP) were measured with a force transducer at days 0, 5 and 10 post-infection.

Results: Infection was confirmed through observation of localized Cpn inclusion bodies in the smooth muscle layer by immunofluorescent staining. Furthermore, there was a significant increase in Cpn-hsp60 protein expression from day 0-10 in Cpn infected tissue indicating the presence of metabolically active Cpn within those vessels. Contractile and relaxation responses remained unchanged in Ctrl tissue for all three time points. In comparison to HI treated vessels, Cpn infected vessels showed decreased contractile response of 49% ($p < 0.05$) and 30% ($p < 0.05$) to KCl and U46619, respectively, at day 5 post-infection and decreased relaxation response of 37% ($p < 0.05$) to Bk at day 10 post-infection. No change in relaxation response to SNP was observed between treatments at both time points.

Conclusion: Our data suggest Cpn infection leads to an impairment of overall contractile function and endothelium-dependent relaxation.

The Use of Psychopharmacotherapy in out of Home Care: A Psychopathological Portrait of Children Living in Therapeutic Foster Care and Group Homes

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Psychotropic drug use in children is a recent phenomenon, which few studies have explored. Knowledge remains limited as to the prescription motive of such drugs, the profile of medicated youth and the importance given to this practice in treating psychosocial problems. Children residing in foster care, because of their high rates of mental health problems, seem to fit the category of a population which appears to be particularly subject to psychopharmacotherapeutics. Although rates of psychotropic drug prescription are very high in foster children, varying between 13% and 77%, few studies have taken interest in this phenomenon (Brelan-Noble & al., 2004; Raghavan & al., 2005; Zima & al., 1999). The present study aims to identify socio-demographic and psychopathological characteristics associated to psychotropic medication use in children living in foster care. A semi-structured interview was carried out with children, ages 6 to 12, residing in group homes in order to assess their symptoms (n=60). Furthermore, educators were asked to complete a questionnaire to provide information regarding prescribed medication and to assess the child's symptoms and overall functioning. Moreover, children's files were studied in order to gather information concerning the child's traumatic experiences, the original motive for placement and the placement stability. These research steps will allow us to gain an in-depth portrait of the potential factors associated with psychotropic drug prescription. The furthering of knowledge will certainly follow from the results of this large study, in turn stimulating reflection on the increasing practice of medication and on the specific clinical needs of the children in question.

Breaking Down Barriers to Program Participation

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Background: While it is clear that some children live in circumstances which make it difficult for them to develop the health status and capacities needed to succeed later in life, it is less clear how to reduce this inequity. Previous research has focused on determining the factors that place children at risk for impaired childhood outcomes or on evaluating specific programs. Little research has tapped into the collective wisdom of service providers across multiple programs.

Methods: During the first phase of this qualitative policy study, 28 service providers from 23 programs in Regina were interviewed. As part of the interview they were asked to outline the barriers they believe impede target families' participation in programs and the strategies that could address these barriers. Interviews were audio-taped, transcribed and analyzed using NVIVO⁷ software. During the second research phase, three focus groups of parents from the target population were asked to provide feedback regarding the barriers and strategies identified by the service providers. These parents confirmed the themes and provided personal accounts of similar situations they had faced. Throughout this project we employed a participatory approach designed to enhance knowledge transfer. Preliminary Phase One results were presented to local organizations at meetings in order to elicit their feedback regarding the interpretation of the findings and to seek their advice on how they would like the results disseminated. The organizations recommended that results be provided to them in a format that would be useful for program planning and preparation of grant proposals and be distributed to policy-makers at various levels of government.

Results: Service providers described two main categories of barriers: psychosocial barriers (e.g. fear) and structural impediments to participation (e.g. transportation). Strategies to address these fell into the same two categories.

Conclusion: The structural barriers that prevent these families from accessing potentially helpful programs are closely tied to the everyday challenges they face. Overcoming them will require substantial resource reallocation. Addressing the psychosocial barriers, on the other hand, will require more of a shift in organizational attitude and approach.

The Cost-effectiveness of Population-based Breast Cancer Screening

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In Canadian women, breast cancer continues to lead in incidence and is the second leading cause of cancer mortality. It has been estimated that 1 in 9 women will develop breast cancer in her lifetime, and 1 in 27 will die from it. Organized population-based breast cancer screening programs have been implemented in most provinces and territories across Canada since 1988. All programs screen women aged 50-69 with biennial two-view mammography, however some provinces screen women outside this age range and more frequently. There is an assumption that the process by which early detection can change prognosis of disease is through stage-shifting, where the higher proportion of screened detected cases would have disease stages with better prognosis. When proposing the implementation or change of a population-based cancer screening program, the tradeoffs between the benefits (increased number of early-stage tumours detected, reduced disease-specific mortality) and harms (false positive and negative tests, radiation exposure, anxiety, and costs) should be examined. The purpose of this research is to systematically review the economic evaluations literature of population-based breast cancer screening programs. This work is aimed to specifically inform the development of a modeling study of the cost-effectiveness of breast cancer screening policies within the Canadian context. There is conflicting evidence regarding the cost-effectiveness of breast cancer screening, often relating to context that include varying burden of disease profiles (rates of disease and mortality), disease etiology, and health care system characteristics of the population of interest. Challenges in critically assessing and comparing economic analyses include the differences across study elements such as the studies' research question, model type used, analytic perspective, interventions being compared, type of analysis, data sources, study population, costs included, discount rate, currency used, time horizon, and the approach used to assess uncertainty.

DNA Binding Independent Regulation of the p21 Gene by MEOX2 in Endothelial Cells

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Introduction: The mesenchyme homeobox genes, *MEOX1* and *MEOX2*, encode transcription factors that contain an evolutionarily conserved DNA binding domain termed the homeodomain. *MEOX2* is a key regulator of vascular cell function that controls cell proliferation via induction of the cyclin-dependent kinase inhibitor, *p21*. It has been shown that ectopic *MEOX2* expression induces apoptosis in proliferating vascular smooth muscle cells and that this apoptotic cell death can be exacerbated with hypoxic treatment. *Meox1/Meox2* gene knockout studies in mice have shown that the MEOX proteins can partially compensate for one another during development, however *MEOX1* function in the vasculature has not yet been studied. The objectives of our study in endothelial cells were to: 1) Establish the requirement of the homeodomain in *MEOX2* activation of the *p21* gene. 2) Determine whether *MEOX1* can also activate transcription of the *p21* gene. 3) Determine whether ectopic *MEOX1* and *MEOX2* expression results in apoptotic cell death.

Methods: MEOX proteins were expressed in primary human umbilical vein endothelial cells (HUVEC) via adenoviral transduction. Cell viability was determined using an MTS assay while apoptosis was assayed by TUNEL staining. Hypoxia was achieved using a chamber containing 95% nitrogen and 5% carbon dioxide at 37°C. The ability of MEOX proteins to regulate *p21* gene expression was determined by luciferase reporter gene assays, western blot analysis and quantitative real-time PCR.

Results: *MEOX2* increased transcription from a *p21* promoter 2-fold compared to an empty vector control and correspondingly increased endogenous *p21* protein levels when expressed in HUVECs. *MEOX1* was also able to activate transcription from a *p21* promoter, however adenoviral transduction of *MEOX1* did not significantly increase endogenous *p21* protein expression in HUVECs. Interestingly, a DNA binding deficient, but not a homeodomain deleted, version of *MEOX2* was able to induce endogenous *p21* protein expression in HUVECs. At the level of ectopic MEOX expression achieved by adenoviral transduction, we did not observe any significant changes in cell survival or apoptosis. In addition, hypoxic treatment of HUVECs did not increase apoptotic cell death in these cells.

Conclusions: Taken together, we conclude that *MEOX2*, but not *MEOX1*, plays a role in cell cycle control via transcriptional regulation of the *p21* gene in endothelial cells. Furthermore, *MEOX2* regulation of the *p21* gene occurs via a mechanism that requires the homeodomain, but is independent of DNA binding. Finally, we conclude that the MEOX proteins do not modulate cell death in primary endothelial cells.

IL-17 Enhances IL-1 β Mediated CXCL-8 Release From Human Airway Smooth Muscle Cells

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Introduction: Recent studies into the pathogenesis of airway disorders such as asthma have revealed a dynamic role for airway smooth muscle cells in the perpetuation of airway inflammation via secretion of cytokines and chemokines. In this study, we evaluated whether IL-17 could enhance IL-1 β mediated CXCL-8 release from human airway smooth muscle cells (HASM) and investigated the upstream and downstream signaling events regulating the induction of CXCL-8.

Methods: CXCL-8 mRNA and protein induction were assessed by real-time RT-PCR and ELISA from primary HASMC cultures. HASMC transfected with site mutated AP-1/NF κ B CXCL-8 promoter constructs were treated with selective p38, MEK-1/2 and PI3-K inhibitors.

Results: We demonstrate IL-17 induced, and synergized with IL-1 β to up-regulate CXCL-8 mRNA and protein levels. Erk-1/2 and p38 modulated IL-17 and IL-1 β CXCL-8 promoter activity however IL-1 β also activated the PI3-K pathway. The synergistic response mediating CXCL-8 promoter activity was dependent on both MAPK and PI3-K signal transduction pathways and required the cooperation of AP-1 and NF κ B cis-acting elements upstream of the CXCL-8 gene.

Conclusion: Collectively, our observations indicate MAPK and PI3-K pathways regulate the synergy of IL-17 and IL-1 β to enhance CXCL-8 promoter activity, mRNA induction and protein synthesis in HASMC via the cooperative activation of AP-1 and NF- κ B trans-acting elements.

Characterization of Histone H3 Kinases, MSK1 and MSK2

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Introduction: Stimulation of the Ras-MAPK signal transduction pathway by growth factors (EGF) or phorbol esters (TPA) in parental (10T^{1/2}) and oncogene (*H-ras*)-transformed (Ciras-3) mouse fibroblasts induces rapid phosphorylation of histone H3. Phosphorylation of H3 occurs on Ser¹⁰ and Ser²⁸ in the NH₂-terminal tail. This phosphorylation event is implicated in the regulation of immediate early genes such as *c-fos* and *c-jun*. Constitutive activation of the Ras-MAPK pathway in *ras*-transformed mouse fibroblasts increases phosphorylation of H3 at Ser¹⁰ and Ser²⁸ and we have shown that this increase is due to enhanced activity of histone H3 kinase, mitogen- and stress-activated protein kinase 1 (MSK1).

Methods: Functional characterization of the MSK complex will be undertaken in 10T^{1/2}, Ciras-3 and HEK293. Chromatin remodeling and histone acetyltransferase (HAT) activity assays will be used. Chromatin remodeling activity of the MSK complex will be investigated by isolating the MSK complex from 10T^{1/2}, Ciras-3 and HEK293 (cycling, serum-starved, TPA/EGF treated) cells and performing HAT and mononucleosome disruption assays.

Results: MSK1 is associated with SWI/SNF ATPase (Brg1) and histone H3 acetyltransferase (HAT) [PCAF], as well as with c-Fos/c-Jun, p65 sub-unit of NFκB, 14-3-3 proteins, but not with HDAC1. Furthermore, HAT activity assay results suggest that the MSK complex contains histone acetyltransferase activities.

Conclusion: Ras-MAPK activated histone H3 kinase MSK1 associates with chromatin modifying activities. Since the Ras-MAPK signaling is frequently deregulated in cancer (30% of human cancers contain aberrant *ras*), characterization of the Ras-MAPK activated MSK complex and its associating activities could provide a basis for the assessment of MSK as a novel therapeutic target to treat cancer.

Elaidic and Vaccenic Trans Fatty Acids have Different Effects on Atherosclerotic Development

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Introduction: Epidemiological evidence has associated trans fatty acids (TFA) in the diet to coronary heart disease. The source of TFAs in our diet has been largely focused on hydrogenated fats like elaidic acid but it is known that dairy products also contain substantial amounts of naturally occurring TFAs like vaccenic acid. These TFAs may have a direct effect on atherosclerosis. The purpose of this study was to determine the effects of consuming TFAs from two sources, either a commercially hydrogenated vegetable shortening rich in elaidic TFA (C18:1t-9) or a butter rich in vaccenic TFA (C18:1t-11), on atherosclerosis in the presence or absence of elevated dietary cholesterol.

Methods: LDLr^{-/-} mice were fed one of 8 experimental diets for 14 weeks: regular fat (RG), elaidic shortening (ES), regular butter (RB), vaccenic butter (VB) or an atherogenic diet containing 2% cholesterol, alone (CH) or supplemented with elaidic shortening (CH+ES), regular butter (CH+RB), or vaccenic butter (CH+VB). The extent of atherosclerosis was quantified by en face examination of the dissected aortae.

Results: Serum CH levels were elevated with either butter or CH feeding, whereas serum triglyceride (TG) levels were elevated in mice consuming either butter or a combination of CH and ES, RB, or VB. Serum CH and TG levels were significantly lower in the CH+VB group as compared to the CH+RB group. Atherosclerosis was limited if the diet was not supplemented with CH. However, the addition of ES to the diet did stimulate atherogenesis. VB did not induce an increase in atherosclerotic plaque formation. Atherosclerosis was extensive following CH feeding. The addition of ES to the CH diet did not advance atherosclerosis beyond CH alone. In contrast, the CH+VB diet significantly reduced atherosclerosis as compared to the CH, CH+ES, and CH+RB diets.

Conclusion: In summary, consuming manufactured TFAs rich in elaidic acid initiates atherosclerotic development but not beyond the effects of dietary cholesterol alone. However, consuming a vaccenic acid rich butter protects against hyperlipidemia and atherosclerosis in the presence or absence of additional dietary cholesterol.

Protective Effect of Sphingosine 1-Phosphate (S1P) in the Cerebral Microvasculature

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Introduction: Sphingosine 1-phosphate (S1P), an endogenous phospholipid, is involved in pre-conditioning responses in the heart. The present study evaluated whether exogenous S1P could have a similar protective role in the cerebral microvasculature following hypoxic insult.

Methods: Human brain microvessel endothelial cells (HBMECs) were exposed to oxygen-glucose deprivation (OGD) for 0-6 hours. Cell viability was assessed using MTT assay immediately after OGD or following a 24-hr re-oxygenation period. The effects of S1P on OGD-mediated cell toxicity was determined by pre-treating the cells with various concentrations of S1P (0-10 μ M) for 0, 1, or 24 hours.

Results: Exposure of HBMECs to OGD resulted in a significant decrease (approximately 20%) in cell viability when assessed immediately after OGD. Pre-treating HBMEC cells with S1P for 1 hr prior to OGD provided a dose dependent protection, with 1.0 and 10 μ M S1P completely reversing the loss in cell viability associated with OGD at all time points examined. However, the protective effects of S1P were not apparent in the 24-hour pre-treatment protocol.

Conclusion: Activation of S1P receptors within the brain microvasculature prior to hypoxic events may help prevent toxicity to the cerebral microvasculature.

The Role of Calmodulin in the Activation of Human Platelet Rac1

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Introduction: Rac1 belongs to the Rho family of small GTP-binding proteins and has been implicated in lamellipodia formation. Calmodulin (CaM), a ubiquitous Ca²⁺-binding protein, has been shown to interact with and regulate the activity of several small GTP-binding proteins. In the present study, we have investigated if CaM interacts with Rac1 and regulates the activation step of this GTPase.

Methods: Rac1 was expressed as a GST fusion protein (GST-Rac1). *In vitro* pull down assay using GST-Rac1 was used to assess interaction between pure CaM and endogenous CaM in platelet lysate. In the reverse experiment, CaM coupled to Sepharose beads was used to pull down Rac1 from platelet lysate. The role of CaM in thrombin mediated activation of platelet Rac1 activation was assessed in the absence and presence of W7, a calmodulin inhibitor.

Results: Both endogenous and pure CaM interacted with Rac1 in a Ca²⁺-dependent manner. Using CaM database analysis a 14 amino acid sequence in Rac1 was identified as the CaM-binding region. The peptide representing the putative CaM-binding region in Rac1 bound CaM while the scrambled form of the peptide did not bind CaM. The role of CaM in Rac1 activation was investigated using freshly isolated human platelets. Maximal activation of platelet Rac1 in response to thrombin occurred at ~60 sec. Incubation of platelets with the CaM inhibitor, W7, prior to thrombin challenge, abolished thrombin induced activation of Rac1.

Conclusion: The results demonstrate that in platelets, binding of CaM to Rac1 increases its activation and that CaM may play an important role in the regulation of cytoskeleton remodelling.

Evaluation of a Distance Education Palliative Care Course in Cape Town, South Africa

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Introduction: In 2000, responding to a dearth of palliative care training opportunities for doctors in Africa, the University of Cape Town (South Africa) began a post-graduate distance education program in the field of palliative medicine. The primary goal of this research project was to evaluate the educational experiences of both current students and graduates of this program. These cohorts of trainees were surveyed, in order to gain insight into the efficacy and the application of this approach in shaping the provision of palliative and end-of-life care in South Africa.

Methods: To assess the impact of this educational initiative, data was collected using a General Survey and a Graduate Survey, both designed to evaluate specific aspects of the course and trainee impressions. The General Survey evaluates competence in five areas: Palliative Care Activities, Pain, Assessment and Management, General Concepts in Palliative Care, and Changes in Treatment. The Graduate Survey assesses the impact of the course in areas such as End-of-Life in South Africa, Team Approach, Clinical Assessment, and General Aspects of Palliative Care. The General Survey and Graduate Survey scores can range from 0-100, with higher scores reflecting higher perceived competence. Survey data was analyzed using SPSS 11.0. Qualitative analysis of open-ended responses as well as quantitative analysis of survey data was completed.

Results: Of the 125 current or past students invited to complete the General Survey, 83 (66.4 %) participated and of the 67 graduates of the program, 41 (or 65.7%) returned a completed Graduate Survey. The mean score for the General Survey was 75.0 (SD 12.9) with scores ranging from 34.0 to 93.0. In all survey sections, males (n=35) had higher scores than did females (n=47), yet the scores were not significantly different [$t(80)=1.5$, $p=0.1$]. An analysis comparing scores of graduates to current students determined that the total scores for graduates was higher [current students- mean 71.4 (SD 14.2); graduates- mean 79.0 (SD 9.9); $p < 0.01$]. Graduates demonstrated significantly higher scores than current students in all categories (p values ranged from 0.01 to 0.05), except "Assessment and Management". A thematic analysis of the Graduate Survey identified critical elements of the distance education curriculum, changes evident in practitioners, and improvements in patient and family care.

Conclusion: The results of both the Graduate Survey and General Survey provide evidence that the palliative care distance education course is effectively training physicians in South Africa.

PROX1 Determines the Lymphatic Phenotype in Endothelial Cells via Distinct Mechanisms

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Introduction: PROX1, a homeodomain protein, induces and maintains the lymphatic phenotype in endothelial cells by down regulating the Blood Endothelial Cell (BEC) markers, while upregulating the Lymphatic Endothelial Cell (LEC) markers at the same time. To explore the mechanism by which PROX1 establishes endothelial cell fate, we compared PROX1 mediated activation of vascular endothelial growth factor receptor-3 (VEGFR-3) to its regulation of fibroblast growth factor receptor-3 (FGFR-3) genes.

Methods: We generated and cloned versions of Prox1 lacking putative functional domains in the pCMV-Tag 4A expression plasmid. We also produced 2 different adenoviral vectors containing either wild type Prox1 (wt) or DNA binding domain deleted Prox1 (HDPD Δ) to infect primary endothelial cells. Using luciferase gene reporter assay and immunoblotting, we compared the ability of different Prox1 constructs to activate either the VEGFR-3 or FGFR-3 genes at mRNA and protein levels.

Results: DNA binding deficient Prox1 versions, which were unable to activate the FGFR-3 promoter, still efficiently activated transcription of the VEGFR-3 promoter. We also showed that nuclear receptor domains (NR1 and NR2) and the glutamine rich domain (Q rich) of PROX1 are necessary for full activation of the FGFR-3 promoter, but not required for activation of the VEGFR-3 promoter. Our immunoblotting results in Human Umbilical Vein Endothelial Cells (HUVECs), which were infected with either wt or HDPD Δ adenoviral vectors, showed that both constructs induced significant increases in VEGFR-3 protein levels, but only the wt construct was able to increase the level of FGFR-3 protein in these cells.

Conclusion: In total, our data demonstrates that in endothelial cells PROX1 utilizes two distinct mechanisms to activate two key endothelial growth factor receptors.

Alteration of C-mannosylation Sites on Ebola Virus Glycoproteins Affects Particle Entry

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Ebola virus (EBOV) produces multiple soluble products from its glycoprotein gene including sGP, delta-peptide, ssGP, GP₁ and GP_{1,2delta}™ in addition to the membrane-bound viral spike protein GP_{1,2}. We have identified a novel form of glycosylation, termed C-mannosylation, as being present on multiple EBOV glycoproteins. This is the first description of this modification on any viral protein. A mannose residue, linked via a carbon-carbon bond, was identified on sGP as well as GP₁ on its predicted motif – WXXW. This motif is also present at two adjacent locations (WTGWRQW) on GP₂; however, it is still unclear whether these tryptophans are actually modified. The alteration of the C-mannosylation motifs on GP₂ significantly reduced the ability of infectious virus-like particles (iVLPs) to enter target cells but did not affect the budding or assembly of these iVLPs. Infectivity was partially restored by replacing the tryptophan with a phenylalanine, which has been previously described as allowing a reduced level of C-mannosylation to occur. While it is still unclear whether this effect on entry is due to the presence of the C-mannose or the alteration of the tryptophan, it is clear that these tryptophan residues, located near the transmembrane domain of GP₂, serve an important role during virus entry. To determine the significance of these observations, reverse genetics was used to generate these mutations in the full-length infectious clone in order to determine whether they affect the ability to rescue these viruses. Further characterization of the function of C-mannosylation of EBOV glycoproteins may provide insight into the role of this post-translational modification in virus entry.

Gambling Among Immigrants in Quebec and the Rest of Canada

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Gambling is an activity that cuts across barriers of race, gender, and culture. Although historically not all cultures have gambled, currently few if any are considered non-gambling cultures. The majority of Canadians gamble and consider gambling a form of distraction and entertainment. Gambling is legal in all 10 provinces and three territories in Canada and most parts of the United States. For most who gamble, the games are a form of harmless entertainment, but for others gambling becomes excessive and problematic. The increased popularity of gambling and the proliferation of gambling venues have also brought an increase in the prevalence of problem gambling. Current prevalence studies suggest that pathological gamblers represent 2-4% of the population. Generally, vulnerability to gambling pathology is unequally distributed within a population. Certain members of a given community are more likely to gamble, and more likely to succumb to gambling addiction. Factors that likely contribute to proclivity for gambling and the development of related pathology include lower income and lower education. Few general population surveys have examined associations between problem and pathological gambling and immigration status in Canada. Yet, the population of Canada is composed of a large proportion of immigrants. Immigrants to Canada have a higher likelihood of finding themselves in situations of chronic low income and experience a large gap in their earnings compared to their non-immigrants counterparts and face deterioration in their economic status. Despite the dramatic rise in educational attainment of entering immigrants, the more recent immigrants are more likely to begin their lives in the new country with low incomes. This study examines the gambling behaviors of immigrants in Canada and compares them to the non-immigrant population. It also compares immigrants and non-immigrants gambling engagement in across the 10 provinces in Canada in order to assess inter-provincial differences. The findings of this study do not support the hypothesis that immigrants are more likely to gamble, or to develop gambling problems, despite earning significantly lower incomes than their Canadian counterparts. The role of immigrants' educational achievement is discussed as a possible mediating factor.

Development of an *in vivo* Rat Model for Induction of Spermidine/Spermine N¹-Acetyltransferase

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Introduction: Amantadine is acetylated to N-acetylamantadine (ACA) solely by spermidine/spermine N¹-acetyltransferase (SSAT1). Diethylnorspermine tetrahydrochloride (DENSPM) and alcohol are representative agents reported to induce SSAT activity *in vitro*.

Methods: In five experimental series, rats were exposed to three concentrations of alcohol in their drinking water, DENSPM alone, or DENSPM combined with alcohol. After each exposure amantadine HCl solution (3mg/kg) was injected i.p. and total urine was collected over 0-6 and 6-24 h. The volume and the pH of all urine samples were measured; then the samples were frozen at -20°C until analyzed for ACA by liquid chromatography/mass spectrometry. Pre- and post-intervention controls were completed where the rats were allowed to drink water for one week and then dosed with amantadine HCl to determine urine content of ACA.

Results: Both alcohol and DENSPM induced SSAT1. The control production of ACA was absent or present only in trace amount in rat urine. Exposure to alcohol increased the amount of ACA production which was proportional with increasing concentration and duration of alcohol in drinking water. The amount of ACA in the 6-24 hr urine collection was greater than in the 0-6 hr sample, however, the rate of ACA produced/hr is more in 0-6 hr samples. In post DENSPM study the amount of ACA produced and the rate of its production in the 0-6 hr specimen was greater than in the 6-24 hr specimen and DENSPM was more powerful inducer than alcohol. Combined exposure to alcohol and DENSPM caused greater than expected increase in urinary ACA excretion.

Conclusion: Longer exposure to alcohol combined with DENSPM administration would appear to provide the greatest potentiation of SSAT1 activity which indicates high likelihood of synergy between alcohol and DENSPM. This model will be valuable to determine the contribution of SSAT1 to acetylation of drugs administered as therapy in various disease states where SSAT1 is induced.

Agonist-Induced Actin Polymerization in Neonatal Pulmonary Arterial, But Not Aortic Myocytes is Independent of Rho and Rac

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Introduction: Normal postnatal disassembly of pulmonary arterial myocyte actin cytoskeleton is impeded by hypoxia. Actin polymerization stabilizes myofilaments and increases smooth muscle force generation. We compared naïve and agonist-induced actin polymerization (APM) in contractile and synthetic myocytes from pulmonary artery and aorta, under hypoxic and normoxic conditions.

Method: Primary cultured myocytes from neonatal porcine descending aorta (Ao) or pulmonary artery (Pa) were serum fed (S+, synthetic) or serum starved (S-, contractile) and grown in hypoxia (HM, 10% O₂) or normoxia (NM, 21% O₂) for 7d, with or without Clostridium C3 transferase (Rho inhibitor) or NSC23766 (Rac inhibitor) for 3d. Cells were challenged with 10⁻⁶M thromboxane mimetic U46619 or diluent for 30 min prior to fixation. F/G actin ratio was quantified in digital images captured under standardized conditions to visualize filamentous actin (Texas red-phalloidin) and globular actin (Oregon green-DNAse).

Results: Hypoxia induced 20-fold APM in S- Pa myocytes. Inhibition of either Rho or Rac in Pa myocytes decreased hypoxic APM in S-. U46619 stimulation markedly increased APM in hypoxic and normoxic S- Pa myocytes; neither inhibition of Rho nor Rac attenuated this effect. Hypoxia increased APM in S- Ao myocytes 5-fold, attenuated by Rac inhibition; in S+, inhibition of Rho or Rac increased APM. Aortic APM increased on agonist exposure of S+ HM, but decreased in agonist challenged S- HM. Rho inhibition did not alter this relationship; Rac inhibition decreased agonist-induced APM in all S+ cells and in S- HM, while increasing it in S-NM. All cited comparisons have p<0.001.

Conclusion: Pulmonary arterial myocytes exhibit marked APM to both hypoxia and vasoconstrictors. Hypoxic pulmonary arterial APM requires Rac and Rho, while agonist-induced APM is independent of both Rac and Rho signaling. Hypoxic aortic myocytes polymerize actin to a lesser degree, and depolymerize to agonist; all aortic APM owes to both Rac and Rho.

The Molecular Details of Mucopolysaccharidosis IIIB Revealed by the Structure of a Bacterial α -N-acetylglucosaminidase

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Mucopolysaccharidosis III (MPS III), or Sanfilippo syndrome, has four forms (A-D) that result from build up of an improperly degraded glycosaminoglycan, heparan, in lysosomes. MPS IIIB is attributable to the decreased activity of a lysosomal α -N-acetylglucosaminidase (*HsGH89* or NAGLU). The structure, catalytic mechanism, and inhibition of a close bacterial homolog of *HsGH89* from *Clostridium perfringens*, *CpGH89*, will be described. The structure enables the generation of a homology model of human *HsGH89*, an enzyme which has resisted structural studies despite having been studied for over 20 years. This model reveals which mutations that give rise to MPS IIIB map to the active site and which map to regions distant from the active site. The identification of potent inhibitors of *CpGH89* and the structures of these inhibitors in complex with the enzyme suggest small molecule candidates for use as chemical chaperones. These studies therefore illuminate the genetic basis of MPS IIIB, provide a clear biochemical rationale for the necessary sequential action of heparan degrading enzymes, and open the door to the design and optimization of chemical chaperones for treating MPS IIIB arising from mutations distant from the active site.

EEG-derived Source Density Analysis (sLORETA) of Auditory Hallucinations in Schizophrenia Across Different Background Noise Conditions

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Purpose: Given the heterogeneity of schizophrenia, it has been suggested that studying individual symptoms, rather than global syndromes, may elucidate more focused information on the nature of this disease. Auditory hallucinations (AH) are a pervasive symptom, and one that may usurp valuable cognitive resources within the brain. We examined the impact of AHs on cortical response to three sound environments of different complexity.

Methods: Participants were 12 schizophrenia patients with auditory hallucinations (HP), 12 schizophrenia patients without auditory hallucinations (NP) and 12 healthy controls (HC). All participants were exposed to 3 auditory stimulation conditions (speech only, speech + white noise, speech + traffic noise) while EEG was recorded from 32 channels. EEG was processed offline using standardized Low Resolution Brain Electromagnetic Tomography (sLORETA).

Results: Within the speech-only condition, HPs showed significantly ($p < .05$) increased delta activity (vs NPs) at the left middle frontal gyrus (BA10), the left superior frontal gyrus (BA11) and the left anterior cingulate (BA32).

Within the speech + traffic noise condition, HPs showed more theta activity than HCs at the right inferior parietal lobule (BA40), and postcentral gyrus (BA2) which was significant at the trend ($p < .1$) level, while NPs showed significantly ($p < .05$) more theta activity than HCs at the left inferior parietal lobule (BA40).

Within the speech + white noise condition, HPs showed significantly ($p < .05$) more theta activity than HCs at the left middle temporal gyrus (BA39) and left superior temporal gyrus (BA22).

Conclusion: Schizophrenia patients with auditory hallucinations show a unique pattern of cortical activation during sound exposure, including deactivation of the left frontal lobe in the presence of speech (vs. NPs), deactivation of the right inferior parietal lobule in the presence of speech and complex noise (vs. HCs) and deactivation of the left auditory cortex in the presence of speech and white noise (vs. HCs).

Linking DLX Transcription Factors to the WNT/ β -Catenin Signaling Pathway: Implications for Intestinal Development and Colorectal Carcinoma

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Objectives: Colorectal cancer (CRC) is the fourth-most common cancer in Canada with a high mortality rate. An early morphologic change known as aberrant crypt foci (ACF) ultimately leads to adenomatous polyps. Genetic changes are well described in the adenoma-carcinoma progression model. Familial adenomatous polyposis (FAP) is a hereditary form of CRC; FAP patients carry germline mutations of the tumour suppressor gene adenomatous polyposis coli (APC). However, the majority of sporadic adenomas and CRC have somatic APC mutations. The function of DLX genes in intestinal development has not been previously explored.

Methods: We will assess DLX1 and DLX2 expression throughout intestinal development, identify the specific intestinal cell types in which DLX genes are expressed, and determine the intestinal phenotype of the *Dlx1/Dlx2* double knockout mouse. Using a chromatin immunoprecipitation (ChIP) assay of intestinal mucosa, we will isolate a region of the *Apc* gene promoter bound to DLX2. We will characterize this DLX2 protein-*Apc* gene interaction using gel-shift and reporter gene assays, and assess APC expression in *Dlx* loss- and gain-of-function models. We will also cross the *Dlx1/Dlx2* mutant mouse with a Wnt signaling reporter mouse to determine the effect of loss of *Dlx* function on the Wnt signaling pathway *in vivo*. We will determine DLX2 expression in rat ACF, intestinal polyps and tumours of *Apc*^{MIN} mice, CRC cell lines and in CRC tumour specimens.

Results: We have found that DLX2 is highly expressed in mouse embryonic and adult intestinal epithelia. Moreover, DLX2 is expressed in a carcinogen-induced rat ACF model as well as in some human colorectal cancer cell lines and tumours. Of particular interest, we have discovered that DLX2 is bound to the APC gene promoter *in vivo*, co-expressed with APC, and that there is decreased APC expression in the *Dlx1/Dlx2* mutant mouse.

Conclusion: Unlike detection of APC mutations, regulation of APC expression is poorly understood. Ultimately, restoring APC expression may be a novel strategy towards preventing progression of intestinal polyps to adenocarcinoma. This research will contribute to our knowledge of the genetic and epigenetic regulatory pathways that control intestinal development, mucosal self-renewal and CRC.

CD8 α positive dendritic cell (DC) is more protective against mycobacterial infection compared with CD8 α negative DC

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Objective: Tuberculosis infection is a serious public health problem worldwide. The World Health Organization (WHO) estimates that about one third of the world's population is currently infected with *Mycobacterium tuberculosis*. Protective anti-mycobacterial immunity relies on type-1 T cell responses. DC is important for the activation and differentiation of T cells. We investigated the role of DC subsets in initiating protective immunity against mycobacterial infection

Methods: DC subsets from the spleen of BCG infected mice were isolated using MACS columns and were analyzed for surface markers, cytokine production and *in vivo* function after adoptive transfer to syngeneic mice followed the BCG infection.

Results: Although both DC subsets (CD8 α ⁺ and CD8 α ⁻) from BCG-infected mice showed increase in the expression of surface markers, but CD8 α ⁺DC exhibited high levels of mRNA for TLR-2, TLR-9, ICOS-L and IFN- α than CD8 α ⁻ DC. Moreover, CD8 α ⁺ DC from infected mice (CD8 α ⁺ DC_{i,f}) produced significantly higher IL-12p40 but lower IL-10 than the CD8 α ⁻ DC_{i,f}. In addition, our data showed less BCG growth and inflammation in the lung and liver of the mice adoptively transferred with CD8 α ⁺ DC_{i,f} following BCG challenge infection compared to those with CD8 α ⁻ DC_{i,f} adoptive transfer or without cell transfer but having the same challenge infection. Spleen cells from CD8 α ⁺DC_{i,f} adoptively transferred mice produced more IFN- γ than those transferred with CD8 α ⁻ DC_{i,f}.

Conclusion: The data demonstrate that DC subsets have different capacity in inducing protective immunity against mycobacterial infection and that CD8 α ⁺ DC is more potent in this capacity than CD8 α ⁻ DC. The finding provides new insight into the mechanism of host defense against tuberculosis infection and may be helpful in guiding the development of prophylactic and therapeutic approaches to tuberculosis.

Development of a Mammalian Cell Based Recombinant Antibody Library Against HIV-1 Envelope Proteins from Human B cells

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Introduction: Analysis of broadly neutralizing monoclonal antibodies (mAbs) to HIV-1 is a crucial step in the development of a prophylactic HIV-1 vaccine. The majority of existing HIV-1 mAbs were picked from a phagemid library generated from subtype B infected individuals. Herein, we describe the initial development of a mammalian cell based recombinant antibody library selection system which utilizes high-throughput cell-based screening with the B cell repertoire of a high-titre HIV-1+ve individual from Nairobi, Kenya.

Methods: This B cell library was derived from human peripheral blood mononuclear cell (PBMC) samples isolated from an HIV-1 seropositive individual from Nairobi with high titer IgG to HIV-1 envelope proteins. The immunoglobulin VH and VL gene cDNA were cloned into mammalian cell expression vectors following RT-PCR amplification from total RNA. The cDNA was amplified using isotype specific oligonucleotide anti-sense primers paired with Fr-1 specific sense primers with restriction sites on board to facilitate cloning. The variable heavy and light chain binding domains were fused in frame to either human IgG1, kappa or lambda constant regions present in the plasmids. A library of full-length antibody secreting cells was created by co-transfection of the two plasmids containing the VH and VL respectively into HEK-293 cells. For a positive control, the potent human monoclonal antibody (mAb) IgG1b12 was also built into the expression system as a full length IgG1/kappa.

Results: Initial tests of the library have included spiking the library with VH or VL of IgGb12, and confirmation of the expression of the positive control. Screening was set up for clonal cells expressing human antibody using ClonePixFL technology. Secondary screening versus monomeric gp120 and gp41, as well as native envelope on the surface of cells was carried out using high-throughput homogenous cell or bead based assays with the ABI-8200 system identifying any HIV-1 Env binding mAbs. Analysis of the neutralization ability and epitope characterization will be carried out for the most potent of the Env binding mAbs.

Conclusion: Screening a fully functional HIV-1 mAb library with native HIV-1 envelope proteins will facilitate the generation of new HIV-1 broadly neutralizing antibodies providing a more comprehensive picture of the epitope and antibody structures involved in the host response to HIV-1, in particular in sub-Saharan Africa.

Autophagy Protects Against Simvastatin-p53 Mediated Apoptosis in Airway Mesenchymal Cells

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Introduction: Airway remodeling, including a marked accumulation of airway smooth muscle and fibroblasts, correlates with duration and severity of asthma. Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase that have pleiotropic anti-inflammatory and anti-oxidant effects, inhibit human airway smooth muscle (HASM) proliferation, and induce apoptosis in many cell types. Statin use has been linked with improved lung health and controlled clinical trials for their use in asthma and COPD are underway.

Methods and Results: We tested the effects of simvastatin on primary cultured HASM and human airway fibroblast (HAF) cell death, and assessed its associated molecular mechanisms. Using MTT and FACS-based propidium iodide uptake assay, we observed a 40% ($P < 0.001$) increase in cell death and DNA fragmentation. Simvastatin suppressed *de novo* cholesterol synthesis, and its effects on cell death were blocked ($P < 0.05$) by downstream products of the HMG-CoA reductase pathway, including mevalonate, farnesylpyrophosphate (FPP), and geranylgeranylpyrophosphate (GGPP). Western blotting confirmed simvastatin activated caspase-3 and -7 and induced poly (ADP-ribose) polymerase (PARP) cleavage in the absence of caspase-8 activation of tBid formation, suggesting involvement of intrinsic apoptosis signaling. We observed a concomitant loss of mitochondrial transmembrane potential ($\psi\Delta m$), increased phosphorylation of p53 at Ser-15 and -37, and increased in Bax, PUMA and NOXA expression. Simvastatin also increased Bax-Bcl2 interaction (immunoprecipitation) and localization of Bax to mitochondria (immunocytochemistry). Our data also revealed that simvastatin induced autophagy as confirmed by: increased LC3- β cleavage, Atg12-Atg5 complex formation, increased beclin-1 expression (immunoblot), and increase in volume and number of cytoplasmic lysosomes and autophagosomes. Autophagy inhibitors, 3-methyladenine (3-MA), bafilomycin-A1 (BAF-A1), and okadaic acid (OKA) nearly doubled cytotoxic/apoptotic effect of simvastatin, and caspase-3/7 and caspase-9 activation was significantly higher in simvastatin exposed HASM and HAF co-treated with BAF-A1 or OKA ($P < 0.05$).

Conclusion: Collectively our results provide first time evidence that simvastatin induces both apoptosis and autophagy in airway mesenchymal cells, and that autophagy appears to suppress the cellular apoptosis. These results have therapeutic implications, as a goal of asthma treatment is to reverse accumulation of smooth muscle and fibroblasts - our data suggest that combined effects of statins and compounds that could selectively inhibit autophagy may be effective in promoting reversal of airway remodeling in chronic lung disease.

Proteomic Approaches to Membrane Characterization

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Membrane proteins are crucial for cellular processes and as such they represent the targets for more than 70% of current biopharmaceuticals. Biochemical and proteomic approaches to the identification and characterization of membrane proteins are still limited by the difficulties associated with their isolation and analysis. We undertook to develop proteomic based methods to identify membrane proteins and to apply them to the analysis of the natural killer-like cell line, YTS. Isolated membranes were treated under conditions designed to remove peripheral proteins. The membranes were trypsin digested and the tryptic peptides were separated by 2D-LC MSMS technique. A total of 2054 proteins were identified and approximately 50% of these could be annotated as membrane proteins based on prediction algorithms or citations. An additional 31 proteins were shown to be acylated using spectral matching criteria. Evidence was also provided for glycosphosphatidylinositol (GPI) linked proteins. However, a significant number (46% of the population) of tightly associated proteins could not be accounted for by any of these mechanisms implying that there may be other modes of tight association. In summary we have developed a strategy for the isolation and characterization of membrane proteins. A complete analysis of these proteins and a clear definition of their interaction modes will provide a better understanding of membrane organization and also may identify new potential drug targets.

Cognitive, Social, and Emotional Aspects of Self-Regulation in Young Children

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This study explored the relations among cognitive, social, and emotional aspects of self-regulation. Current theorizing about the development of self-regulation suggests that different aspects of self-regulation covary during development. One hundred thirty preschoolers (ages 3-5) completed a battery of tasks measuring cognitive control (i.e., executive functions), emotional control (i.e., emotion regulation), social cognition (i.e. false belief understanding), and language ability. Parents completed additional measures of temperament, emotion regulation, and temper tantrums. As predicted, temperamental sadness and anger mediated the relations between emotion regulation and temper tantrums. Social understanding was a significant predictor of both temper tantrums and emotion regulation. Contrary to our predictions, cognitive aspects of self-regulation did not predict emotion regulation or temper tantrums, and general cognitive ability was not related to emotion regulation or temper tantrums. These results suggest that cognitive and emotional aspects of self-regulation may be domain specific rather than domain general. Given that social understanding was related to emotion regulation, these results suggest that over and above the influence of cognitive self-regulation, social experience may be an important factor in the development of emotional self-regulation. This research study represents a first step toward understanding the nature of the relations between temper tantrums and the development of self-regulation in young children. Furthermore, given the importance of emotional regulation for social and academic competence, these results have implications for parenting, school readiness, peer victimization, and the social-emotional health of young children. For example, early intervention programs may need to target specific aspects of self-regulation rather than assuming that practice within one domain will influence deficits within a different domain of self-regulation.

Mirror, Mirror on the Wall: Self-Perceptions, Medical Severity, and Depressive Affect in Youth with a Hidden Congenital Deformity

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Currently, there is little research on self-perception and the psychological impact of concealable deformities such as pectus excavatum (PE; aka funnel chest). The current study addresses this gap by comparing self-perceived severity, actual physical severity, and depressive symptoms in youth with PE. Substantiating concerns by parents and physicians, participants' scores on the Child Depression Inventory and Youth Self-Report depression subscales were significantly higher than non-clinical normative samples, indicating more depressive symptoms. Supporting the importance of subjective self-perception, youths' perceptions of severity correlated strongly with depressive affect (.67 and .86) while there were no significant correlations between a medical index of severity (the Haller Index) and depressive affect. Interestingly, an interaction effect was found between the actual severity of deformity and the self-perceived severity on the subscale that measured anxious/depressed affect. Therefore, self-perceptions mediate the relation between the Haller Index and the anxious/depressed symptoms. These results suggest a) that youth with PE are at greater risk for depressive disorders, and b) that self-perception, not actual physical severity, is the better predictor of the psychosocial impact of the deformity. The results of this study are supported by research that has consistently found that youth with deformity may be more distressed about their appearance, and as a result are more likely to suffer the adverse affects of self-consciousness, such as depression and anxiety problems. This data suggests that social-emotional functioning should be considered when parents, surgeons, and youth are deciding whether to pursue corrective surgery. This is in contrast to the current sole reliance on physiological measures, such as the HI, to determine eligibility for corrective surgery. Further longitudinal research is being conducted to determine whether corrective surgery actually improves youth's psycho-social functioning.

Src Family kinase-dependent Disruption of Endothelial Barrier Function by *Plasmodium falciparum* Merozoite Proteins

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Cerebral malaria and pulmonary edema are two of the most severe complications of *P. falciparum* malaria. The etiology of both conditions is incompletely understood, but is believed to involve a component of vascular leakage. Cerebral edema is not clinically demonstrable, but functional studies have indicated a subtle but definite compromise of the blood brain barrier in both adult and pediatric patients. Malarial lung complication bears the cardinal features of the acute respiratory distress syndrome (ARDS) of which vascular leakage is the major pathology. In this report, we examined the effect of clinical *P. falciparum* isolates on barrier function of primary dermal and lung microvascular endothelium *in vitro*. We showed that parasite sonicates, but not intact infected red blood cells, disrupt endothelial barrier function. The increased vascular permeability is associated with the disruption of endothelial junctional proteins in a Src family kinase-dependent manner, resulting in gap formation. There was no evidence of direct cellular cytotoxicity. The active component in the sonicates appeared to be a merozoite protein. Increased permeability was also induced by purified *P. falciparum* GPIs. These results demonstrate that parasite products can directly alter endothelial barrier function, and thus contribute to the pathogenesis of severe falciparum malaria.

Realist Approach to Theory and Implementation of Knowledge Translation: The Case of a Patient Safety Intervention within an Adult Acute Care Setting

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Background: The knowledge translation field acknowledges that the gap between knowing and doing continues and is even more pronounced where front line health care management occurs. The literature indicates that change is possible but not predictable and models have not been validated. Calls have been made for more theory and context based research approaches. The research question directing this thesis is: How do front line managers working in urban adult acute-care hospitals within the Calgary Health Region implement an evidence based package of patient safety policies?

Methods: A realist approach was used to investigate what works for whom under what circumstances and why and aimed for explanation and understanding. Two causal mechanisms were examined to determine the effect modification of local context in order to arrive at a generative understanding of causality. This explains whether the mechanisms were likely to be activated in different contextual situations and hence if new knowledge was used. Theory and implementation were studied side by side.

Results: The Context-Mechanism-Outcome analysis indicated that the educate mechanism appeared to be broadly activated when wide consultations and a workshop were provided. However, the delegate mechanism appeared very sensitive to local conditions and was only fully activated in day units with limited manager-physician interactions and where standard training times and staff were readily available. Several agency and structural factors appear to disrupt the delegation action.

Discussion: The revised delegation theory suggests that local unit management realities especially between managers and physicians are important but not always reflected in the organizational charts. Implementation efforts need to address and support multi-team decision making processes as decision making autonomy amongst many front line managers is far more variable than the official hierarchy would suggest.

Epidermal Growth Factor Cytoplasmic Domain is a Major Functional Component of the Membrane-Anchoring Region of proEGF and a Novel Regulator of Elastin Invasiveness of Human Thyroid Carcinoma Cells

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Introduction: Epidermal Growth Factor (EGF) is a polypeptide growth factor originally discovered in the mouse submaxillary gland and is important for eyelid opening and early incisor eruption in the newborn rodents. EGF is the founding member of EGF-like growth factors. EGF-like growth factors have essential functions during cell proliferation, cell migration, tissue differentiation and cell survival. All EGF-like family members are synthesized as transmembrane proforms consisting of an extracellular, transmembrane and cytoplasmic domain. EGF is cleaved from the extracellular domain of this membrane-anchored proform and acts as a soluble growth factor by binding to membrane-bound receptor tyrosine kinases of the Epidermal Growth Factor Receptor family (erbB1-4). Overexpression and ligand-induced activation of EGFR correlates with increased *in vivo* invasiveness of tumor cells and enhanced *in vitro* migration of cell lines. Increasing evidence suggests an active physiological role of transmembrane proEGF-like growth factors and diverse novel functions for the cytoplasmic domain of EGF-like precursors. We are studying the role of the cytoplasmic domain of human proEGF in human thyroid carcinoma. Thyroid carcinoma is the most frequent endocrine tumor in humans and can present as three histological types with different clinical therapy and prognosis: papillary, follicular and anaplastic thyroid carcinoma. Human thyroid carcinoma cell lines present an established model to study the role of growth factors during thyroid carcinogenesis and provide a unique opportunity to study cellular events and regulatory mechanisms impacting on tumor cell growth and invasiveness.

Methods: PCR cloning and expression of proEGF constructs in human thyroid carcinoma cells (FTC133), Western blot analysis, migration and motility assay, Immunofluorescence, siRNA study

Results: All Epidermal Growth Factor-like molecules are synthesized as membrane-anchored proforms. We and others have shown that the cytoplasmic domains of EGF-like ligands have important biological functions. Employing stable transfectants of the human follicular thyroid carcinoma cell line FTC133 expressing membrane-anchored (FTC133-proEGFctF) and soluble cytoplasmic domain of proEGF (FTC133-proEGFcyt)

we show that proEGFcyt is the essential functional element responsible for the transcriptional up-regulation of the lysosomal hydrolases cathepsin- (cath-) B and -D. ProEGFcyt also affects the processing of cath-L. Cath-L has strong elastinolytic activity and was the only cathepsin to be secreted by all FTC133 transfectants. Of all components of the membrane-anchoring region, we identified proEGFcyt to be sufficient in significantly impairing the ability of FTC133 to migrate through elastin matrices when compared with FTC133 stably transfected with the empty plasmid and a natural proEGFsplice mutant construct. Decreased migration through elastin matrix resulted from decreased secretion of cath-L in the FTC133 clones. A similar reduction in elastinolytic activity was observed when FTC133 control or proEGFsplice form transfectants were incubated with a specific cath-L inhibitor suggesting that this elastinolytic activity detected was largely mediated by cath-L. Down-regulation of cath-L in FTC133proEGFctF and -proEGFcyt involved the upregulation of the t-SNARE component SNAP25 as determined by siRNA knock-down of SNAP25 mRNA. Incubation of FTC133-proEGFcyt with soluble EGF reversed this effects and this antagonistic EGF action was mediated by the EGFR.

Conclusion: In summary, we provide first evidence for the suppressive role of proEGFcyt on the ability of thyroid carcinoma cell to invade elastin matrices and identify novel opposing modular functions of soluble EGF and the cytoplasmic domain of human proEGF.

The Novel Compound Pancratistatin Induces Apoptosis Selectively in Colon Cancer Cells by Targeting Mitochondria and Reduces Growth of Human Colon Tumor Xenografts

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Introduction: Current options for the treatment of prostate cancer include surgery, radiation therapy, hormone therapy and chemotherapy. Radiation therapy, like chemotherapy, targets components essential to all cells and are not selective to cancer cells. Hormone therapy is more targeted, but has no effect on hormone-independent cancer cells. Recently, a major effort in cancer research is being made to develop anti-cancer drugs that target components of a biochemical pathway that will induce apoptosis in cancerous cells. Several natural products have proven effective in inducing apoptosis in cancer cells, but with limited specificity. Pancratistatin is a natural compound isolated and characterized fifteen years ago that shows potential as a novel anti-cancer compound with high specificity for inducing apoptosis in cancer cells with low toxicity to non-cancerous cells.

Methods: Response to PST treatment *in vivo* was studied in the human colon cancer cell line HT-29 and normal human fibroblast cells (NHFs) using various biochemical assays. Additionally, the response of 8 Nu/Nu mice with sub-cutaneous human colon tumors to PST treatment was studied *in vivo* by biochemical and histochemical methods.

Results: Our investigation of the biochemical mechanism of action of PST indicates that it specifically and effectively induces apoptosis in colon cancer cells. Previous results from our laboratory suggest that the target of PST is non-genomic. We found that phosphatidyl-serine exposure and activation of caspase-3 occurs within 24 hours of PST treatment, and generation of ROS was a later event. Our *in vitro* results prompted *in vivo* experimentation to evaluate PST treatment on human colon cancer xenotransplants. Sub-cutaneous tumors were established and PST treatment was administered twice weekly for 5 weeks at a dosage of 3mg/kg.

Conclusion: Intra-tumor PST treatment drastically inhibited tumor growth compared to Placebo. Histochemical and biochemical analysis confirmed that tumor cells were apoptotic and that major organs were not affected by PST. These exciting preliminary results present a significant platform for clinical trial testing and the development of non-toxic anti-cancer therapies.

The Effects of IL-12/IL-23p40 Peptide-Based Vaccines in the Down-regulation of Murine Chronic Colitis

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Introduction: Over-expressed IL-12 and IL-23 that share subunit p40 are key cytokines in the pathogenesis of Crohn's disease. Blocking IL-12/IL-23p40 using monoclonal antibodies has shown to be effective in the treatment of Crohn's disease. We sought to develop IL-12/IL-23p40 peptide-based virus-like particle vaccines which induce long-lasting autoantibodies to IL-12/IL-23, and to test the effects of the vaccines in the down-regulation of intestinal inflammation in a murine model of chronic colitis, induced by intrarectal administrations of trinitrobenzene sulfonic acid (TNBS), which exhibits features of Crohn's disease.

Methods: Three antigenic peptides were selected from the p40 subunit based on the occurrence of amino acid residues in experimentally known segment epitopes and DNASTAR software. Recombinant p40 vaccines were constructed by inserting selected peptides into the carrier protein (hepatitis B core antigen) using gene engineering methods, expressed using *E. coli*, and purified. Mice were subcutaneously immunized 3 times with each vaccine, carrier and saline. Two weeks after the final immunization, mice were intra-rectally administered with increasing doses (1.0-2.3 mg) of TNBS for 7 times. Symptoms and body weight were monitored daily. An *in vitro* inhibition test of sera from vaccine-immunized mice was performed using IL-12 induced IFN- γ secretion of splenocytes. Colon inflammation was evaluated by histological examination using H&E staining. Collagen deposition in colon tissues was semi-quantitatively examined using Masson's trichrome staining. Soluble collagen in colon tissues was quantitatively measured using a Sircol Collagen Assay Kit. Serum IL-12 specific-IgG and colon tissue cytokine levels were measured by ELISA.

Results: Three recombinant p40 peptide-based virus-like particle vaccines (C, D and F) induced higher levels of IgG antibodies to IL-12. Their antisera inhibited the IL-12-induced secretion of IFN- γ from splenocytes dose-dependently. Vaccines F and C had higher inhibition percentages than vaccine D. After the administrations of TNBS, mice that had previously been immunized with vaccine F or vaccine C, but not mice with vaccine D, had less body weight loss, decreased inflammation and collagen deposition and reduced expression of IL-12p40, IL-23, IL-17, IL-13, and TGF β in colon tissues, compared with saline and carrier groups ($p < 0.05$).

Conclusion: Administration of IL-12/IL-23p40 peptide-based vaccines induces higher levels of antibodies to p40, leading to the improvement of chronic intestinal inflammation, suggesting that this may be a potential therapeutic approach in the treatment of Crohn's disease.

Modulation of mesenteric vascular tone via splenic afferent nerve activation of mesenteric efferent nerves, vasopressin and angiotensin II release.

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Outflow from splanchnic vascular beds is impeded in portal hypertension. We hypothesized that increased splenic intravascular pressure may initiate a neural reflex to increase mesenteric arterial tone and reduce blood flow into the congested mesenteric vascular bed. Splenic venous pressure was selectively increased by partial splenic vein occlusion (SpVL, 7.0 ± 0.4 to 22.6 ± 0.2 mmHg, $n=60$) in anesthetized rats. During the initial 3 minutes of occlusion, mesenteric conductance fell (-0.01 ± 0.002 mmHg/mL min⁻¹; $p < 0.05$, $n=7$), an effect which was unchanged by renal denervation (-0.01 ± 0.009 mmHg/mL min⁻¹; $n=6$), exacerbated by mesenteric denervation (-0.02 ± 0.004 mmHg/mL min⁻¹; $p < 0.05$, $n=7$) and abolished by splenic denervation (-0.002 ± 0.002 mmHg/mL min⁻¹; $p < 0.05$, $n=6$). Although the increase in mesenteric efferent nerve activity observed during SVL (22.2 ± 2.8 to 27.9 ± 3.8 spikes/sec, $p < 0.05$, $n=13$) was abolished by splenic denervation (32.4 ± 2.4 to 31.2 ± 1.6 spikes/sec, $n=7$), the fact that mesenteric denervation did not also prevent the SpVL-induced fall in mesenteric conductance prompted us to hypothesize that there may be a humoral component to the reflex. We investigated the potential contribution of vasopressin by infusing V_{1a} receptor antagonist SR49059 i.v. prior to SpVL. In mesenteric denervated animals, V_{1a} receptor blockade restored mesenteric conductance to the level observed with SpVL alone (-0.008 ± 0.01 mmHg/mL min⁻¹; $p < 0.05$, $n=6$), but still did not prevent the fall. In contrast, blockade of angiotensin II AT₁ receptors by i.v. infusion of Losartan in intact animals abolished the fall in conductance (-0.001 ± 0.004 mmHg/mL min⁻¹; $p < 0.05$, $n=9$). We propose that the spleen is integral in modulating mesenteric vascular tone via the combined activation of mesenteric efferent nerves, vasopressin and angiotensin II release. However, we propose that splenorenal reflex release of angiotensin II is the main mediator of this response.

A High-Resolution Method for Characterizing Killer Immunoglobulin-Like Receptor (KIR) Genes and Novel Alleles and their Associations with HIV-1 Infection in Kenyan Sex Workers

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Introduction: Natural killer (NK) cells are key players in the innate immune response against viruses. Once thought to be non-specific, it is now known that NK cells have surface receptors that impart specificity to their cytolytic activity, including Killer Immunoglobulin-Like Receptors (KIRs). KIRs inhibit or activate NK function, depending on the presence/absence of their HLA class I ligand. Like HLA genes, KIR genes are polygenic and polymorphic, providing a basis for natural selection and suggesting a significant role for KIR variability in immunity and infection. KIRs have recently been implicated in anti-HIV immunity, but current PCR sequence specific (SSP) typing methods are time-consuming, lack allele-specificity and cannot identify novel alleles. We hypothesize that 1) comprehensive sequence-based KIR-typing will reveal new KIR alleles; 2) KIRs play a role in HIV-1 resistance in HIV-1 resistant sex workers in Nairobi, Kenya.

Methods: DNA was isolated from 1154 women defined as HIV-1 resistant or HIV-1 susceptible. KIR genes were PCR amplified using primers in introns flanking the most polymorphic exons (each corresponding to a receptor Ig domain/region): 4, 5, and 6 for KIR 2DL2/2DL3 and exons 2 and 3 for KIR 3DL1/3DS1, then sequenced with nested primers. Taxonomy-based sequence genotyping software CodonExpress™ was used to genotype KIR genes. HLA class I ligands were genotyped. TA-cloning of the whole 6kb 3-exon genomic typing region was performed to confirm identification of new KIR alleles.

Results: A novel method was developed for typing at both gene and allele levels. It was used to type over 1000 women and of these 10% had only 2DL2 genes and alleles, 35.3% had both 2DL2/2DL3 and 3DL1/3DS1, 46.3% had only 2DL3 and 7.3% had no sequence. We identified new alleles in ~10% of women. Alleles which were associated with HIV-1 status will be further studied in combination with their HLA class I ligands.

Conclusions: This method is superior to SSP typing because it is rapid, highly specific, requires less template and fewer reactions, co-amplifies 2 genes and all alleles of each gene simultaneously, and can identify new alleles. This typing strategy can be applied to all KIRs, which will facilitate large-scale associations of KIR hap-

lotypes and susceptibility to HIV, as well as providing new insight into the link between innate and adaptive immunity. Association of KIRs with HIV-1 emphasizes the importance of cell-mediated cytotoxicity in response to HIV-1.

The Role of TRIM32 in Limb-girdle Muscular Dystrophy Type 2H (LGMD2H)

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Introduction: Limb-Girdle Muscular Dystrophy type 2H (LGMD2H) is a mild myopathy with autosomal recessive inheritance commonly found among Hutterites. It is due to a D487N point mutation in the TRIM32 (tripartite motif) protein. We postulate that TRIM32 is an E3-Ubiquitin ligase that recognizes target proteins for degradation by the 26S proteasome. Specifically, mutation(s) in *TRIM32* lead(s) to an overabundance of target protein(s) and cause(s) premature muscle cell death underlying the myopathy.

Methods: Mouse monoclonal antibodies against TRIM32 were developed and screened for this study. Their specificity was established by western blot analysis using mouse wild type and *Trim32* knock-out tissues. These anti-Trim32 antibodies were used to characterize the following aspects of Trim32: tissue distribution, molecular size of Trim32 and solubility of Trim32 in cell lysates. Proteasome inhibitor treatment assays were performed to test whether Trim32 is ubiquitinated. In order to investigate the interacting partner(s) of TRIM32, a pull-down approach using protein G beads coupled with antibodies was used to purify TRIM32 and its interacting protein(s). A Tandem Affinity Purification (TAP) system was adapted to further purify TRIM32 and to look for its interacting proteins.

Results:

1. The specificity of anti-Trim32 antibodies were verified using *Trim32* knock-out mice tissues. Trim32 of uniform molecular size was found to be expressed in brain, skeletal muscle, brown fat, thymus, kidney, lung, spleen, heart, and liver tissue of wild type mice but not in *Trim32* knock-out animals.
2. Most Trim32 was found to be insoluble in BHK cells. However, in C2C12 cells, more soluble Trim32 was found, sufficient for Trim32 purification and protein interaction studies.
3. TRIM32 was found to be post-translationally modified, possibly by the addition of ubiquitin.
4. The interaction between endogenous Trim32 and endogenous Piasy was confirmed by pull-down experiments.
5. TRIM32 was found to be co-purified with its interacting proteins by TAP method. Several differential protein bands were detected on a silver stained gel between purified lysates of BHK cell transfected with TAP-*TRIM32* and TAP-*TRIM32*-D487N constructs.

Conclusions:

1. Trim32 of uniform molecular size is expressed in various tissue types of wild type mice including brain, skeletal muscle, brown fat, thymus, kidney, lung, spleen, heart, and liver.
2. Trim32 is post-translationally modified, possibly by the addition of ubiquitin. Endogenous Trim32 interacts with endogenous Piasy.
3. The TAP method appears promising to further study Trim32 interacting protein(s).

Post Tympanostomy Tube Otorrhea: A Meta Analysis

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Introduction: Post-tympanostomy tube otorrhea is the most common complication of tympanostomy tube placement. The incidence of this problem varies from 3.4 to 74%. Trials that study post-tympanostomy tube otorrhea may involve valid randomization “by patient” or “by ear.” In an attempt to define “best practice,” we conduct a meta-analysis to quantify the benefit of using topical prophylactic antibiotic drops in the post-operative period. We then compare our findings with previous results found in the literature.

Methods: We selected randomized studies for which antibiotic drops had been used for at least 48 hours after tympanostomy tube insertion. Nine studies, three “by ear” and six “by patient,” met our inclusion criteria. The odds ratio and 95% confidence intervals were calculated for each to conduct the meta-analysis.

Results: Overall, prophylaxis appears to be effective at reducing the incidence of post tympanostomy tube otorrhea. The mean odds ratio was 52% suggesting that prophylaxis may reduce the incidence of post tympanostomy tube otorrhea by half. Individually, however, none of the three “by ear” studies and only three of the six “by patient” studies were statistically significant.

Conclusion: This meta-analysis suggests that routine post tympanostomy tube prophylaxis is beneficial, but this finding is dependent on selection criteria used.

Homocysteine Stimulates Monocyte Chemoattractant Protein-1 Expression in the Kidney via NF-Kappa B Activation

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Introduction: Hyperhomocysteinemia, a condition of elevated blood homocysteine levels, is associated with cardiovascular disorders. Although renal disease is an important factor causing hyperhomocysteinemia, the direct effect of homocysteine on the kidney is not well documented. One of the important features in kidney diseases is the infiltration of monocyte/macrophage in the kidney. Monocyte chemoattractant protein-1 (MCP-1) is a potent chemokine that stimulates monocyte migration into the tissue. Nuclear factor kappa-B (NF- κ B) plays an important role in upregulation of MCP-1 expression. The aim of this study was to investigate the effect of hyperhomocysteinemia on MCP-1 expression and the underlying mechanisms in rat kidneys.

Methods: Eight-week-old male Sprague-Dawley rats were fed a control diet (Control), a high-methionine diet (Met) or a high-cysteine diet (Cys) for 12 weeks (n=12 per group).

Expression of MCP-1 mRNA and protein in rat kidneys were measured by real-time PCR detection system and ELISA, respectively. MCP-1 protein distribution in rat kidneys was examined by immunohistochemical staining. NF- κ B activation in rat kidneys was determined by EMSA. One group of rats fed a Met diet was treated with pyrrolidine dithiocarbamate (PDTC, 100mg/kg, daily) for 3 days prior to euthanasia. Data were analyzed by two-tailed independent Student t-test. P < 0.05 was considered statistically significant.

Results: NF- κ B activity, the levels of MCP-1 mRNA and protein were significantly increased in the kidneys of hyperhomocysteinemic rats. Pretreatment of hyperhomocysteinemic rats with a NF- κ B inhibitor completely abolished hyperhomocysteinemia-induced MCP-1 expression in the kidney. This further confirmed the causative role of NF- κ B activation in hyperhomocysteinemia-induced MCP-1 expression.

Conclusion: Taken together, these results suggest that diet-induced hyperhomocysteinemia can stimulate chemokine expression in the kidney via NF- κ B activation. Such an inflammatory response may contribute to renal injury and chronic systemic inflammation associated with hyperhomocysteinemia.

From Alpha to Beta: Identifying Amino Acid Residues Required for Bundlin N-Acetyllactosamine Lectin Activity

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During the early stages of infection, EPEC produce bundle-forming pili, which are polymers of the bundlin protein. Two divergent bundlin alleles, α and β , are expressed by diverse EPEC isolates. Synthetic *N*-acetyllactosamine (LacNAc) glycosides coupled to BSA competitively inhibit early localized adherence (LA) of EPEC strains expressing α but not β bundlin alleles to HEp-2 cells. Purified α bundlin specifically binds LacNAc, but β bundlin does not. α and β bundlin proteins differ at two surface-exposed regions on the protein: to investigate the LacNAc-specific lectin activity of α bundlin, block mutations were introduced in α bundlin to convert it to β bundlin these regions. Four mutants were produced: RMH1 (GENNI \rightarrow SPDST at position 131); RMH2 (DQA (TSTN, at position 139); RMH3 (S52N, D55N and T57N); and RMH4 (N91A and A95N). All mutants expressed bundlin and assembled BFP. RMH1 were LA negative, RMH2 and RMH3 displayed intermediate levels of LA, and RMH4 LA was the same as wild type. The early LA phenotype in EPEC is dependant on the ability of bundlin to bind to LacNAc-containing receptors on the host cell surface. In this study, we identified GENNI as the key amino acids responsible for the difference in LacNAc binding between α and β bundlin. These amino acids lie in close proximity to the only surface-exposed aromatic amino acid in α bundlin, tyrosine 57. This also supports our hypothesis that this region of the bundlin molecule is responsible for LacNAc-binding, as aromatic residues have a high propensity to interface with sugars, due to hydrophobic interactions between the hexose ring of the sugar and the planar surface of the amino acid side chain.

Development of a Relevant Mouse Model to Study Acute Airway Inflammation and Hyperresponsiveness

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Introduction: Common murine models of asthma, which are characterized by eosinophilic airway inflammation, use ovalbumin (OVA) as an allergen for immune-sensitization and subsequent inhalation challenge. OVA is isolated from egg yolk, making its use as an inhaled allergen artificial, and necessitating development of alternate allergen exposure protocols. We tested the utility of whole house dust mite (HDM) (*Dermatophagoides pteronyssinus*) extract as allergen for murine models of acute airway inflammation and hyperresponsiveness. HDM is a common human aerosol allergen linked to asthma exacerbation. It is unique from OVA as it includes multiple protein and non-protein components that induce allergic reactions, inflammation, and have proteolytic activity.

Methods: We compared effects of the duration (3, 5 or 10 days) of daily intranasal administration HDM (35 μ L, 0.71 μ g/ μ L), and effects of prior sensitization with subcutaneous injections of HDM (100 μ L, 1 μ g/ μ L) on airway inflammation and respiratory mechanics in female BALB/c mice (8-10 wks). We also compared effects of HDM exposure to those induced by standard acute OVA sensitization and challenge. To quantify airway inflammation bronchoalveolar lavage fluid (BALF) was collected and total inflammatory cell number and differential distribution was evaluated using a hemacytometer and light microscopy (modified Wright-Giemsa stain). To evaluate respiratory mechanics we used a Scireq *flexiVENT* small animal ventilator, employing low frequency forced oscillation maneuvers to derive changes in central airway (Rn) and lung tissue (G) resistance, and lung elastance (H) induced by increasing concentrations (0-50mg/mL) inhaled methacholine (MCh).

Results: Inhaled HDM challenge for 10 days was required to see a significant increase (391.66 \pm 4.41%, P <0.001) in total inflammatory cells compared to the saline challenged mice. Eosinophils and neutrophils comprised 44.8 \pm 1.42% and 21.4 \pm 1.56% of inflammatory cells, respectively. No augmentation of total inflammatory cell number, or any change in relative numbers of eosinophils and neutrophils was observed in mice receiving pre-challenge HDM sensitization. In contrast to the predominantly eosinophil inflammation observed HDM challenged mice, only 25.6 \pm 1.27% BALF cells from OVA treated animals were eosinophils, and

in these animal neutrophils were the predominant cell type, accounting for $34.1 \pm 1.42\%$ of all BALF cells. Compared to control animals, 10 days of HDM challenge induced a significant increase in large and small airway reactivity ($146.86 \pm 45.6\%$ and $274.49 \pm 22.4\%$ for Rn and G), and tissue elastance ($169.06 \pm 37.79\%$ for H). Pre-sensitization with HDM had little impact on the effects of 5 days of inhaled HDM on lung function parameters. These animals and 10-day HDM challenged animals also exhibited equivalent airway responsiveness to that we observed for OVA sensitized/challenged mice.

Conclusion: Daily intranasal exposure of mice to HDM induces robust eosinophilic airway inflammation and accompanying airway hyperresponsiveness. HDM is more effective at inducing eosinophilic inflammation than commercially available OVA, which can be contaminated with pro-neutrophilic compound, endotoxin. The murine model we have developed using HDM offers outstanding potential as a platform for future acute and chronic studies of airway physiology and biology and for pre-clinical testing of new therapies for chronic allergic respiratory disease.

Prolongation of Mouse Islet Allograft Survival by local Expression of Indoleamine 2,3 Dioxygenase (IDO)

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Introduction: The success of transplantation of the insulin-producing islets of Langerhans as a promising therapeutic method for diabetic people mostly depends upon long-term use of systemic immunosuppressive drugs whose adverse side effects are one of the main obstacles in clinical islet transplantation. Here we report, a novel approach for preventing islet graft rejection through which local induction of the immunosuppressive factor, indoleamine 2, 3-dioxygenase (IDO), a tryptophan catabolizing enzyme, in syngeneic fibroblasts generates a tryptophan deficient microenvironment in which infiltrated immune cells, but not allogeneic islets, are unable to survive, proliferate and destroy the engrafted islets.

Materials and Methods: Three-dimensional composite islet grafts were prepared by embedding Balb/c mouse islets and adenovirally transduced IDO-expressing or control C57Bl/6 mouse fibroblasts in collagen matrix. These engraftments were then transplanted into renal subcapsular space of streptozotocin induced diabetic C57Bl/6 mice. The graft recipient mice were monitored for graft function by measuring blood glucose levels until occurrence of hyperglycemia, as a sign of graft rejection was observed. Graft survival rates were plotted and compared between experimental groups using Kaplan-Meier survival analysis method with log rank (Mantel-Cox) test.

Results: Normoglycemia occurred in all experimental groups on the first day post-transplantation. Blood glucose levels in mice receiving engraftments with control fibroblasts or islets alone increased as early as the second week post-transplantation and all of these grafts were rejected by day 17. However, mice which received grafts with IDO-expressing fibroblasts maintained normoglycemia for a significantly longer period of time (41.6 ± 7.2 days). Analysis of survival rates of islet grafts in experimental groups showed a significant prolongation of islet graft survival in the group receiving IDO expressing fibroblasts (41.6 ± 7.2 vs. 13.8 ± 2.6 days, $p=0.002$).

Conclusion: The findings of this study show that local expression of IDO by syngeneic fibroblasts significantly prolongs allogeneic islet graft survival. The duration of this protective effect closely correlates with the

expected length of transient IDO expression caused by adenoviral transduction. Thus, this new approach of using autologous cells expressing IDO as a local immunosuppressive factor to protect allogeneic islet transplantation would be significant. This is because it may eliminate the need for immunosuppressive drugs in transplant patients if a stable and persistent local IDO expression by other means such as the use of IDO lenti-viral transfection is used.

Involuntary Volume Stacking in Young Patients with Neuromuscular Disease

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Introduction: Incentive spirometry and deep breathing exercises can prevent or reverse atelectasis in patients with shallow breathing due to neuromuscular disorders (NMD). However, children with NMD often cannot understand or perform the required maneuvers. Data from cooperative adults with NMD suggest that volume stacking can exceed maximum spontaneous vital capacity.

Methods: To investigate the effectiveness of a volume-stacking mask in children with NMD, we studied 5 boys and 4 girls, median age 6 y (range 3.5-18 y), weight 25 kg (11.7-73.9 kg). One boy was studied during 3 admissions over one year. For involuntary volume stacking (IVS) a one-way valve and pneumotach were attached to a cushioned full-face mask that was held to the face of the child, ensuring a tight seal. Flow and volume signals were acquired to computer (AcqKnowledge, BIOPAC Inc.). Tidal flows and volumes were recorded 30 s before and again after 15 s of valve closure during which expiration was prevented.

Results: The median length of recording was 75.9 s (73.1-86.2 s), with a median duration of valve closure of 15.4 s (12.8-19.6 s). The median tidal volume (V_t , normalized to body weight) before valve closure was 9.3 mL/kg (2.8-21.6 mL/kg) and the spontaneous respiratory rate was 24/min (15-42/min). The median volume increase during stacking, relative to the average V_t before valve closure, was 217% (55-607%). The median number of stacked breaths was 5 (range 0-9).

Conclusion: Our findings show that a substantial increase in lung volumes above V_t can be achieved by involuntary stacking. The procedure was well tolerated. However, not every child was able to stack breaths above V_t , and the latter finding became obvious only during data analysis. Our investigation is continuing to identify best candidates for IVS and to evaluate the effectiveness of this intervention.

Phosphorylation and Cleavage of Presenilin-Associated Rhomboid-like Protein (PARL) Promotes Changes in Mitochondrial Morphology

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Primary mitochondrial activities like respiration, calcium signaling and apoptosis impinge on the mechanisms regulating the shape and structure of the mitochondrial network. Consequently, the molecular mechanisms controlling biogenesis and homeostasis of the organelle are central to the development of major neurodegenerative disorders, including Parkinson's disease and optic nerve atrophy. Therefore, it is imperative to understand how mitochondria morphology remodeling is regulated and which signaling pathways link physiological transitions to structural changes of the mitochondrial architecture. In this study we show that the activity of a central regulator of mitochondria morphology remodeling, the rhomboid protease PARL, is governed through phosphorylation and cleavage of its N-terminus. Phosphorylation of three residues embedded in this domain, Ser-65, Thr-69, and Ser-70, impair a cleavage at position Ser(77)-Ala(78) that is required to initiate PARL-induced mitochondrial fragmentation. These findings provide the first example of how mitochondria biogenesis is regulated in the mammalian system and indicate that the pathway triggering structural remodeling of mitochondria shape is based on the activity of a kinase/phosphatase switch.

Investigation into Potential Cross-Talk Between Cx43-Induced Inhibition of DNA Synthesis and the TGF β Pathway

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Introduction: The membrane phospho-protein connexin43 (Cx43) has anti-oncogene activity and inhibits cardiomyocyte DNA synthesis. Mitogens such as FGF-2 counteract Cx43-mediated growth inhibition by promoting its phosphorylation at serine (S) 262, a protein kinase C (PKC) target site. Recently, Cx43 was reported to activate intracellular signals and genes traditionally associated with the TGF β signal transduction.

Methods and Results: We thus investigated the hypothesis that growth inhibition induced by Cx43 overexpression in neonatal cardiomyocytes is dependent on engaging components of the TGF β pathway. TGF β signaling requires the activity of receptors TGF β R1, TGF β RII, and the downstream activation of Smad2/3. Pharmacological inhibition of TGF β R1 by SB431542, while effective in preventing Smad2 phosphorylation (and thus its ability to signal by translocating to the nucleus) had no effect on Cx43-mediated growth inhibition. Similarly, neither the overexpression of dominant-negative TGF β RII nor dominant-negative Smad2 (or Smad3) could restore DNA synthesis in Cx43-overexpressing cardiomyocytes. On the other hand, TGF β , prevented the PKC-mediated, phosphorylation of Cx43 at serine S262, an event that is necessary for DNA synthesis

Conclusion: Taken together our data suggest a cardiomyocyte growth inhibition scenario in which TGF β acts upstream of Cx43, promoting its inhibitory potential by preventing PKC-mediated phosphorylation at S262-Cx43. Subsequently, Cx43-inhibition does not require downstream engagement of TGF β signals.

Natural Killer Cells Modulate the Infection-mediated Inhibition of Asthma-like Reaction via Modulating Function of Dendritic Cell

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Introduction: Asthma is one of the most common chronic diseases in Canada, with the symptom of coughing, wheezing, chest tightness, shortness of breath or even death. Epidemiological as well as experimental data show that the rapid increase of incidence of allergic diseases is inversely associated with the dramatic decrease of incidence of certain infections in the past several decades. Therefore, it is of great significance to understand the nature of the protective infection and the mechanism underlying the infection-mediated inhibition of allergic diseases, including asthma. In our lab, we have shown that lung *Chlamydia muridarum* (MoPn) infection exerts a dramatic protective effect on the subsequent allergic reactions induced by allergen exposure. Furthermore, we have found that dendritic cell (DC), one of the most potential antigen presenting cells, is a central mediator in the infection-mediated inhibition of allergic diseases. But little is known as to how DCs are modulated by other innate cells in this process. Natural Killer (NK) cells are one of the key components of innate immune system. NK-DC interaction has been shown to be important in determining the subsequent adaptive immune responses in some infectious diseases and tumor insults. However, to our knowledge, there is no report on the mechanism by which NK cells modulate function of DC in the situation of infection-mediated inhibition of allergic diseases.

Methodology: Polyclonal anti-asialo-gangli-N-tetraosylceramide (anti-asialo-GM1) antibody was used to deplete NK cells *in vivo*. C57BL/6 or BALB/c mice were injected with 30 μ l of anti-asialo-GM1 or control normal rabbit immunoglobulin G (IgG) in 300ul PBS intraperitoneally (i.p.) once every two days, starting from 3 days before chlamydial infection. On day 0, both groups of mice were inoculated intranasally (i.n.) with MoPn [1103 inclusion-forming units (IFUs)] in a volume of 40mL of sucrose phosphate glutamic acid buffer (SPG). Control mice received an i.n. inoculation of 40mL of SPG only without infection. Spleens were aseptically removed from mice at specific time points following *C. muridarum* infection. CD11c positive cells were isolated from single cell suspension prepared according to manufacturer's instruction by using anti-CD11c magnetic beads. The expression of co-stimulatory markers (CD80, CD86) was examined by Flow Cytometry. The cytokines produced by DC was examined by ELISA. The ability of DC to direct the differentiation of naïve

CD4⁺T cells was evaluated in DC-CD4⁺T co-culture at the presence of allergen stimulation. Finally, the *in vivo* ability of adoptively transferred DC to inhibit the development of asthmatic-like reactions was investigated in recipient mice. The airway eosinophilic inflammation and mucus oversecretion were examined by immunohistopathology. The systemic immune responses were evaluated by intracellular cytokine staining.

Results: In the present study, we have demonstrated that NK cells play an important role in modulating microbe-exposed DCs' capacity to inhibit airway allergic responses. We have shown that DCs isolated from NK cells-depleted and MoPn-infected mice failed to inhibit the induced allergic responses in recipient mice, whereas DCs isolated from MoPn-infected mice successfully dampened airway eosinophilic inflammation and mucus over-secretion, which are hallmarks of asthmatic symptoms. To further investigate how NK cells affect the function of DCs during MoPn infection, we analyzed phenotypic and functional changes of DC after NK cells depletion during MoPn infection. Firstly, DC from mice depleted of NK cells exhibited less mature phenotype with dramatically reduced expression of surface co-stimulatory markers (CD80 and CD86) when compared to the wild type (WT) controls following MoPn infection. Secondly, DC from NK depleted mice showed reduced cytokine production. Cytokines from DC serve as an important signal to direct naïve T cell differentiation. In particular, IL-12-producing DC promotes Th1 polarization which has been recognized as an important protective factor for resistance to chlamydial infection. IL-10-producing DC may induce the generation of regulatory T cells that may limit pathology by dampening inflammation. We found that DC from NK depleted mice after *C.muridarum* infection (NK⁻/MoPn/DC) produced significantly lower levels of both IL-12 and IL-10 than the WT mice. Further, NK⁻/MoPn/DC showed reduced ability to direct antigen-specific CD4⁺T cell responses. Finally, adoptive transfer experiments showed that NK cells play a key role in the DC-mediated protection against allergic responses in recipient mice.

Conclusion: The present study demonstrates the important role of NK cells in *C.muridarum* infection-mediated inhibition of allergic responses by modulating DC maturation and function.

Linkage of Innate and Adaptive Immunity via Modulation of Dendritic Cell Function by NKT Cell Activation Induces Strong Protective Immunity to an Intracellular Bacterial Lung Infection

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Introduction: *Chlamydia pneumoniae* infection is a leading cause of a wide variety of respiratory diseases in humans and also has also been implicated in the pathogenesis of chronic inflammatory diseases such as atherosclerosis and multiple sclerosis. Natural killer T (NKT) cells are a specialized CD-1 reactive innate T cell subset implicated in the regulation of innate and adaptive immunity. We previously reported that NKT cells play a protective role in host defense to *C.pneumoniae*. In this study, we further investigated the mechanism by which NKT influence the T cell adaptive immunity through examining the effect of NKT cell activation in modulating DC function during this infection

Methods: We used a combination of different approaches including gene knock out mice, specific cellular activation, co-culture and adoptive transfer. NKT-KO and wild type control (C57BL/6) mice were intranasally infected with *C.pneumoniae*. Dendritic cells (DC) were isolated from these mice and analysed for co-stimulatory molecule expression, cytokine production and naïve T cell priming pattern. α -Galcer, the synthetic NKT ligand was used for specific activation of NKT cells. Further, DC adoptive transfer was performed to evaluate induction of protective immunity against *C.pneumoniae* infection.

Results: DC isolated from NKT-KO mice (KO DC) showed significantly decreased costimulatory molecule expression, most notably CD40 and reduced IL-12 production compared to that from the WT mice (WT DC). In contrast, specific *in vivo* NKT activation using α -Galcer greatly enhanced CD40 expression and IL-12 production by DC in the WT mice following infection. Further, DC from the infected NKT-KO mice had significantly reduced capacity to polarize organism-specific type-1 T cell responses *in vitro*, whereas DC from α -Galcer treated mice showed enhanced type-1 CD8 and CD4 T cell priming ability. NKT-DC co-culture experiments revealed that the presence of NKT cells significantly enhanced the production of bioactive IL-12p70 by DC. We further found that the modulating effect of NKT on DC is dependent upon NKT derived signals, CD154 and IFN γ and direct cell-cell contact. Finally, the adoptive transfer of DC isolated from the WT infected

mice, induced strong protective immunity, whereas those from KO mice induced pathology in the recipient mice upon challenge infection.

Conclusion: These findings show a critical role of NKT cells in the functional development of dendritic cells for the subsequent polarization of T cell adaptive immunity for protection against an important human infection and suggest the potential for exploitation of NKT activation to design novel therapeutic strategies for the control of infectious diseases.

Molecular Mechanisms of a GNB3 Polymorphism Affecting HIV Progression

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Introduction: Previous work has demonstrated an association between a SNP in the GNB3 (G protein β subunit 3) gene and delayed HIV disease progression. The GNB3 SNP results in the production of splice variants characterized by increased signalling activity through G protein coupled receptors (GPCRs), a family which includes the HIV co-receptors CCR5 and CXCR4. This project will identify the molecular mechanisms by which the GNB3 SNP affects both the response to HIV infection and subsequent disease progression. We hypothesize that the GNB3 SNP will affect the activation state of immune cells and that increased signal transduction within T cells will activate anti-apoptotic pathways, contributing to slower CD4 decline after HIV infection. The development of an *in vitro* model to characterize the roles of the GNB3 splice variants in T cells will further clarify the role of G protein signalling in HIV infection.

Methods: To characterize the effect of the GNB3 SNP on immune activation, expression of activation markers on CD4 and CD8 cells was analysed by flow cytometry. Characterization of GNB3 subcellular localization and response to chemokine stimulation *in vitro* (T cell lines) and *ex vivo* (PBMCs) was performed by immunostaining and confocal microscopy. T cell lines expressing either wildtype or polymorphic GNB3 are being developed using shRNA-mediated knockdown and subsequent expression of GNB3 cDNA.

Results: Preliminary studies show a trend toward decreased expression of the activation marker CD38 on CD4+ and CD8+ cells in individuals homozygous for the GNB3 SNP. Confocal microscopy has demonstrated differential localization of GNB3 in several T cell lines, ranging from predominantly cytosolic localization to strong membrane localization. Chemokine stimulation of cells leads to recruitment of GNB3 to the membrane from the cytosol. CD4- T cell lines with reduced GNB3 expression are currently being developed using shRNA vectors.

Conclusions: The GNB3 SNP may play an important role in HIV infection and disease progression. These results indicate that the SNP may affect activation of CD4 and CD8 cells, suggesting that the slower CD4 decline could be mediated by reduced cytotoxic activity. Additionally, the distribution of GNB3 in T cells appears to be membrane localized and responsive to chemokine stimulation.

Temperature Sensitivity - a Screening Factor for Avian Influenza Viruses More Apt to Crossing the Species Barrier?

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Introduction: Influenza A viruses are naturally circulating viruses of aquatic birds, however the emergence of highly pathogenic strains poses a pandemic threat if they are successfully introduced into the human population. Pigs may play an important role in this process as cells lining their respiratory tract contain both α -2,3-linked and α -2,6-linked sialic acid receptors for avian-tropic and human-tropic viruses, respectively. Both human and avian influenza viruses can therefore infect porcine cells, generating isolates with altered receptor specificity or recombinant viruses that can cross the species barrier. Such viruses must possess human receptor specificity as well as the ability to replicate at human respiratory tract temperatures ranging from 33 °C - 37 °C, compared to the avian core temperature of 41°C.

Methods: Using the immortalized porcine alveolar macrophage cell line 3D4-31, seven viruses from varied species origin, including two human, one swine, and three avian viruses, were tested for their ability to replicate at 33 °C, 37 °C, and 41 °C over a 48-hour time-course. Experimental viruses included A/WS/33 (H1N1), A/SouthCarolina/1918/ (H1N1), A/Swine/Iowa/15/1930 (H1N1), A/Chicken/Vietnam/14/2005 (H5N1), A/Emu/Texas/39924/93 (H5N2), and A/Chicken/BC/514/2004 (H7N3).

Results: Real-time RT-PCR results showed that all avian viruses grew to equal titers at 37°C and 41°C. The 1918 human pandemic virus also grew well at 41, while the WS/33 human isolate did not. Additionally, while avian viruses were expected to grow poorly at 33°C, the BC isolate, an avian isolate which caused two cases of conjunctivitis in humans, reached similar titers at 33°C and 37°C by 48 hours post-infection.

Conclusions: The ability of viruses to replicate at different physiological temperatures is not always predictable based on the host species from which the virus was isolated. Though a shift in receptor affinity is an absolute requirement for an avian virus to successfully crossover into the human population, the ability to replicate efficiently at physiologically relevant human temperatures does not seem to serve as a screening tool, and likely additional complex requirements are necessary.

Human TH17 Lymphocytes Promote Immune Cell Recruitment into the Brain

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Introduction: The blood-brain barrier (BBB) plays a crucial role in protecting the central nervous system (CNS) by restricting entry of cells and molecules into the brain. In the CNS disorder multiple sclerosis (MS), breakdown of the BBB allows activated leukocytes to infiltrate the brain parenchyma, leading to the formation of the characteristic demyelinated lesions. Interleukin (IL)-17-secreting lymphocytes (T_H17) appear to be essential in the pathogenesis of numerous inflammatory diseases, including MS. In spite of the intensified focus on T_H17 lymphocytes, their contribution to the disruption of the BBB and leukocyte infiltration into the CNS, both important early events in the development of MS, remains unclear.

Methods: We developed and optimized a method to generate human T_H17 lines *in vitro* from peripheral blood lymphocytes of healthy donors and MS patients, analyzed T_H17 cytokine profile by flow cytometry and studied T_H17 lymphocyte migration across the endothelium using an *in vitro* model of trans-BBB migration.

Results: We demonstrate that human T_H17 lymphocytes produce IL-17 and IL-22, cytokines that increase the permeability of the BBB by disrupting tight junction molecules between endothelial cells. We also show that human T_H17 cells transmigrate efficiently across brain endothelial cells and promote CD4⁺ lymphocyte recruitment across the BBB, through the concerted action of IL-17 and IL-22. Moreover, we found IL-17 and IL-22 receptors to be upregulated on inflamed endothelium in MS lesions.

Conclusions: Our study further refines the phenotype of human T_H17 lymphocytes and emphasizes the importance of T_H17 lymphocyte infiltration into the CNS and their consequent involvement in lesion formation in MS.

Diffusion Weighted Imaging of the Optic Tract in a Multiple Sclerosis Model

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Introduction: Diffusion weighted MR imaging (DWI) measures the mobility of water molecules and may provide specificity to ambiguous hyperintense T2-weighted white matter lesions. In this study, we utilized both T2-weighted MR imaging (T2W) and DWI to investigate lesions within the optic chiasms (OX) of myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) mice.

Methods: MR Imaging: T2W and DWI were obtained on a Bruker Biospec spectrometer with a 7T/21-cm magnet using a 24-mm quadrature volume coil. Each animal was imaged at pre-disease, onset and peak states of EAE corresponding to motor function assessment. Protocol: Female C57BL/6 mice were induced with MOG, weighed and scored daily to monitor disease progression. Cardiac perfusions with either 10% formalin for basic histology, or 2% glutaraldehyde for electron microscopy (EM) were performed.

Results: MOG-induced animals showed signs of EAE. Changes in average apparent diffusion coefficients (ADC) corresponded to the development and progression of hyperintense lesions within the OX of T2W images of EAE animals. HE stains showed aggregates of small round cells (lymphocytes) mainly on the surface of the OX while SC stains of EAE animals suggested reduced amounts of myelin. EM examination of the optic chiasma from EAE animals confirmed the presence of lymphocytes and neutrophils on the surface, and showed glial and axonal swelling, scattered axons lacking myelin, scattered degenerating axons, scattered apoptotic oligodendrocytes, and extracellular edema.

Conclusions: The development and progression of MOG-induced EAE disease course varied in all mice. Hyperintense T2W lesions were detected in the OX of MOG-induced EAE mice and corresponded with ADC changes in DWI. Microscopic examination suggests that the change in SC staining is due to the volumetric dilution of myelin rather than demyelination per se. MOG-induced EAE disease is unpredictable in the OX, therefore emphasizing the need for longitudinal *in vivo* studies.

Characterization of the Mechanism of Xenin-Induced Anorexia in Mice

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Introduction: Xenin is a 25-amino acid peptide and is produced in gastric mucosa. We and others have shown that peripheral and central administration of xenin reduces food intake in rodents. However, the mechanism by which xenin reduces food intake is not well understood. A number of gut hormones reduce food intake by delaying gastric emptying. Effect of xenin on gastric motility is partly mediated through neurtensin receptor 1 (Ntsr1). Furthermore, we have recently found that xenin injection stimulates hypothalamic c-fos mRNA expression in mice, suggesting that the effect of xenin on food intake is mediated through the hypothalamic signaling pathways. Therefore, we hypothesized that (i) xenin reduces food intake by delaying gastric emptying and (ii) the anorectic effect of xenin is mediated through Ntsr1, and (iii) xenin regulates food intake by modulating the activity of its downstream targets in the hypothalamus. The present study addressed these hypotheses.

Method: To examine the effect of xenin on gastric emptying, C57BL/6J mice were fasted overnight and provided with food pellets for 1 hour before an intraperitoneal (i.p.) injection of xenin (50 μ g/g b.w.). The mice were sacrificed immediately after injection or 2 hours after injection. Gastric emptying rates were calculated by subtracting the stomach weight at 2-h time point from that sacrificed immediately after injection. To determine if the anorectic effect of xenin is mediated through Ntsr1, wild type and Ntsr1 knockout mice were fasted overnight and injected intracerebroventricularly (i.c.v.) with xenin (1 μ g/mouse) or artificial cerebrospinal fluid (aCSF). Cumulative food intake was measured up to 24 hours after injection. To identify the downstream targets of xenin in the hypothalamus, C57BL/6J mice were injected i.p. with xenin (50 μ g/g b.w.) and sacrificed 30 or 90 minutes later. Hypothalamic RNA was extracted and expression levels of metabolism-related genes were measured using a PCR-array technique and a semi-quantitative real-time PCR.

Result: The i.p. injection of xenin significantly reduced the rate of gastric emptying compared to saline-injected control group. The i.c.v. injection of xenin significantly reduced food intake in wild-type mice. In contrast, the anorectic effect of xenin was significantly and markedly attenuated in Ntsr1-deficient mice. Hypothalamic mRNA levels of interleukin-1 beta (IL-1 beta) and orexin receptor 1 were significantly increased after the i.p. xenin injection compared to saline injection.

Conclusion: Xenin reduces food intake as a result of delayed gastric emptying, and the anorectic effect of xenin is at least partly mediated through Ntsr1. Furthermore, our findings suggest that xenin regulates feeding by modulating hypothalamic signaling pathways involving IL-1 beta and orexin receptor 1.

Immune Correlates of Treatment Induced Depression in Chronic HCV Infection

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Chronic Hepatitis C Virus (HCV) infection is treated with pegylated interferon- α (IFN) and ribavirin. The main reason for treatment discontinuation is depression. Clinical depression has been shown to correlate with the presence of pro-inflammatory cytokines IFN- α , IL-1 β , IL-6 and TNF- α and a decline in the anti-inflammatory cytokine IL-10. The purpose of this research is to determine whether the balance of cytokine activity at baseline (pre-treatment) can predict susceptibility to treatment-induced depression. Cytokine activity was evaluated by culture of peripheral blood mononuclear cells (PBMC) with IFN alone or with HCV proteins Core and NS3. Supernatant cytokine levels, evaluated by ELISA, were analyzed against whether the individual experienced no change (NDS) or an increase (IDS) in depression scores. Compared against the NDS cohort, PBMC from the IDS cohort exhibited increased IL-1 β (11 fold) and IL-6 (3 fold) upon the addition of IFN. Core and NS3 augmented IL-1 β and IL-6 synthesis in all cases. In contrast, Core and NS3 markedly enhanced IL-10 production in the NDS cohort. To date, these data suggest that an increase in the balance of pro-inflammatory over anti-inflammatory cytokine activity at baseline associates with increased depression scores during treatment. In this ongoing study, cytokine mRNA expression during treatment is also being examined. Being able to predict IFN- α induced depression in individuals with chronic HCV infection will lead to better management of side effects and subsequently better treatment outcomes.

An Epidemiologic and Laboratory Based Study to Define the Association Between Exposure to Cyanobacterial Hepatotoxins and the Prevalence of Hepatocellular Carcinoma in Canada

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Blue green algae, also known as cyanobacteria produce a variety of toxins some of which have been implicated in the pathogenesis of acute liver injury and hepatocellular carcinoma. As cyanobacterial contamination of fresh water lakes and rates of hepatocellular carcinoma continue to increase worldwide, it is important to enhance our understanding of cyanobacterial growth, toxin production and features, the risk toxin exposure poses to human health and what measures need be undertaken to limit such threats.

This study will address the following questions: 1) Whether an epidemiologic correlation exists between the rising rates of hepatocellular carcinoma in Canada and cyanobacterial contamination of drinking water. 2) Whether chronic, low dose exposure to cyanobacterial hepatotoxins *in vitro* promotes and/or induces malignant transformation of healthy hepatic stem cells. This study will also address water quality and treatment, and whether the World Health Organization limits of permissible toxin concentrations in the drinking water are appropriate. Due to global climate change, increased industrialization and altered watersheds which all contribute to increasing cyanobacterial growth, there is an urgent need to document the potential adverse effects of toxin exposure on the health of Canadians. This study represents an important step towards addressing these questions, and providing a guideline for safe water management.

Mucosal Immunity in the Female Genital Tract of Sex Workers (FSW) of Benin

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Objective: Over three-quarters of HIV/AIDS cases occur in third-world countries through heterosexual transmission. We estimate at 17.7 million women infected with HIV-1. In Africa, women constitute nearly 60% of new infections. The women are a vulnerable population concerning HIV infection. Since the genital tract is a major route of entry for HIV, understanding how the genital mucosal immune system might prevent or facilitate HIV infection is important. The main objective of our project is therefore to analyse the immunity of the mucosal genital tract in African FSW. A first step is to characterise and compare the presence of some cytokines in the cervico-vaginal lavage (CVL) and the serum of FSW.

Methods: CVLs and serum from average 60 HIV-1 uninfected FSW, 60 HIV-1 infected FSW and 60 low-risk HIV-1 uninfected non-FSW have been analysed qualitatively and quantitatively for the presence of IL-2, IL-4, IL-6, IL-10, TNF and IFN- γ using BD™ Cytometric Bead Array (CBA) technology Th1/Th2 kit II technology.

Results: We report that HIV-1 infected CSW have higher levels of TNF- α and IFN- γ in their CVL when compared to HIV-1 uninfected women. We also observed a diminution of all the cytokines levels tested in the serum of the HIV-1 infected FSW when compared to the HIV-1 uninfected women.

Conclusion: Our results demonstrate the important differences between the systemic and mucosal compartment and the importance of well understanding the FGT mucosal immunity in order to develop effective preventive strategies as microbicide.

HIV-1 Proviral Hypermutation Associates with Decline in CD4 Count in HIV Infected Kenyan Women

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Introduction: APOBEC3G is an important innate immune molecule that causes HIV-1 hypermutation, which can result in detrimental viral genome mutations. The Vif protein of wildtype HIV-1 counteracts APOBEC3G activity by targeting it for degradation and inhibiting its incorporation into viral particles. Additional APOBEC cytidine deaminases have been identified, such as APOBEC3F, which has a similar mode of action but different sequence specificity. A relationship has been proposed between APOBEC3G/3F and HIV disease progression. We hypothesized that hypermutation could be identified in a cohort of HIV-infected subjects from Nairobi, Kenya and that this hypermutation could be correlated with clinical measures of HIV disease progression.

Methods: Proviral DNA was isolated from 240 Kenyan women infected with HIV-1 from a high risk commercial sex worker cohort, as well as a lower risk group, and used as a template for sequencing the vpu gene and the first 349 nucleotides of env. Plasma RNA of this region and proviral vif was additionally sequenced for a subset of patients. The resulting sequences were examined for hypermutation. Where available, CD4 cell count was determined. Mann-Whitney and test for Correlation statistical analyses were performed using GraphPad Prism 4.

Results: Thirteen hypermutated proviral vpu/env sequences were identified out of the 240 that were examined. Sequences derived from plasma virus, however, lacked hypermutation, as did proviral vif. When examining correlates of disease progression, subjects with hypermutated provirus were found to have significantly higher CD4 counts compared to the other subjects ($p = 0.0052$). Furthermore, hypermutation as estimated by elevated adenine content positively correlated with CD4 count for all the study subjects ($p = 0.042$). The sequence context of the observed hypermutation was statistically associated with APOBEC3G/3F activity.

Conclusions: In contrast to previous studies, this study demonstrates that higher CD4 counts correlate with

increased hypermutation in the absence of obvious mutations in the APOBEC inhibiting Vif protein. This strongly suggests that host factors such as APOBEC3G/3F are playing a protective role in these patients, modulating viral hypermutation and host disease progression. These findings support the potential of targeting APOBEC3G/3F for therapeutic purposes.

Mobilization of Recycling Endosomes and Golgi Disassembly through a Novel Cdc42- and Rab11a-regulated Pathway in Nonapoptotic Cell Death Signaling

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Introduction: Cytoskeletal dynamics and membrane trafficking were shown to influence the commitment of cells to different death programs, but the mechanisms involved remain largely undefined. The nonapoptotic death program induced by the adenovirus early region 4 ORF4 (E4orf4) necessitates an increase in *de novo* actin polymerization on perinuclear membrane vesicles associated with markers of the endocytic recycling compartment (ERC). This process depends on Cdc42 and drives large-scale remodeling of the actin cytoskeleton followed by caspase-independent cell death. How these changes in actin dynamics perturb membrane trafficking and engage the death program is unknown.

Methods: Single-cell imaging methods and three-dimensional computer analyses were used to study dysfunctions of organelles related to recycling endosomes (RE) trafficking. The role of key regulators of actin polymerization (Rho GTPases) and membrane trafficking (Rab GTPases) was assessed by siRNA.

Results: Using E4orf4 as a model, we uncovered a novel role for actin, Cdc42 and Rab11a in upstream death signaling events, linked to the early dispersal of tubulovesicular ERC elements and disassembly of the Golgi complex. Loss of Golgi integrity, inhibition of post-Golgi protein transport and cell death all relied on the concerted actions of Cdc42 and Rab11a, which together with actin, promoted the fragmentation of ERC-derived membrane vesicles and their delivery to medial-*trans* Golgi membranes. Increased membrane fusion events between recycling endosomes and Golgi membranes stimulated the local remodeling of Golgi tubules that underwent stretching and fission. The subsequent loss of Golgi integrity contributed to cell death commitment, since inhibition of Golgi disassembly downstream of ERC dispersal significantly impaired E4orf4-induced nuclear condensation. Consistent with a general function in death signaling, actin, Cdc42 and Rab11a were also found to regulate ERC dispersal, Golgi fragmentation and nuclear condensation during staurosporin-induced caspase-independent apoptosis.

Conclusion: Collectively, our findings strongly suggest a novel role for actin, Cdc42 and Rab11a in the mobilization of ERC- and Golgi-derived membrane vesicles during programmed cell death signaling.

Peripheral, but not Central, Tolerance nor Level of Autoantigen Expression is Responsible for the Gender Bias in Susceptibility to Experimental Type 2 Autoimmune Hepatitis

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Introduction: Autoimmune hepatitis (AIH) is a disease of unknown aetiology characterized by a progressive destruction of the hepatic parenchyma. In type 2 AIH, 90% of cases are women and 80% are diagnosed before the age of 18. Recently, we have developed an experimental model of type 2 AIH based on xenoinmunisation with human autoantigens (CYP2D6 and FTCD) in C57BL/6 mice. Therefore, using this model, we aim to study the influence of sex and age on AIH development.

Methods: AIH was induced in 7 week-old male and 4, 7 and 14 week-old female C57BL/6 mice by DNA xenoinmunisation. To study the influence of central tolerance on disease development, male and female B6.129S2-**Airetm**1.1Doi (+/o) mice were used. ALT levels and LKM1/LC1 autoantibody titers were monitored for 8 months. Mice were sacrificed and liver histology, FACS and B and T cells responses were analysed. Thymic and liver autoantigen expression was assessed using qPCR.

Results: Female mice vaccinated at seven weeks of age showed higher serum levels of ALT, increased liver inflammation ($p < 0.01$), higher specific T cell cytotoxicity and elevated autoantibody titers ($p < 0.01$) compared to other groups. Titers of anti-LC1 autoantibodies correlated with disease activity. B6.129S2-**Airetm**1.1Doi (+/o) mice showed a similar gender bias in disease susceptibility as C57BL/6 mice. Liver and thymic expression of AIH autoantigens was similar in mice of both sexes. Regulatory T cell levels in PBMC and spleen were higher in males C57BL/6 mice with AIH and in liver infiltrates of male B6.129S2-**Airetm**1.1Doi mice compared with females ($p < 0.01$).

Conclusions: As in humans, age and sex influence the development of type 2 AIH in our experimental model. B cell responses against murine FTCD (anti-LC1) could be involved in AIH pathogenesis. A difference in peripheral tolerance but not of central tolerance nor of hepatic expression of autoantigens is responsible for the observed gender bias in disease susceptibility in our model of type 2 AIH. The identification of specific mechanisms responsible for the observed gender bias in human AIH could help us understand female preponderance in autoimmune diseases in general.

Diffusion Tensor Imaging of Children with Fetal Alcohol Spectrum Disorders

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Introduction: Children with fetal alcohol spectrum disorders (FASD) may have a variety of cognitive, behavioral, and neurological impairments, including structural brain damage. White matter provides the brain connections necessary for proper cognitive function; however, little is known about how these connections are affected in FASD. This study used diffusion tensor imaging (DTI), an advanced magnetic resonance imaging technique that may be more sensitive to white matter changes than conventional imaging, to examine microstructural white matter differences in children with FASD.

Methods: Subjects were 24 children aged 5-13 years previously diagnosed with FASD and 95 healthy children over the same age range. Diffusion tractography, a novel technique capable of virtually reconstructing white matter pathways *in vivo*, was used to delineate ten major white matter tracts in each individual. Fractional anisotropy (FA), an indicator of white matter integrity, and mean diffusivity (MD), which measures the magnitude of water diffusion, were assessed in each of the ten brain structures and compared between groups using a MANCOVA controlling for age. White and gray matter and total brain volumes were also compared between groups.

Results: DTI revealed significant diffusion differences (FA and/or MD) between groups in seven of ten white matter tracts: the genu and splenium of the corpus callosum, cingulum, corticospinal tracts, inferior fronto-occipital fasciculus, and inferior and superior longitudinal fasciculi. Gray matter, white matter, and total brain volume were significantly reduced in children with FASD.

Conclusions: These results indicate reduced white matter integrity in children with FASD in a variety of brain areas, supporting previous findings of diffusion abnormalities in the corpus callosum and inferior longitudinal fasciculus, and describing further diffusion changes for the first time. These results suggest that white matter tracts with commissural and temporal connections are particularly sensitive to prenatal alcohol exposure, and may help to further understand the structural changes underlying cognitive deficits in FASD.

Soluble Expression of a Recombinant Human Epididymal Sperm-binding Protein

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Introduction: Mammalian sperm undergo several maturation steps before acquiring fertilization competence. The initial events take place during epididymal transit, whereas the final modifications occur inside the female genital tract during capacitation. We demonstrated the importance of Bovine Seminal Plasma (BSP) proteins in sperm capacitation. Recent studies identified a BSP-homologous sequence in the human genome (*hBSPH1*) and revealed epididymal mRNA expression. This study sought to characterize recombinant *hBSPH1* with regard to its binding properties and role in sperm functions.

Methods: *hBSPH1* cDNA was cloned into the pET32a vector and expression was induced in OrigamiB(DE3) *E.coli* cells. Protein was purified by Ni²⁺ affinity chromatography, dialyzed and lyophilized. Binding properties were assessed by heparin-sepharose affinity chromatography, Lipo-Gel electrophoresis, as well as liposome- and human sperm-binding.

Results: hBSPH1 contains tandem type-II domains, each consisting of 2 disulfide bonds. Since proteins containing these domains are very difficult to express in the soluble form, our first attempts at expressing His-tagged or GST-tagged hBSPH1 resulted in protein accumulation in inclusion bodies. Production of soluble hBSPH1 was achieved upon fusion to thioredoxin in OrigamiB(DE3) cells. This system allowed the purification of approximately 5-6 milligrams of metal-affinity purified protein from 1 L of bacterial culture. Purified recombinant protein was shown to be active as judged by its ability to bind to heparin, phosphatidylcholine liposomes, low-density lipoproteins and human sperm.

Conclusions: The availability of large quantities of soluble and active hBSPH1 protein will aid us in establishing the role of this novel epididymal protein in human sperm functions and fertility.

Effects of Voluntary Exercise on Structure, Function, and Stem Cell Activation in Skeletal Muscle with Age

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Introduction: The huge impact of age-related sarcopenia led to this research to re-examine the effects of age and voluntary exercise on factors correlated with maintenance of muscle, namely muscle function, strength, fiber size, and precursor cell proliferation.

Methods: Several baseline variables (body mass, muscle mass, CSA, and satellite cell activation) were obtained from studies of normal C57BL6 mice at 3, 6, 8, 12 and 18 months of age. Mice of 8 and 18 months of age were randomized into an exercise or control group to determine functional changes in muscle. Distance traveled during voluntary wheel running over a 24 hr period was recorded from a wheel apparatus and grip strength was measured using a Chatillon strain gauge. Immediately after euthanasia, extensor digitorum longus (EDL) and gastrocnemius (GAST) muscles were isolated and prepared for analysis of DNA synthesis and fiber CSA, respectively. Scintillation counts of tritiated thymidine incorporation were used to determine levels of DNA synthesis. Fiber CSA was acquired from 5 μm sections of GAST using NIH ImageJ software. Data were compared using 2-way ANOVAs and Least Significant Difference statistical tests ($P < 0.05$).

Results: Results indicated body mass (g), muscle mass (mg) and fiber CSA (μm^2) rose significantly up to 6 mo of age and then declined. Body mass was also significantly greater at 8 mo compared to 3, 12 and 18 mo. By contrast, grip strength ($\text{g} \times 10/\text{g BW}$) was greatest at 3 mo. DNA synthesis was significantly greater in 8 mo compared to 18 mo mice but was not changed by exercise. At 18 mo of age, distance run increases over a 3 week training period and body mass decreases.

Discussion: Further experiments on changes in skeletal muscle with age are in progress to determine if various drug treatments can minimize, or eliminate sarcopenic changes in muscle.

β 1-integrin is a Novel Prognostic Biomarker and a Target in HER-2-overexpressing Trastuzumab Resistant Women with Breast Cancer

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Introduction: Overexpression of Human epidermal growth factor receptor-2 (HER-2)) correlates with poor prognosis in ~30% of breast cancer. Trastuzumab (Herceptin™), a HER-2 targeting antibody, is the standard of care for HER-2 overexpressing metastatic breast cancer (MBC) patients, though ~70% show primary resistance to trastuzumab therapy. Increasing evidence suggests that HER-2 functionally interacts with integrins, adhesion molecules promoting tumor-cell survival and invasiveness. Interestingly, HER-2 is overexpressed and co-localized with β 1-integrin in JIMT-1, a trastuzumab resistant cell line. We hypothesize that β 1-integrin may functionally contribute to trastuzumab resistance.

Methods: β 1-integrin inhibiting antibody, A1B2, was used to assess whether trastuzumab sensitivity could be restored to JIMT-1 by blocking β 1-integrin function *in vitro* and *in vivo*. The functional relationship between HER-2 and β 1-integrin was assessed by overexpressing β 1-integrin in trastuzumab sensitive SKBR-3 cells. The prognostic value of β 1-integrin expression in 78 HER-2 positive MBC patients treated with trastuzumab was assessed by immunohistochemistry.

Results: Combination of trastuzumab and A1B2 reduced β 1-integrin expression, decreased phosphorylation of pro-survival mediators AKT and ERK1/2, and decreased proliferation and clonogenic survival of JIMT-1 cells. Combination treatment significantly delayed growth of JIMT-1 mammary tumors *in vivo*, with no toxicity. Strikingly, overexpression of β 1-integrin in trastuzumab sensitive SKBR-3 cells decreased HER-2 levels and increased resistance to trastuzumab. Furthermore, high β 1-integrin membrane staining was an independent negative prognostic marker of time to tumor progression ($p=0.0089$) in 78 patients.

Conclusion: Inhibition of β 1-integrin restores trastuzumab sensitivity *in vitro* and *in vivo*. High β 1-integrin staining is a prognostic marker for trastuzumab resistance in MBC patients.

Molecular Characterization of HIV-1 Gag Quasispecies by Ultra-deep Pyrosequencing

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Introduction: New generation Sequencer utilizing Pyrosequencing technologies can produce millions of bases of nucleotide sequences in one run. It has sparked interest of microbial genomics researchers for its potential to sequence viral quasispecies that are challenging using traditional approach, such as the Human Immunodeficiency Virus (HIV). It can sequence mixed population of genomic materials without cloning and with a very high coverage (ultra-deep). The technology has been used to rapidly sequence microbial genomes and detect minor mutations. In this study, we systematically investigated the potential of ultra-deep pyrosequencing in characterization of HIV-1 gag quasispecies.

Methods: HIV gag was amplified by PCR from 96 patient genomic DNA. The purified PCR products were cloned and average 32 clones/per patient were sequenced with Sanger sequencing method and used as references. Pyrosequencing of the purified PCR products was conducted with 454 Life Science Genome Sequencer 20 platform (GS20). Sequence reads with high quality of scores were mapped to references by Wu-Blast. The coordinate of each read to reference was recorded for building consensus and calculating frequency of nucleotide/or amino acid at each position of the reference by in-house developed Perl scripts. Quasi analysis was conducted on consensus determined from clone sequences and pyrosequencing reads.

Results: Over 2% nucleotide consensus mismatches were identified between sequences from clones and sequences generated by pyrosequencing per sample and more than 60% of the mismatches overlap regions targeted by host cytotoxic T-cell responses, neutralizing antibody responses, and involved in virus assembly/packaging. More than two fold sequence variations were detected in pyrosequences than that of clones in most of HIV-1 gag genes. Quasi analysis shows that 11.25% of positively selected sites are different between sequences of clones and pyrosequencing.

Conclusion: Ultra-deep pyrosequencing is a promising sensitive tool to characterize HIV-1 gag genetic diversity, especially to detect minor virus populations and to analyze positive selection pressures exerted by host immune response. Our study provided detailed assessment of using pyrosequencing technology to investigate viral quasispecies.

The p110 δ Isoform of Phosphoinositide 3 Kinase (PI3K) Controls the Quality of Secondary Anti-Leishmanial Immunity by Regulating Expansion and Function of Memory T Cell Subsets

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We previously showed that mice with an inactivating knock-in mutation in the p110 δ isoform (p110 δ ^{D910A}) of the phosphoinositide 3 kinase (PI3K), displayed enhanced primary resistance to *Leishmania major* despite mounting paradoxically impaired T cell responses. Here, we show that despite this enhanced primary resistance, p110 δ ^{D910A} mice that healed from their primary *L. major* infection are dramatically impaired in their *Leishmania*-specific recall responses *in vitro*. Following secondary virulent *L. major* challenge, these mice exhibited impaired delayed-type hypersensitivity (DTH) response and weaker parasite control when compared to their wild-type counterpart mice. Using adoptive transfer of immune cells into congenic naïve recipients, we show that T cells from p110 δ ^{D910A} mice were significantly impaired in their proliferation and effector cytokine (IFN- γ) responses upon virulent *L. major* challenge. This impaired secondary response was not due to parasite clearance because we isolated comparable numbers of parasites in primary infection site in healed p110 δ ^{D910A} and wild-type mice. Interestingly, *Leishmania*-reactive T cells from healed p110 δ ^{D910A} mice have much lower CD62L^{low} (effector memory) and higher CD62^{hi} (central memory) T cells than those from healed wild-type mice. Since effector memory T cells control rapid anti-*Leishmania* memory response, the significantly impaired secondary immunity in p110 δ ^{D910A} mice may be related to impairment in expansion and function of this population of memory T cells.

Distinct Roles of IL-10 in Regulation of Peanut Allergy

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Introduction: Enhanced serum and T cell derived IL-10 is thought to underlie clinical tolerance to food antigens. Here, we evaluate its role in regulation of peanut-driven cytokine production in peanut allergic, sensitized, and clinically tolerant human populations.

Methods: Eighteen clinically peanut allergic (history +, sp IgE +, skin test +), 8 sensitized (ingest peanut without clinical symptoms, skin test +) and 29 peanut non-allergic (clinically tolerant) individuals between 6-45y were studied. PBMC were stimulated with peanut Ag alone, and in the presence of rIL-10 or anti-IL-10R neutralising Ab in short-term primary cultures. Peanut-driven Type 1, Type 2, and regulatory cytokine response profiles were quantified by ELISA.

Results: Contrary to expectation, IL-10 production in response to peanut-specific stimulation was higher in peanut allergic than non-allergic or peanut sensitized individuals. Exogenous rIL-10 added to culture abrogates peanut-driven cytokine production in peanut allergic and clinically tolerant individuals. Blocking endogenous IL-10 function has no impact on recall IFN γ , IL-9 or IL-13 responses, arguing against an essential role in maintenance of clinical tolerance. Among peanut allergics, anti-IL-10R treatment during allergen stimulation enhanced both Th2 (35-90% $p < 0.001$) and Th1 (90-250%, $p < 0.0001$) recall responses, suggesting a role in limiting maladaptive immunity.

Conclusion: IL-10 and the IL-10R play no detectable role in preventing initiation of peanut specific food allergy, but may be important in limiting the intensity peanut specific Th2 and pro-inflammatory responses in peanut allergic individuals.

Mechanisms of c-Myc Dependent Gene Amplification

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Introduction: Cancer is a disease of genomic instability to which c-Myc contributes during its initiation and progression. The transforming activity of c-Myc is generally thought to lie in its ability to modulate the expression of a series of genes, among them certain proliferation-promoting genes. Moreover, c-Myc is a multifunctional protein that does not require a mutation to affect the stability of the genome and initiate neoplastic transformation. The term genomic instability refers to genetic and/or epigenetic changes that alter the normal organization and function of genes and chromosomes. Deregulated c-Myc expression generates genomic instability by initiating intra and extrachromosomally locus specific gene amplification, gene rearrangements and karyotypic instability. One way for the development of gene amplification is illegitimate replication. Replication is considered illegitimate if it takes place either in a wrong phase of the cell cycle or when more than one replication fork fires from a single replication origin simultaneously or repeatedly. Recent data from our group showed that upon c-Myc deregulation the firing of four replication forks at the ribonucleotide reductase 2 (*R2*) gene was observed and preceded *R2* gene amplification.

Methods: Two cell lines exogenously expressing c-Myc were considered for the study; both are immortalized but stably diploid: Mouse PreB cell line and rat fibroblasts. Cells were synchronized at G1/S boundary. c-Myc was activated and cells were harvested at specific time points. Whole cells lysate were analyzed using Immunoprecipitation technique to identify protein/protein interaction taking place between Myc and replication initiation proteins. Results were confirmed using immunofluorescence. Isolated genomic DNA was analyzed using two dimensional gels for detecting replication intermediates.

Results: We show a common mechanism by which experimentally deregulated c-Myc induces the initiation of illegitimate replication intermediates at the specific Myc gene amplification targets dihydrofolate reductase (DHFR), cyclin D2 (CCND2) and *R2*. The replication intermediates included rereplicating origins and multiple replication forks. We also report protein-protein interactions involving c-Myc and the members of the early replication initiation complex primarily with Cdc 6 and consequently with Orc1 and Cdt1 but not Mcm7 or PCNA. Those interactions stabilize between the replication initiation complex and the replicating DNA element and lead to re-firing of the origins.

Conclusion: These findings establish a novel explanation for the initiation of gene amplification in absence of a significant DNA damage effect. Understanding this mechanism will facilitate controlling gene amplification initiated by this pathway which in turn will decrease rates of recurrence and improve prognosis.

The Role of AngII in the Modulation of SMC Phenotype

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Introduction: Coronary heart disease can be treated by interventions such as angioplasty or bypass graft surgery, but these procedures also result in injury to the blood vessel. Upon vascular injury, the smooth muscle cells that surround blood vessels change their properties and shift from the resting state to a dividing state. This phenomenon, besides being a cause of recurrent coronary heart disease, is also a challenge for subsequent heart surgeries. The underlying mechanism typically takes the form of either hypertrophic (certain forms of hypertension) or hyperplastic (atherosclerosis, restenosis) cell growth, processes that involve many different genes and proteins, the most significant of which are associated with cell proliferation. Angiotensin II (AngII) is a critical element in the development of hypertension and atherosclerosis. Angiotensin receptor blockade with losartan (AT1 receptor antagonist) effectively prevented cellular proliferation and migration and consequently neointimal formation. Previous data suggested that AngII triggers signalling that includes the cyclic AMP response element binding protein (CREB) and forkhead transcription factors (FOXO). In this study we investigated the possibility that crosstalk between AngII AT1-R and IGF1-R involving a Src kinase-dependent pathway mediates CREB and FOXO activation.

Methods: Porcine primary smooth muscle cells isolated from the left descending coronary artery were made quiescent and were subsequently treated with inhibitors for hypothesized members of Ang II-dependent signalling pathways. This was followed by treatment with either Ang II or IGF-1. Cells were then lysed and protein extracts prepared. Protein extracts were analyzed by Western blotting using antibodies for putative signalling pathway members to monitor protein levels or phosphorylation status.

Results: AngII signaling activates CREB *via* two possible mechanisms, involving either the PI3K/AKT or IGF1-R/MAPK pathways. Furthermore, the data suggest a member of the Src-tyrosine kinase family mediates IGF1-R transactivation upon AngII stimulation.

Conclusions: Understanding the signalling pathways that control the switch in smooth muscle cell phenotype that enables cell proliferation will help to identify targets suitable for therapeutic intervention. The ability to control this phenomenon will in turn decrease the chance of recurrent coronary heart disease.

TLR Responsiveness is Deficient in Late Preterm Neonates

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Background: Late preterm neonates (34-36 weeks) have historically been grouped with full term neonates; however this group have been recognized as having increased risk of infection and respiratory distress at birth. Our objective was to assess how gestational age affects the pattern and intensity of innate immune responses at birth.

Methods: Cord blood samples were collected following vaginal delivery from full-term (<37weeks, n=77), early (<33 weeks, n=7) and late preterm (34-36 weeks, n=40). Cord blood mononuclear cells (CBMCs) were cultured with Toll-like receptors (TLR)1-9 for 24 hours. Expression of cytokines and chemokines in supernatants was measured via ELISA. The frequency of CBMCs producing IL-6 and IL-10 was measured via ELISPOT analysis. Flow cytometry was utilized to quantify absolute number of CD14+ monocytes. Quantitative Real-Time PCR was employed to assess the mRNA levels of various TLRs and related signaling molecules.

Results: We examined a broad range of responses to TLR antigens for both viral and bacterial antigens at different gestational ages. In general, proinflammatory markers (IL-1 β and CCL2), IL-6 and IL-10 were all markedly reduced in the late preterm group following TLR stimulation. Frequencies of IL-6 and IL-10 producing cells and the absolute number of CD14+ monocytes were not different in the preterm and full term groups. However, the expression of TLR2,4,6,7,8, CD14, MyD88 and TRIF mRNA was significantly reduced in the late preterm compared to the full term population.

Conclusions: Late preterm neonates demonstrate an impaired innate immune response to both bacterial and viral stimuli. Our data suggest that CBMCs from late preterm neonates may possess intrinsic deficiencies in TLR signaling pathways which could contribute to the hyporesponsiveness observed in this population.

The Effect of HSV-1 Infection on the Host miRNA Expression Profile

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MicroRNAs (miRNAs) are small, non-coding RNA species (~22 nt in length) that are involved in post-transcriptional gene regulation. MiRNAs bind to complementary regions in the 3'-UTR of protein-coding mRNA and mediate either translation repression or transcript degradation. Hundreds of miRNAs have been identified, each with a potential to regulate numerous transcripts. MiRNAs represent one of the largest classes of gene regulatory mechanisms and understanding the function and roles of these small RNA molecules during an infection are essential for gaining insights into the pathobiology of disease.

Currently, miRNA research centered on viral diseases has primarily focused on identifying the role of viral encoded miRNAs. For example, Herpes Simplex Virus Type 1 (HSV-1) has been shown to encode miR-H1 and miR-LAT. However, there is limited knowledge about relevance of host miRNA response during HSV-1 infection. Our goal in this particular study was to determine host miRNA response during an HSV-1 infection. This knowledge should complement what is already known about the role of viral miRNAs and therefore allow us to gain a better understanding of the disease mechanism.

In this work, Vero cells were infected with an HSV-1 strain F at a multiplicity of infection of one. RNA samples were collected at timed intervals and using miRNA specific microarrays and multiplex qRT-PCR we were able to identify numerous host cell miRNAs that showed altered expression throughout HSV-1 infection. Subsequently, by using a consensus-based target finding approach we identified potential genes regulated by these differentially expressed miRNAs. We complemented the bioinformatic approach with functional studies on a subset of these miRNAs through *in vitro* over-expression experiments to detect their target genes.

In this presentation, we will report on host miRNAs that are aberrantly expressed in response to HSV-1 infection and subsequent target genes that are altered.

Adolescents Taking Exploratory Action to Enhance Sexual Health Education

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Introduction: A Canadian study indicates that 50% of Grade 9 students believe a HIV/AIDS vaccine exists. Presumably, school-based health educators would debunk such a myth; however, they rely upon curricula that are restricted to information, skills, and attitudes that ‘others’—such as health teachers, administrators, and health policy makers—warrant essential for adolescents. This approach to curriculum development is problematic because it is done to rather than with students. My doctoral research responds to this concern by asking: 1) What are adolescents’ sexual health educational perspectives? and 2) How does the education system respond to students’ sexual health educational perspectives?

Methodology: I adopt participatory action research, premised on the belief that research is conducted with rather than on those immediately affected by a problem. I invite eight Grade 12 students to assume the roles of co-researchers, and thereby conceptualize, collect, analyze, and present research findings. To assist them with this effort, I guide them through the ethical research process, ensuring they possess the requisite knowledge and skills to conduct semi-structured interviews with three peers. These interviews are transcribed and analyzed by the student co-researchers in order to inform the design and development of a Sexual Health Education Action Plan. Subsequently, this plan is presented to their teachers, administrators, and curriculum consultants so the education system may not only acknowledge, but also respond to students’ sexual health educational perspectives.

Results: The Sexual Health Education Action Plan is a multi-media presentation designed and developed by the student research team. Notably, it communicates potential enhancements that educators, at various levels of the education system, can consider when modifying the existing sexual health education offered at their school.

Conclusion: Students—typically at the bottom of the education organizational structure—can offer substantive insights into their own sex education. This study underscores that educators must recognize, respect, and, ultimately, respond to students’ sexual health educational perspectives.

Stimulated Down-Regulation of Fc γ R1la in Human Neutrophils : Role of the Ubiquitin Ligase c-Cbl

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Little is known about the mechanisms that arrest Fc γ R1la signalling in human neutrophils once engaged by immune complexes or opsonised pathogens. In our previous studies, we observed a loss of immunoreactivity of antibodies directed against Fc γ R1la following its cross-linking. Here we report on the mechanisms involved in this event. A stimulated internalisation of Fc γ R1la leading to the down-regulation of its surface expression was observed by flow cytometry. Immunoprecipitation of the receptor showed that Fc γ R1la is ubiquitinated after stimulation. MG132 and clasto-lactacystin β -lactone inhibited the loss of immunoreactivity of Fc γ R1la, suggesting that this receptor was down-regulated via the proteasomal pathway. The E3 ubiquitin ligase c-Cbl was found to translocate from the cytosol to the plasma membrane following receptor cross-linking. Furthermore, c-Cbl was recruited to the same subset of high-density detergent-resistant membrane fractions as stimulated Fc γ R1la itself. Silencing the expression of c-Cbl by siRNA decreased Fc γ R1la ubiquitination, prevented its degradation without affecting the internalisation process. It also prolonged the stimulation of the tyrosine phosphorylation response to the cross-linking of the receptor. We conclude that c-Cbl mediates the ubiquitination and degradation of stimulated Fc γ R1la *via* the proteasomal pathway and thereby contributes to the termination of Fc γ R1la signalling and the down-regulation of neutrophil activation through this receptor.

Stochastic Resonance in the Cross-modal Sensory Integration and in the Motor System

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Introduction: The Stochastic Resonance (SR) is a phenomenon that increases the detection of weak stimuli by a particular level of noise added to one system. SR type effects have been demonstrated in diverse sensory systems in which the signal and noise applied were of the same sensory modality. However, there are neither studies related with the cross-sensory modality systems nor with cross-system responses. The aims of this study were to analyze changes in the ability of humans to detect a weak visual stimulus when a continuous auditory noise was applied, and to explore if the SR phenomenon is present in the motor system, specifically in the monosynaptic Ia-motoneuron reflex in the spinal cord of the cat.

Methods: We performed a series of psychophysical experiments in 15 young subjects, and we applied them a constant sub-threshold visual stimulus during six different levels of auditory noise. In order to analyze the SR in the motor system, we examined changes in the monosynaptic reflex amplitude in the ventral root L7 in the spinal cord of decerebrate cats. The MSR were evoked by periodic electrical stimulation of the medial gastrocnemius nerve during different intensities of noisy stretches applied in the tendon of the lateral gastrocnemius and soleus muscle.

Results: Our results related to the cross-sensory modality between auditory noise and a weak visual stimulus suggests that a different noisy sensory modality from a particular sensory system improves the performance of the last one. In our case, the correct percent detection of a sub-threshold visual signal is improved by an intermediate level of auditory noise. On the other hand, our results showed an increase in the monosynaptic reflex amplitude during an intermediate level of mechanical noise and a reduction of this amplitude for high level of mechanical noise. This result indicates that the Stochastic Resonance phenomenon is present in the Monosynaptic Reflex Pathway in the spinal cord of the decerebrate cats. This study is the first direct evidence of the Stochastic Resonant phenomenon in a motor system.

Conclusion: Collectively, these finding suggests the noise improves the performance in sensory and motor systems, even though the noise is from a different modality from the periodic signal. Furthermore, we propose that facilitation and occlusion mechanisms are involved during the optimization and depression of the monosynaptic reflex amplitude, respectively.

Trajectories of Internalizing Psychopathology Across Childhood: A Longitudinal Study on the Impact of Cognitive Functioning in “At Risk” Children

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Rates of internalizing problems (i.e. depression and anxiety) increase across childhood. Factors that precipitate this increase have not been closely examined. The current study examines the longitudinal trajectories of internalizing problems and their associations with IQ in 91 disadvantaged children. Internalizing was assessed at three time points, from early to late childhood. Hierarchical linear modeling revealed that mother- and teacher-reported internalizing increased over the course of childhood, with time accounting for 29% of the within-subjects variance. Subsequently, level 2 analyses were performed to examine whether child’s IQ, and demographic variables were associated with the development of internalizing problems in children over time. After controlling for child age, gender, maternal education, and family income, lower IQ was associated with increases in internalizing across childhood. High anxiety combined with low IQ had a negative effect on internalizing. These results support the involvement of cognitive ability in the developmental course of child psychopathology.

Cultural Implications of Plagiocephaly

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In order to reduce the number of babies who die from Sudden Infant Death Syndrome (SIDS) in Canada, Health Canada, the SIDS Foundation, the Canadian Institute of Child Health and the Canadian Paediatric Society formed a coalition and launched the national “Back to Sleep” campaign in 1999, promoting the supine sleep position (Canadian Institute of Child Health, 2007). The “Back to Sleep” campaigns had phenomenal success in decreasing the incidence of SIDS; however research is now showing this change in sleep position has brought a new set of concerns, including the development of positional plagiocephaly (Davis, Moon, Sachs, & Ottolini, 1998). Plagiocephaly is defined as a malformation of the skull producing the appearance of a twisted and lopsided head, caused by irregular closure of the cranial sutures, commonly referred to as “flat head” (Thomas, 1997). There is beginning evidence to suggest the long term impacts of plagiocephaly, mainly neurodevelopmental delays including cerebral development and psychomotor development and impaired visual field development which ultimately impact capacity to learn (Kordestani, Patel, Bard, Gurwitch & Panchal, 2006; Miller & Clarren, 2000; Siatkowski et al., 2005).

In Northern Areas, Pakistan, plagiocephaly is widely observed. During discussions with community members in Northern Areas in May 2006, I found that mothers of newborn infants rub the back of their babies’ heads in efforts to deliberately flatten them (J. Akbar, personal communication, May 30, 2006). A flattened head is valued and is a sign of beauty.

The purpose of the study is to assess cultural implications of plagiocephaly. Canadian health research that examines diverse population groups is still in its early stages. As a result, there is a need for knowledge about how best to approach health issues of immigrants with cultural sensitivity (Christensen, 2001). Cultural competence in health care provision is emerging as an important area of investigation, moving from a previous era of speculation on the exotic behaviour of non-Western people to using sophisticated research methodology to determine the impact culture has on health (Al-Issa, 1995).

A Novel Requirement for the Rb/E2F Cell Cycle Signaling Pathway in Regulating Neuronal Migration

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Aberrant migration of interneurons during nervous system development has been hypothesized to contribute to the etiology of disorders such as autism, mental retardation, and epilepsy, however the mechanisms regulating neuronal migration are not fully understood. While the role of the cell cycle regulatory Rb/E2F pathway is well appreciated as a key regulator of neural precursor proliferation, here we describe a novel role for the Rb pathway in neuronal migration. Using cortical slice co-culture assays we identified a cell autonomous requirement for Rb in regulating migration of ventrally derived interneurons in the developing telencephalon. Next, we sought to determine the mechanism by which Rb mediates proliferation and migration in neurogenesis. Members of the E2F transcription factor family are key Rb interacting factors, well known for mediating cell cycle regulation, whose activity is deregulated in the absence of Rb. Using mice with compound null mutations of Rb and E2F1 or E2F3, we asked to which extent either E2F interacts with Rb in neurogenesis. Here, we report that E2F1 and E2F3 are both functionally relevant targets in neural precursor proliferation, cell cycle exit, and laminar patterning. Neuronal migration, however, is specifically mediated through E2F3, beyond its role in cell cycle regulation. Through microarray based screening methods we have identified neogenin, a member of the neogenin-netrin pathway as a candidate E2F responsive gene, and through in vitro explant cultures observe a disrupted netrin mediated migration response in Rb deficient interneurons. Together, we have identified a novel role for the classical Rb/E2F cell cycle pathway regulating neuronal migration. Further, we have identified a new molecular pathway mediating migration of interneurons during nervous system development.

Fracture Incidence Among Canadian Women and its Association with the Osteoporosis Risk Assessment Instrument

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Osteoporosis is a progressive skeletal disease commonly affecting postmenopausal women and is characterized by decreased bone mineral density (BMD) leading to an increased risk of fracture. The Osteoporosis Risk Assessment Instrument (ORAI) is an effective osteoporosis risk factor screening tool developed to identify postmenopausal women at increased risk of low BMD who should undergo dual energy x-ray absorptiometry (DXA) screening. The aim of this study is to determine whether ORAI scores indicating high risk of low BMD is associated with fracture incidence. Analysis was based on weighted data from 19,651 Caucasian women over 60 years of age from the Canadian Community Health Survey (Cycle 2.1). Chi-square and logistic regression analysis were used to determine the association between ORAI risk level and fracture incidence of the hip, thigh, or spine. Calculated risk scores showed 89.8% of the women were at high risk of low BMD. Of those who reported fracture, 92.3% had ORAI scores indicating high risk of low BMD, while 89.7% of women with no fracture were also at high risk. It was determined that women who reported fracture were more likely to have high risk levels than those who did not report fracture ($p = <0.001$). ORAI scores indicating high risk of low BMD were associated with fracture incidence (OR = 1.37, 95% CI: 1.31, 1.43; $p = <0.001$). Results suggest the majority of Canadian women over 60 years of age are at increased risk of osteoporosis. The ORAI is a useful screening tool to identify women who are at high risk of low BMD who have suffered fracture and would most benefit from DXA screening.

Identification of a New Soluble Glycoprotein (ssGP) During Ebola Virus Infection

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Introduction: Ebola virus (EBOV) contains an editing site in its glycoprotein gene which encodes the soluble glycoprotein (sGP) from a single open reading frame while the transmembrane glycoprotein (GP) requires transcriptional editing resulting in a +1 shift in the open reading frame after the editing site. The production of a small soluble glycoprotein (ssGP) has been proposed as a result of transcriptional editing leading to a -1 or +2 shift in the open reading frame at the editing site.

Methods: GP mRNA from EBOV infected Vero E6 cells was reverse transcribed, and a fragment covering the editing site was cloned into the TOPO plasmid and subsequently sequenced to determine the ratio of different transcripts. BALB/c mice were infected with EBOV and blood and liver samples were collected 4 days post infection to determine if ssGP transcripts were also produced *in vivo*. The open reading frame encoding for ssGP was cloned into the eukaryotic expression vector pDisplay for transient transfection in 293T cells. 72 hours post-transfection the supernatant was collected and purified by affinity chromatography. The glycosylation status and tertiary structure of ssGP was characterized using biochemical and immunological methods. The identification of ssGP during Ebola virus infection is currently being analyzed by 2D-electrophoresis and mass spectrometry.

Results: From infected Vero cells mRNA species encoding for GP, sGP and ssGP were found in a ratio of 27%, 68% and 5%, respectively. Similar ratios of mRNA were found in the liver from EBOV infected mice; however, the transcript ratio in blood samples was 8%, 89% and 3%, respectively. Recombinant expressed ssGP appears as a disulfide linked dimer that is exclusively N-glycosylated similar to sGP.

Conclusions: During infection small amounts of ssGP transcripts are produced indicating that ssGP is produced and represents a new secreted Ebola virus protein.

Effect of Docosahexaenoic Acid Supplementation on Retinal Function in ELOVL4 Mice

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Introduction: The ELOVL4 gene is thought to be linked with the production of DHA, which humans can synthesize but only in small amounts. Functional improvement in vision has been reported in patients with a mutation in ELOVL4 who were given DHA in their diet. For this study we have examined how DHA supplementation might delay functional decline both in ELOVL4 and wt mice from 1 to 3 months of age.

Methods: ELOVL4 mice (n=11) and wt littermates (n=13) at one month of age (line 2 from Dr. K Zhang, Moran Eye Center, Utah) were each divided into two groups, one fed a 1 % DHA diet (E4+, WT+), the other fed the same diet without DHA (E4-, WT-). Under xylazine-ketamine anesthesia, ERGs were recorded (Espion E2, Diagnosys LLC). After two months of dietary manipulation standard ISCEV bright flash and photopic tests were recorded, as well as the cone specific test scotopic double-flash ERG. In order to perform the statistical analysis, the single higher responsive eye was selected. Groups E4-/E4+ and WT-/WT+ were compared as pairs using one tailed Mann-Whitney U test.

Results: The E4+ group showed statistically significant higher mixed a-wave amplitudes ($p=0.0411$). In addition, pure cone amplitudes isolated with the double flash protocol also demonstrated statistically significant elevations in amplitude values ($p=0.0260$) when compared to E4-. No differences were demonstrated in either mixed scotopic or isolated cone implicit times. Standard ISCEV scotopic bright flash b-wave and photopic b-wave amplitudes in the E4+ and WT+ groups resulted in a tendency for higher values in contrast to E4- and WT-, but these values did not reach statistical significance.

Conclusion: ELOVL4 mice under a DHA supplemented diet demonstrated protection of retina function (mixed scotopic a-wave and pure scotopic cone b-wave) from one to three months of age. Preservation of amplitude values suggests DHA may potentially have a neuroprotective role on the retina.

Correlation of the Novel HIV Inhibitor Elafin with Biological Confounders and HIV-1 Disease Progression

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Introduction: There are currently more than 33 million people infected with HIV-1 worldwide. A subset of sex workers from Nairobi, Kenya remain HIV-1 uninfected despite repeated HIV exposure. Preliminary studies of a small sample group showed Trappin-2/elafin, an anti-leukoprotease, and RANTES, a CCR5 tropic chemokine are over expressed in cervicovaginal lavage (CVL) samples from HIV resistant sex workers. The aim of this study was to test a larger group of samples in order to confirm the over-expression of Trappin-2/elafin in genital tract secretions. Additionally, Trappin-2/elafin was measured in plasma to assess the systemic role of Trappin-2/elafin in altered susceptibility to HIV infection and to determine if Trappin-2/elafin levels correlate with disease progression in HIV positive individuals. Finally, Trappin-2/elafin levels were correlated with a number of biological variables to determine relationships between levels of protein and biological confounders such as douching practices.

Methods: RANTES and Trappin-2/elafin levels were measured in CVL samples of HIV positive, HIV negative, and HIV resistant members of the cohort using ELISA. Levels of Trappin-2/elafin and RANTES were compared across the various study groups and with various epidemiological confounders.

Results: We confirmed that Trappin-2/elafin levels are over expressed in HIV resistant subjects in comparison to HIV positive subjects ($p=0.0172$). We also detected levels of trappin-2/elafin in serum samples, and found higher levels in resistant women than in HIV negative women ($p=0.003$). When HIV positive subjects are divided into subgroups based on disease progression we found no differences in Trappin-2/elafin levels. Trappin-2 levels are also higher in pre-menopausal women than in post-menopausal women ($p=0.0119$).

Implications: Knowledge of Trappin-2/elafin expression patterns in HIV resistant women could potentially inform microbicide development, currently the best hope to curb the spread of HIV.

Towards a Sociology of Physical Activity in Congenital Heart Disease (CHD): The Social Determinants of Physical Activity and Health in Children and Youth with CHD

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Background: Rapid advances in medicine have dramatically altered the illness course for youth with congenital heart disease (CHD) and a growing number of these youth will become young adults. Despite reduced mortality, the psycho- social burden of CHD persists; the field of Pediatric Cardiology is concerned with maximizing health related quality of life, and the mediating role of physical activity. Although physical activity is associated with numerous physiological benefits, levels of participation and self -perceptions towards active pursuits remain low. By drawing on novel, child- centred methods, this study explored how social and contextual factors may detract from the attainment of health and physical activity participation.

Methods: This study was facilitated by the Labatt Family Heart Centre at the Hospital for Sick Children in Toronto; 17 complex cardiac patients and six allied health professionals participated in this investigation. Semi- structured interviews and critical visual methods were employed in order to examine how variables in “youth’s social lives and worlds” influence health and physical activity; a broad thematic analysis was generated.

Results: The findings indicated that youth with CHD are Embodied Health Actors who seek to garner control over their cardiac health and physical activity. As “health actors,” they contemplated their lives as young adults and understood The Future as an Uncertain Terrain. By articulating What I Wish You Knew, youth with CHD both resisted and confirmed dominant representations of children with chronic illnesses. They drew on the richness of past experience to illustrate the complexity of their Disclosure Dilemmas in health and physical activity, discussed Barriers Encountered in Physical Education, and articulated their Physical Activity Information Needs. Lastly, where physically active settings “threaten and disrupt normalcy,” youth with CHD considered themselves through the eyes of their “able- bodied peers,” and articulated their unresolved struggle to understand themselves as Similar or Different.

Conclusion: Where “disability is often visible conspicuous but socially and politically erased” (Garland Thomson, 2002), this study brought youth with heart disease from the periphery to the center. Findings are

discussed within the context of the existing research base and important implications for health and social scientists, are proposed. As the “experts of their own illness experiences,” youth with CHD rendered visible the barriers in their social lives and worlds that have prevented the attainment of optimal health and physical activity; by informing the conditions required for enjoyable, inclusive, and barrier-free play, they direct the way to structural and social change.

MLH1 Gene Region Haplotypes and Colorectal Cancer Risk

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Colorectal Cancer (CRC) develops primarily via two major genetic pathways. The suppressor pathway is identified by frequent mutations of tumour suppressor genes while the mutator pathway results from a deficiency of mismatch-repair (MMR) genes and leads to genome-wide microsatellite instability (MSI). *MLH1* promoter hypermethylation accounts for the majority of MSI CRCs. We have shown a strong association between the *MLH1* (-93G>A) promoter SNP (rs#1800734) and MSI-high CRCs in two separate populations. In addition, we have also observed its association with *MLH1* deficient tumours and more specifically with *MLH1* promoter methylation. We hypothesize that the *MLH1* -93G>A SNP is a representative SNP for a haplotype that predisposes the region to methylation and consequently *MLH1* deficiency and microsatellite instability. We have performed a haplotype analysis of the *MLH1* gene region using a case-control study design. The analysis was performed using lymphocyte DNA isolated from 929 CRC cases and 1098 controls from Ontario. Selected SNPs were genotyped using TaqMan[®] assay and the 500kb chromosome 3 region surrounding the *MLH1* gene was further enriched using SNPs from the Affymetrix 100K and 500K chips. We have identified a haplotype that is strongly associated with MSI-H tumours when compared to the controls. The SNPs comprising this haplotype were strongly associated with MSI-H tumours, *MLH1* IHC deficiency and *MLH1* promoter methylation. One of the SNPs, rs749072, was associated with the strong family history of CRC based on the Amsterdam Criteria I (P=0.035) and Amsterdam Criteria I and II (P=0.026), *MLH1* expression status (P=0.008), MSI-H tumours (P<0.001), and borderline associated with tumour stage (P=0.07) and tumour grade (P=0.06). We have also identified a number of other SNPs in the *MLH1* and LRRFIP2 genes that are associated with MSI-high tumours and *MLH1* IHC deficiency when compared to the controls. We are currently examining expression of LRRFIP2 gene in a selected group of MSI-H and MSS CRCs and we are replicating our haplotype findings in two additional populations. These results suggest that SNPs in genes surrounding the *MLH1* gene may also contribute to CRC risk and characterization of such SNPs will help to enhance our understanding of CRC development.

Berenil Modulates Immune Response to *Trypanosoma congolense* in Addition to its Trypanocytic Activity

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Trypanosomiasis are diseases of man and livestock caused by different species of extracellular hemoprotozoan parasites that are transmitted by Tse-tse fly bite. *Trypanosoma congolense* are extracellular but intravascular blood parasites and infections result in debilitating severe acute or chronic disease in cattle and other domestic animals. Because of their intravascular nature, the parasites circulate in the bloodstream and evade the host immune responses by antigenic switching of the variant surface glycoprotein. Berenil (Diminazene aceturate) has been widely used as a trypanocytic agent in livestock since 1955. The drug works by inserting in kinetoplast DNA thereby effectively suppressing parasitic DNA replication and is highly effective as it can clear parasites in a mouse model within 24 hours. Although the molecular basis for Berenil's action is well known, its effect on the host immune system has remained relatively unstudied. The highly susceptible BALB/c mice infected with *T. congolense* succumb to the infection due to the development of systemic inflammatory response syndrome (SIRS), which is caused by massive production of proinflammatory cytokines including IFN- γ , IL-1, IL-6, IL-12 and TNF- α by hyper-activated macrophages and T cells. This SIRS results in enlarged capillary bed, decreased blood pressure and early mortality. In the present work, we examined whether Berenil, in addition to its trypanolytic effect, could modulate the host immune response to *Trypanosoma congolense*. First, we show that Berenil suppressed LPS-induced production of proinflammatory cytokines (IL-6, IL-12, IL-1 β , and TNF α) by bone marrow derived macrophages in a dose-dependent manner *in vitro*. Furthermore, the production of IFN- γ and IL-10 by splenocytes from BALB/c mice with infected *T. congolense* and treated with Berenil was lower than those from untreated control mice. Interestingly, the percentage of IFN- γ positive cells from non T (CD3-CD4-) cells was higher in Berenil treated animals than in untreated controls. These results provide preliminary evidence that Diminazene aceturate does in fact influence the immune response of the host in addition to clearing the parasites.

PDGF Induced Activation of ERK1/2 in Oligodendrocyte Progenitors is Time and Concentration Dependent

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Objective: To clarify the role of intracellular signaling in the regulation of oligodendrocyte progenitor cell (OP) migration.

Introduction: Oligodendrocytes are the myelinating cells of the CNS. In the developing brain, they originate as progenitors in the germinal matrix (GM). From there they migrate both radially and tangentially to populate white matter tracts. OP migration is induced in response to numerous local cues including soluble signaling proteins derived from neurons. One such factor, Platelet-derived growth factor (PDGF), is essential for the development of myelin. OP response to PDGF differs according to the signaling pathway activated by receptor phosphorylation, i.e. migration is regulated via activation of the extracellular regulated kinase (ERK) signaling pathway, whereas proliferation is regulated via the PI3K signaling pathway. We have recently shown that PDGF (10ng/ml) induced migration requires transient (<30 mins) exposure, and that this migration is sustained for up to 72 hours. This study furthers that finding by showing that low concentrations of PDGF activate ERK without inducing OP migration.

Materials and Methods: OPs were isolated from neonatal rat pups (P0-P1) as described previously. *ERK activation:* PDGF induced *ERK activation* in OP was assessed by Western Blot analysis. Briefly, OPs were exposed to PDGF at 0.01-10ng/ml for periods from 5 mins to 2 hours. Cell lysates were electrophoresed on a 12% polyacrylamide gel. After electrotransfer to PVDH membrane, blots were blocked in 5% skim milk before being exposed to antibodies against phosphorylated ERK1/2, total ERK1/2 and GAPDH, a standard housekeeping protein. Bands were visualized using an HRP conjugated secondary antibody enhanced with ECL reagent and exposed to X-ray film *Migration:* The agarose drop assay was used to analyze the migration of OP after exposure to PDGF, as described previously.

Results: All concentrations of PDGF at all durations of exposure activated ERK1/2 in OP. Whereas PDGF induced OP migration required either transient exposure (>10 minutes) to high concentrations (10ng/ml), or sustained exposure (<2 hours) to low concentrations (<1 ng/ml).

Conclusion: We show OP migration is only activated at 1ng/ml PDGF (a physiologically relevant concentration), with continuous exposure. Whereas ERK phosphorylation occurs in response to all PDGF concentrations and durations this is supported by previous studies that show that duration of receptor occupancy determines the downstream effects of ligands. We hypothesize that OP migration requires a threshold level of ERK activation that is not achieved by exposing OPC to low concentrations of PDGF for short periods of time. In addition, we hypothesize that ERK activation alone is not sufficient to drive OP migration.

The Induction of Early Growth Response 2 (Egr 2) is Triggered by Neuronal Activity-Dependent NF-κB Activation

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Introduction: NF-κB mediated signaling is complex and plays a critical role in many biological processes. Investigators have reported that NF-κB is activated during the induction of long term potentiation (LTP) and may be a requirement for synaptic plasticity and memory. In an attempt to explore target genes of NF-κB in LTP, we identified early growth response 2 (Egr 2) as one of targets of NF-κB. The present study focused on obtaining evidence of the linkage between the induction of Egr 2 and NF-κB signaling pathway.

Materials and Methods: We analyzed transcriptomes of LTP-induced hippocampal slices (CA1 region) from 2-month-old NF-κB p50 knockout mice (p50^{-/-}) versus its littermate (p50^{+/+}) to identify target genes of NF-κB. LTP was evoked by applying theta-burst stimulation to Schaffer collateral axon in the CA1 region. At 3hr after conditioning, total mRNA samples were extracted from LTP-induced slices and non-stimulated control slices. These mRNA samples were subjected to the DNA microarray analysis (Affymetrix GeneChip® Mouse Genome 430 2.0) and real-time RT-qPCR. We also examined the mRNA and protein expression level of Egr 2 in HeLa cells using real-time RT-qPCR and Western blotting. TNF α was used for activating NF-κB signaling pathway in HeLa cells. The P-Match software was used for sequence analysis of distal promoter region of Egr 2.

Results: There were no significant differences of both basal synaptic transmission and LTP magnitude in p50^{-/-} and p50^{+/+}. We identified early growth response 2 (Egr2) is induced by NF-κB activation during LTP. From the gene-structure analysis, we found several NF-κB consensus binding sites around promoter region of Egr2. In addition, the upregulation of Egr2 mRNA in HeLa cells treated with TNF α, an activator of the NF-κB signaling pathway, has been observed.

Conclusion: These data suggest that Egr2 expression level is controlled by direct transcriptional activity of NF-κB.

MPIP Participates in Innate Host Defense by Modulating the Normal Oral Flora in the Mouse

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Introduction: The mPIP protein, the mouse homologue of the hPIP/GCDFP-15, a marker for abnormal breast transformation, has been found to bind oral bacteria, showing the highest affinity for streptococci. This interaction suggests a potential function of mPIP in the non-immune host defense in the mouse oral cavity. Since the exact functions of mPIP are still unknown, we examined the roles of mPIP both by *in vitro* and *in vivo* studies, focusing on the possible role of this protein in non-immune host response through modulating the oral flora

Methods: The consequences of interaction between mPIP and oral bacteria were examined *in vitro*, to determine whether mPIP plays a role in bacterial aggregation. The *in vivo* studies addressed the roles of mPIP through the analysis of an mPIP knockout mouse model generated in our laboratory, specifically by examining the impact of the lack of mPIP on the mouse oral flora.

Results: Both quantitative and qualitative differences in the oral flora of mPIP knockout mice were identified when compared with wild-type controls. A sexual dimorphism has been identified in the amounts of mouse resident oral flora suggesting a possible hormonal influence to the effect of presence or absence of mPIP in mouse saliva. Also, a higher proportion of the oral bacteria of mPIP knockout mice were found to belong to genus *Streptococcus* and certain genera were absent from the oral cavity of these mice. The effect of saliva from mPIP knockout mice on the aggregation of oral bacteria was compared to wild-type mouse saliva. We found that mPIP contributed to saliva-induced bacterial aggregation.

Conclusion: Oral commensal flora has multiple functions, including protection of the organism against infection with pathogens. This study suggests that mPIP might play a role in the non-innate host defense through modulating the resident oral flora in the mouse.

The Role of Membrane Fusion in Activating the IRF3-Mediated Innate Antiviral Response

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Following the entry of enveloped virus particles, IRF3 is essential for the induction of an antiviral response. Although virus replication induces phosphorylation of C-terminal S/T residues, entry of enveloped virus particles can elicit robust IFN-stimulated gene (ISG) induction in the absence of detectable IRF3 hyper-phosphorylation. Using 2D gel electrophoresis and native PAGE we have initiated the characterization of post-translational modifications of IRF3 following treatment with both RNA and DNA viruses to determine the minimal post-translational modifications required for IRF3 activation and to investigate the differential modification of IRF3 during the various steps of the virus replication cycle. We have previously shown that virus binding and penetration are necessary to initiate the IRF3-mediated antiviral response. To investigate whether membrane fusion, an event required by all enveloped viruses for entry, is necessary and sufficient for IRF3 activation, we took advantage of a group of proteins from Reovirus known as fusion-associated small transmembrane (FAST) proteins. These non-structural proteins are made late during the viral life cycle and act to promote syncytia formation, facilitating virus spread. Here, we show that FAST-mediated fusion is sufficient to elicit ISG induction. Studies are ongoing to elucidate the effect of membrane fusion on IRF3 activation and antiviral state induction.

The Role of Innate Polymorphisms in Drug Selected Reverse Transcriptase (RT) Mutations in HIV-1 and HIV-2 Subtypes

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Background: The presence of innate resistance mutations and polymorphisms in drug naïve, HIV infected persons may impact on subsequent responses to antiviral therapy. Recent findings suggest bidirectional antagonisms between K65R and thymidine analogue mutations (TAMs) in treatment experienced patients. Our studies show facilitated development of K65R in subtype C due to a signature KKK motif at codons 64, 65 and 66. This present study addressed the effects of innate polymorphisms (T69N, V75I, V118I, L210N, T215S and K219E) in HIV-2 on emergent resistance to nucleoside/nucleotide analogues.

Methods: Emergent drug resistance profiles in HIV-2 subtype A (n=3) and B (n=1) were compared to HIV-1 subtypes B and C. Drug resistance was evaluated in cord blood mononuclear cells (CBMCs), using selective pressure with tenofovir (TFV), zidovudine (ZDV), stavudine (d4T), didanosine (ddI), abacavir (ABC) and lamivudine (3TC), and dual combinations of TFV-DDI, TFV-ABC, TFV-3TC, ZDV-3TC, and d4T-ddI. Culture fluids were analyzed weekly for virus production by RT assay. Resistance was evaluated using conventional and ultra-sensitive sequencing approaches.

Results: In agreement with our previous findings, dual drug combinations of TFV, ddI, ABC, d4T, ZDV and 3TC preferentially selected for the K65R mutation in HIV-1 subtype C isolates within 22 weeks. In single drug selections, K65R was observed with TFV and ddI and M184V with 3TC. In HIV-1 subtype B, TFV-3TC and ZDV-3TC selected for M184I and D67N respectively. In stark contrast, selections with all four HIV-2 cultures favored development of M184I in all dual drug combinations that included 3TC. With single drugs, M184V appeared within 6 weeks while some HIV-2 isolates developed S134A, V167I and A174V under TFV selective pressure. Since HIV-2 cultures did not develop K65R, an ultra-sensitive codon-specific real-time PCR assay was developed and the latter distinguished the presence of the K65R mutation from the wild type HIV-2 plasmid by 16 cycles (Δ CT).

Conclusion: These results underscore potential differences in emergent drug resistance pathways in HIV-1 and HIV-2 and show that polymorphisms may determine the resistance pathways that emerge. These studies may have implications in the design of TAM-sparing regimens used against HIV-1 subtype C.

Pathways Regulating Prox1 Induction in Venous Endothelial Cells

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Introduction: In the developing embryo, arteries and veins first develop and it is from these initial vessels that the lymphatic vessels sprout. *in vitro*, Human Umbilical Vein Endothelial Cells (HUVECs) are primary endothelial cells that are able to differentiate into lymphatic like cells following treatment with different growth factors. In previous studies, interleukin-3 (IL-3) and interleukin-7 (IL-7) have been shown to induce expression of Prox1 homeobox gene which is an early marker of lymphatics. These cytokines were shown to be sufficient to convert HUVECs into more lymphatic-like phenotype. The objective of our study is to determine the signalling cascades activated by these growth factors which lead to activation of Prox1 expression. **Methods:** The effects of IL-7 on Prox1 expression of HUVECs were analyzed by treating HUVECs with different doses of IL-7 (0, 0.1, 1.0, 10, 50, 100, 200 ng/ml) for 18 hours and western blotting using a rabbit polyclonal anti-Prox1 antibody.

Results: Prox1 was similarly expressed both in control (0 ng/ml of IL-7) and IL-7 treated HUVECs. Prox1 expression was not dependent on the dose of IL-7 added.

Conclusions: IL-7 did not alter Prox1 expression in HUVECs with the doses that have been used. This inability may be due to lack of other unknown factors that are necessary for Prox1 upregulation by IL-7 in our cells. IL-3 may be a more potent inducer of Prox1 expression.

β arrestin Engagement by the Insulin-like Growth Factor Receptor is Required for V2 Vasopressin Receptor-promoted ERK 1/2 Activation

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Introduction: The V2-vasopressin receptor (V2R) is a G protein-coupled receptor endogenously expressed in the principal cells of the collecting duct where it regulates water reabsorption through the stimulation of the adenylate cyclase. We have recently shown that V2R can also activate the MAPK ERK1/2 through a mechanism involving the scaffolding protein β arrestin, a well known GPCR regulator. We further reported that this activating pathway is independent of *G α s/i/q* or *G β γ* and involves *c-Src* and a metalloproteinase-dependent receptor tyrosine kinase (RTK) transactivation event. To further characterize the transactivation mechanism leading to V2R-mediated ERK1/2 activation, we sought to identify the RTK involved.

Methods and Results: Based on the use of siRNAs, selective pharmacological inhibitors and dominant negative mutants, we report that the V2R-induced ERK1/2 activation relies on the metalloproteinase-dependent transactivation of the insulin-like growth factor receptor (IGFR) that becomes phosphorylated upon vasopressin stimulation. In the HEK293 cells studied, we further found that *c-Src* was required for the metalloproteinase-promoted processing of the ligand precursor. Notably, β arrestin was not necessary for the metalloproteinase-dependent release of the transactivating ligand but is rather involved downstream of the IGFR transactivation event. Given the recent observations that β arrestin can be recruited to RTK in response to their cognate ligands, we next investigated whether β arrestin could also be recruited to IGFR following its transactivation by V2R. In co-immunoprecipitation studies, we indeed observed that β arrestin-1 associates with IGFR in response to V2R activation and that this event is required for the V2R promoted ERK1/2 activation.

Conclusion: These findings substantiate the pleiotropic nature of β arrestin and suggest that recruitment of β arrestin to the transactivated IGFR may be involved in the scaffolding of the downstream MAPK signaling cascade promoted by V2R activation. The present study thus brings to light a previously unappreciated level of complexity in the regulation of signaling by these scaffolding proteins. We now plan to confirm our findings *in vivo* and investigate the physiological role of V2R-mediated ERK1/2 activation.

Examination of Blood-Brain Barrier (BBB) Integrity in a Mouse Brain Tumor Model

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Introduction: The present study examines, on a functional and biochemical level, brain tumor-induced alterations in capillary endothelial cells forming the blood-brain barrier (BBB).

Methods: Tumors were induced by injecting Lewis Lung carcinoma (3LL) cells into the right hemisphere of female Balb/c mice. Blood-brain barrier permeability was assessed at various stages of tumor development, using both magnetic resonance imaging (MRI) and various permeability markers. The expression of the P-glycoprotein (P-gp) in the BBB was also evaluated using Western Blot.

Results: Median mouse survival following 3LL injection was 15 days. Tumor volume progressed from 1.38 mm³ on day 7 to approximately 78 mm³ on day 15. Histological and MRI analysis confirmed that the resulting tumor mass was restricted to the right hemisphere at all time points examined. The permeability of the BBB to ³H-mannitol was similar in both hemispheres at 7 and 10 days post-injection. At day 12 and 15, significant increases in BBB permeability was observed. The alterations in BBB permeability were associated with an increase in cerebral vascular volume. However, P-gp expression was unaffected at all stages of tumor development.

Conclusion: The BBB was largely intact during tumor development with disruptions only observed at the later stages. Furthermore, no change in the BBB expression of P-gp was observed despite an apparent increased angiogenesis in the tumor bearing hemispheres.

Apoptin: A Novel Peptide Based Inhibitor of Bcr-Abl Kinase

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Introduction: The non-receptor tyrosine kinase activity of fusion gene *Bcr-Abl* derived oncoproteins is the key factors responsible for development and progress of Philadelphia positive (Ph+) Chronic Myeloid Leukemia (CML) and Ph+ Acute Lymphoblastic Leukemia (ALL). In the search for a peptide-based inhibitor of Bcr-Abl tyrosine kinase, here I investigated a naturally occurring molecule called apoptin. Apoptin is a 14 kDa viral protein (chicken anemia virus protein-3) and known to induce apoptosis in a wide range of transformed but not in primary cells.

Methods: During the initial phase of my study an array-based analysis demonstrated that apoptin interacts with the SH3 domain of Abl. High stringent pull-down assay; co-immunoprecipitation assay and immunofluorescence studies were applied to confirm this interactions apoptin with Bcr-Abl. I further investigated the role of apoptin on the Bcr-Abl phosphorylation by kinase assays and also used 'MTT Cell proliferation assay', 'FACS analysis' of apoptotic cell death using 'Nicoletti Method' to study the effect of apoptin on human and murine CML cell lines. Finally, I used computational protein modelling algorithms to study the 3D structure of apoptin and its interactions with Bcr-Abl at the molecular level.

Results: I detected the ability of apoptin to inhibit Bcr-Abl kinase significantly and presumably indirectly inhibiting a series of downstream targets (e.g. CrkL, Stat5, c-Myc, etc.). In comparison studies, using Imatinib[®] I discovered that apoptin has a significantly higher killing efficacy on human and mouse CML cell lines expressing Bcr-Abl. I also determined a specific proline rich motif of apoptin (amino acid: 81-86) strongly interacts with the SH3 domain of fusion protein Bcr-Abl by analyzing the interactions of different apoptin and Bcr-Abl mutant proteins. The results obtained from the computational modelling of apoptin structure and interactions were supportive and conclusive.

Development of a DNA-based Vaccine Platform with Broad-Spectrum Efficacy Against Avian Influenza (H5N1)

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Introduction: Avian influenza (H5N1) infects aquatic bird as well as poultry populations and has been associated with a high rate of avian mortality. In recent years, the incidence of poultry-to-human transmission has increased and the potential of human-to-human transmission raises concerns of an emerging global influenza pandemic derived from H5N1. Currently, vaccination is the most effective prophylaxis against influenza infections. The current study evaluates mono- and multivalent DNA vaccines based on four H5N1 (A/Hanoi/30408/2005) antigens for protection against diverging avian influenza strains.

Methods: Potential antigenic targets were chosen based on virion surface exposure and potential for antibody stimulation: haemagglutinin (HA) and neuraminidase (NA), as well as based on sequence conservation: M2 ion channel protein and nucleoprotein (NP). An optimized expression cassette for each vaccine was generated through gene synthesis and insertion into a DNA-based platform. Immunized BALB/c mice were challenged with homologous (A/Hanoi/30408/2005) and heterologous (A/Hong Kong/483/1997) avian influenza (H5N1) viruses and serum samples were obtained for the evaluation of haemagglutination inhibition (HI) and neutralizing antibody (NAB) levels. T-cell responses in BALB/c and C57BL6 were assayed post-vaccination through detection of interferon- γ (IFN γ) production.

Results: Challenge experiments suggest that the HA-based DNA vaccine offered full protection against both homologous and heterologous challenges. Humoral and cell-mediated immune responses were evaluated for each antigen and several immunodominant epitopes were identified in mice following peptide re-stimulation. A multivalent approach identified the combination of HA and NA as optimal against homologous challenge, while HA and NP offered the best protection against heterologous challenge.

Conclusion: Our studies suggest that cross-protection against diverging strains of avian influenza (H5N1) is achievable in mice through optimal combination of antigens.

Analysis of the Partial Phenotypic Rescue of *Cyp26b1* Null Mice Following Ablation of *Rarg*

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Proper embryonic patterning requires the precise spatio-temporal regulation of retinoic acid (RA) activity. Morphogenesis can be regulated at the level of RA distribution, mainly via its synthesis and catabolism by the RALDH and CYP26 enzymes respectively, and at the level of signalling by activating its cognate nuclear receptors (RAR α , β , and γ). Knocking out *Cyp26b1* leads to various phenotypic abnormalities by increasing local concentrations of RA in tissues such as the limb and craniofacial structures. These defects include phocomelia (shortening of the limbs), adactyly (missing digits), micrognathia (shortened lower jaw), and open eyes at birth. In addition, embryos have a paucity of vibrissal (whisker) and pelage (hair) follicles. We have previously shown that ablating the gene encoding RAR γ in a *Cyp26a1* null background could rescue the caudal abnormalities associated with increased levels of RA during development by limiting aberrant RA signalling to downstream genes involved in caudal development. In the current study, the ablation of *Rarg* in a *Cyp26b1* null background is shown to reduce the severity of defects associated with both forelimb and hindlimb development, with the exception of zeugopod formation. Moreover, in *Cyp26b1/Rarg* double knockout mice whiskers are present, the eyelids are fused, and various aspects of skull development are rescued. This double-null murine model illustrates that RAR γ plays a specific role in transducing the RA signal within tissues that are affected by the loss of CYP26B1. Further analysis of the pathways responsible for directing limb bud outgrowth and eyelid development are currently underway. This study will not only provide insight into the pathways regulated by RAR γ in the rescued tissues, but will ultimately influence our understanding of RA-regulated tissue (dys)morphogenesis and resultant embryonic development.

Molecular Characterization of Trimethoprim Sulfamethoxazole Resistant Urinary Tract Infection *Escherichia coli* Isolates in Northern Saskatchewan Communities

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Objective: Antimicrobial resistance in urinary tract infections (UTIs) is of concern as it limits treatment options. Approximately 80% of UTI cases are caused by uropathogenic *E. coli* (UPEC). The objective of this study was to characterize trimethoprim-sulfamethoxazole (TMP-SXT) resistant *E. coli* identified from three northern Saskatchewan communities.

Methods: Isolates were collected through the Northern Antibiotic Resistance Partnership (NARP) over a 6 month period from July 2006 – December 2006. Susceptibilities to 28 antibiotics were determined using microdilution broth assays following CLSI guidelines. Pulsed-field gel electrophoresis (PFGE) using *Xba*I was used to type the isolates. Class I integrons among isolates were identified by PCR and DNA sequencing.

Results: A total of 342 UPEC isolates were collected (Site 1=211, Site 2=104, Site 3=27). Most of the UPEC isolates were from female patients (females = 90%, males = 4%, no data = 6%). The highest percentage of UTI patients were between the ages of 20-24 years old (13%). TMP-SXT resistance was observed in 111 isolates (Site 1= 33%, Site 2 = 26%, Site 3 = 56%). From the isolates that were TMP-SXT resistant, 15% were resistant to only TMP-SXT, 34% were resistant to one other antibiotic class, 39% were resistant to 2 classes, and 12% were resistant to > 3 classes. PFGE data showed that there were no large outbreaks of TMP-SXT resistant UPEC within or between the sites. Class 1 integrons were detected in 96 (86%) of the TMP-SXT resistant isolates. Ten different cassette arrangements were identified within the integrons which contained *dfr*, *aadA*, or a combination of the two cassettes. Trimethoprim resistance cassettes identified were *dfrA1*, *dfrA7*, *dfrA12*, *dfrA17*, and *dfrV*. Streptomycin/spectinomycin resistant cassettes identified were *aadA1*, *aadA2*, and *aadA5*.

Conclusion: The high rate of resistance to TMP-SXT observed among the sites, suggests that a possible re-evaluation of first line therapies for UTI treatment should be considered. The lack of strain clonality and the large number of resistance cassettes observed suggests resistance in the community is diverse and unlikely to have a common origin.

The Role of Leptin in Granulosa Cells Steroidogenesis in the Human Ovary

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Context: Obesity has been linked with increased risk of female infertility. Leptin, an adipocytokine that is elevated during obesity, may influence the gonadal function through the modulation of granulosa cells steroidogenesis.

Objective: The present study aimed to investigate the cellular mechanisms underlying the leptin-regulated steroidogenesis in human granulosa cells.

Methods: Levels of progesterone was determined by ELISA. Real-time PCR and Western blotting were used to detect mRNA and protein levels of steroidogenic enzymes and MAPK signaling molecules under the administration of leptin. Pharmacological inhibitors were used to monitor the involving signaling molecules and siRNA was used to knockdown the endogenous leptin receptor in granulosa cells.

Results: Leptin inhibited the 8-bromo cAMP-stimulated progesterone production in a dose dependent manner and inhibited the cAMP-stimulated steroidogenic acute regulatory protein (StAR) expression. Leptin induced the phosphorylation of ERK1/2, p38 and JNK but only PD98059 (ERK1/2 inhibitor) and SB203580 (p38 inhibitor) reversed leptin inhibition on cAMP-stimulated StAR protein expression and progesterone production. Using siRNA for leptin receptor abolished the effect of leptin on cAMP-induced StAR protein expression and progesterone production.

Conclusion: Leptin acted through MAPK pathway to downregulate cAMP-induced StAR protein expression and progesterone production in SVOG-4M cells. These results point to a possible mechanism by which gonadal steroidogenesis could be suppressed in obese women.

Mapping Brain Metals in Friedreich's Ataxia with Rapid-Scanning Synchrotron X-ray Fluorescence Spectroscopy

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Friedreich's ataxia (FRDA) is a progressive neurodegenerative disease inherited as an autosomal recessive trait that affects 1:50,000 Caucasians. FRDA typically presents before 25 years of age with progressive loss of coordination (ataxia) and loss of deep tendon reflexes. Later in the disease, swallowing and breathing difficulties as well as cardiomyopathy, diabetes mellitus and skeletal deformities are present. The neurological features of FRDA can be explained by the neuropathological findings characteristic to this disease: neurodegeneration of the dorsal root ganglia, posterior columns, corticospinal and spinocerebellar tracts, superior vermis and deep cerebellar nuclei. The biochemical basis of FRDA is deficiency of frataxin, a nuclear encoded mitochondrial protein. Frataxin deficiency impairs heme and iron-sulfur cluster synthesis leading to iron accumulation and oxidative stress. In humans, iron accumulation has been observed in FRDA fibroblasts, hepatocytes and spleen cells, but there are no human studies linking spinal cord and brain neurodegeneration to iron accumulation. Moreover, even though early Canadian work suggested a possible dysregulation of copper and zinc metabolism in FRDA, the possible involvement metals other than iron in FRDA neurodegeneration has not been investigated. Rapid scanning X-ray fluorescence (XRF) spectroscopy is a novel synchrotron technique developed to rapidly and simultaneously map multiple metals in large whole tissues. We applied this technique to map multiple metals in FRDA human brain and spinal cord. Formalin fixed FRDA and control tissues were obtained from the Human Brain and Spinal Fluid Resource Centre, Los Angeles, CA, USA, Brain and Tissue Bank for Developmental Disorders of the National Institute of Child Health and Human Development, Baltimore, USA and Douglas Hospital Research Centre General Brain Bank, Montreal, Quebec, Canada. The diagnosis of FRDA was based on the clinical phenotype and neuropathology report. Using this new technique, we show that iron, copper and zinc are elevated in spinal cord, medulla and certain areas of the forebrain of FRDA patients. Our data show that not only iron, but also copper and zinc may play a role in FRDA physiopathology and suggest that these metals should be considered as possible new therapeutic targets.

Motoneuron Excitability is Enhanced During Fictive Scratch by Voltage Threshold Hyperpolarization and Reduced Afterhyperpolarization Amplitude

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Introduction: Hindlimb motoneuron firing is facilitated at the onset of fictive locomotion in the decerebrate cat by a lowering of the voltage threshold (V_{th}) for action potential initiation and a reduction of the post-spike afterhyperpolarization (AHP). Whether these state-dependent changes in motoneuron excitability occur during other motor behaviours or is dependent upon brainstem stimulation in the decerebrate cat is not known.

Methods: Fictive scratch was induced by topical application of curare to the left C1 dorsal root entry region and light stroking of the left side of the face in adult decerebrate cats following neuromuscular blockade. Fictive scratching (fictive due to immobilization of the limb) is an example of a cyclical hindlimb motor behaviour that can be elicited without electrical stimulation of the brainstem, which is different from the brainstem evoked fictive locomotion. Measurements of the V_{th} and AHP of spikes evoked by intracellular injection of depolarizing ramp currents or square wave pulses were compared during control and fictive scratch in discontinuous current clamp mode.

Results: Recordings were made from 31 antidromically identified motoneurons in intact cats. V_{th} became hyperpolarized in 26 of 31 (mean hyperpolarization -6.3 ± 4.4 mV; range -1.1 to -17.2 mV) with the onset of fictive scratch. In two cats a complete transection of the spinal cord at C1 was made. In 12 of 13 motoneurons in this preparation fictive scratch resulted in V_{th} hyperpolarization (mean hyperpolarization: -7.1 ± 1.4 mV; range: -1.3 to -26.2 mV). In addition to changes in V_{th} , there was a robust reduction of AHP amplitude during both the approach (tonic flexion) and rhythmic phases of fictive scratch, there was a robust reduction of the AHP. This was repeatable with successive trials and recovered soon after the cessation of an episode of fictive scratch. AHP reduction was also observed during fictive scratch following spinal transection and was not a result of increased motoneuron conductance.

Conclusion: The results show that motoneuron excitability is enhanced during fictive scratch through a hyperpolarization of the V_{th} for action potential initiation and a reduction in AHP amplitude. These effects are robust and are not dependent on upon electrical brainstem stimulation, providing the first evidence for an intraspinal mechanism capable on enhancing motoneuron excitability during a rhythmic motor output.

Threshold Control of Static Wrist Positions, Intentional Movements and Unloading Reflexes Revealed by Transcranial Magnetic Stimulation of the Motor Cortex

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Motor cortex cells activity usually correlates with mechanical variables or EMG signals. Correlations, however, do not imply causality. We tested the hypothesis that descending M1 signals can influence the output variables while remaining independent of them. The spinal excitability of motoneurons of 4 wrist muscles (FCR, FCU, ECR, ECU) was evaluated by perturbing the wrist, placed in a manipulandum, with a torque motor at two static positions (45° flexion and 25° extension). By applying small loads, EMG was equalized (near zero levels) at these two actively specified positions. Transcranial magnetic stimulation (TMS, single pulses, 1.2 motor threshold, M1 wrist area) was used to evaluate the excitability of cortico-spinal pathways by recording motor evoked potentials (MEPs) from the wrist muscles in 16 healthy subjects. Muscle reflex reactions to perturbations appeared to be position-independent. However, extensors MEPs in extension were substantially bigger whereas flexors responses were smaller than in flexion position. Thus, active changes in wrist position were associated with reciprocal changes in cortico-spinal influences on flexors and extensors motoneurons, independently of EMG levels. When subjects relaxed wrist muscles and the same positions were established passively, the MEPs decreased and became less correlated with position. We also analyzed the changes resulting from unloading of pre-loaded wrist extensors elicited either unexpectedly or by subjects. In both cases, extensors MEPs decreased but the decrease was bigger in self-initiated unloading, even though the final EMG levels were similar. The dissociation between EMG levels and corticospinal excitability suggests that M1 is not involved in the specification of EMG patterns. Rather, M1 primary effect is a change in the threshold position of body segments, i.e. the position at which muscles are silent but are ready to respond to deviations from it. Thereby, to produce a motor action, descending systems reset the threshold position of body segments, whereas EMG patterns emerge following the difference between the actual and the threshold positions. This M1 function should be taken into account while using TMS as a tool in assessing the integrity and reorganization of descending pathways in clinical populations.

Characterization of mice knockout for DNase-X, a muscle-Specific Endonuclease

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Introduction: DNase-X is a DNase-I-like endonuclease which is highly expressed in cardiac and skeletal muscles. The aim of this study is to characterize the mice in which the DNase-X gene is knocked out. We are looking at the functional properties, morphological and histopathological changes as well as gene expression profile in skeletal muscles of these mice.

Methods: Fiber enumeration was performed by acid digestion of freshly-isolated muscles and counting single fibers under dissecting microscope. Immunohistochemistry was conducted on snap-frozen tissues stained with specific antibody against slow myosin heavy chain (MHC type I). Physical activity was tested via both treadmill and voluntary wheel running. Gene expression was analyzed by quantitative real time RT-PCR. Nuclease activity and DNA degradation was assessed via agarose gel electrophoresis.

Results: DNase-X deficient mice exhibit a normal postnatal development but are lighter than the age- and sex-matched wild-type counterparts. Morphological analysis of muscles in these mice showed that the number as well as the cross-sectional area (CSA) of myofibers was diminished in knockout muscles. In soleus, type I and type II fiber composition is similar between knockouts and wild-types, however, there are a large number of damaged fibers with centrally-located nuclei in non-exercised knockout soleus whose incidence was increased in exercised muscles. When tested for running capacity, DNase-X^(-/-) mice exhibited an impaired performance that was significant in older animals. There was no evidence of sex- and age-dependence in the expression of DNase-X, but the amount of other DNases transcripts in this family was upregulated in skeletal muscles of knockout mice. We also found a reduced nuclease activity in knockout muscles, whereas no similar difference was observed in other tissues.

Conclusion: DNase-X is an active component of normal physiology of skeletal muscles lack of which results in impaired exercise capacity in mutant mice and higher incidence of regenerative events in DNase-X deficient muscles. Further investigations, however, need to be performed to unravel the mechanisms contributing to this phenotype.

Insulin-Like Growth Factor Type-I b Subunit Receptor Expression in Cigarette Smoke Extract-Exposed Fetal Rat Lung

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Introduction: Smoking during pregnancy exposes the fetus to 4000 different potentially genotoxic agents of cigarette smoke. In the developing embryo and fetus good evidence supports the role of the Insulin-like Growth Factor (IGF) axis in growth regulation. One component of this axis, IGF-Type I receptor (IGF-IR) is a tyrosine kinase receptor and beta subunit of this receptor is essential in cellular proliferation and survival. The present study is focused on the effects of cigarette smoke extract (CSE) on IGF-IR expression in developing fetal rat lung cells.

Methods: Cell cultures from two lung cell types, fibroblasts and Type II alveolar cells (AECs) were isolated from Sprague Dawley fetal rats at 21 gestational days. Data were derived from 15-18 pooled litters. Isolated cell types were exposed to CSE at concentrations from 1-20% for 24 hours. IGF-IR expression was determined by western blotting on 7% polyacrylamide gels. Subsequently, transfer blots were blocked in 5% skim milk before being exposed to IGF-IR beta rabbit polyclonal antibody at 1:1000 dilution. Antibodies to beta actin were used as loading controls. Microscopic imaging was done to assess CSE induced morphological changes. Cell proliferation and cell viability studies were done concurrently.

Results: Our data indicate that IGF-IR is expressed in both cell types. IGF-IR expression profile is in accordance with the different concentrations of CSE, with no significant change in expression at 1-5%. However, IGF-IR expression was reduced at 10% CSE and above. In addition CSE concentrations above 10% for 24 hours of exposure significantly reduced cellular proliferation and viability in a dose dependent manner.

Conclusions: The level of IGF-IR expression by developing fetal rat lung cell types suggests its importance during cellular replication and differentiation. The cellular exposure to cigarette smoke extract differentially affects fetal lung cell viability, proliferation and expression of a major regulator of growth, IGF-IR. In humans, exposure to primary or secondary smoke products *in utero* may similarly adversely alter fetal lung cell growth and development.

TNF- α , IL-1 β and IL-4 Regulate the High Affinity IgE Receptor Expression in Human Airway Smooth Muscle Cells

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Asthma is a chronic inflammatory disease of the bronchial airways, displaying enhanced bronchial responsiveness, airway obstruction and airway inflammation. Airway smooth muscle (ASM) has been well thought-out to be a key determinant of asthma, owing to their ability to contract in response to proinflammatory mediators. The high affinity IgE receptor (Fc ϵ RI), a tetrameric ($\alpha\beta\gamma_2$) immune receptor is a crucial structure for IgE-mediated allergic reactions. We have previously showed that human airway smooth muscle cells express a functional Fc ϵ RI ($\alpha\beta\gamma_2$), and its activation leads to marked transient increases in intracellular Ca²⁺ concentration, release of Th-2 cytokines and eotaxin/ CCL11. The present study was undertaken to delineate the factors regulating the expression of Fc ϵ RI- α chain (IgE binding domain) in primary human bronchial and tracheal smooth muscle (B/TSM) cells. Incubation of B/TSM cells with TNF- α , IL-1 β or IL-4 resulted in a significant increase in Fc ϵ RI- α chain mRNA expression ($p < 0.005$) compared to unstimulated cells. Furthermore, in contrast to IL-1 β ; TNF- α , IL-4 induced a significant increase in Fc ϵ RI- α protein neosynthesis at 24, 48 and 72 hrs. As a functional outcome, TNF- α sensitized B/TSM cells (hence upregulated Fc ϵ RI- α expression) potentially augmented the eotaxin/CCL11, IL-6, RANTES, IP-10 and IL-8/ CXCL-8 production following IgE stimulation. Collectively, our data suggest that proinflammatory (TNF- α) and Th-2 (IL-4) cytokines-induced regulation of Fc ϵ RI expression in human B/TSM cells could play a critical role in allergic airway inflammation via potentially novel mechanisms involving IgE/ IgE receptors network.

Central Memory CD8+ T Cells Correlate with Slower Disease Progression in HIV Infected Patients.

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Objectives: Human Immunodeficiency Virus-1 (HIV-1) infection induces CD8+ T cell responses that are critical in controlling viral loads. This response ultimately fails in most individuals, and this failure coincides with progression to AIDS. Long Term Non-Progressors (LTNPs) provide a unique opportunity to study protective cell-mediated immunity. Historically, IFN- γ expression levels have been measured to determine HIV-specific CD8+ T cell responses. IFN- γ is an effector memory (Tem) attribute, and so studies have largely ignored central memory (Tcm) proliferation responses, which are thought to be more important in controlling HIV infection. A better understanding of the fine specificity of HIV-specific Tem and Tcm pools, and how these pools interact *in vivo*, is of critical importance to the design of vaccines intended to elicit protective cell-mediated immunity. We hypothesize that there will be differential epitope recognition by CD8+ Tcm and Tem memory responses in infected individuals and further that Tcm responses will correlate with protection.

Methods: Peripheral blood mononuclear cells from HIV infected LTNP and normal progressors were isolated and stimulated overnight and for 6 days with an HIV-1 p24 peptide library. The p24 peptide library consisted of 16 pools of 9 mers overlapping by 8aa. Tcm and Tem responses were evaluated by polyfunctional flow cytometry measuring a variety of cytokines, cytotoxic potential, proliferation and phenotypic markers.

Results: Substantial differences were seen in the specificity of Tem and Tcm responses within individual patients and overall IFN- γ and proliferation responses were inversely correlated. Phenotypic markers used to identify memory subsets reveal that LTNPs have higher amounts HIV-specific Tcm responses than normal progressors.

Conclusions: This data suggests a disconnect between the specificity of CD8+ T-cells when measuring Tcm and Tem responses to HIV-1 and that LTNPs are more likely to elicit Tcm responses against HIV. Numerous HIV-specific responses would have been missed if IFN- γ production were the sole functional readout and future vaccine trials should monitor more than IFN- γ as an indication of the immune response and pay closer attention to Tcm responses.

The Contribution of K^+ and Ca^{2+} Channels to the Proliferation of Breast Cancer Cells

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Cancer cells are characterized as cells that undergo uncontrolled growth. This growth can be influenced by many hormonal factors in the body. Estrogen is one factor that has been implicated in both the development and progression of breast cancer. The role of ion channels in the development and progression of cancer has recently gained much attention, mainly because inhibition of specific channels can lead to reduced cell proliferation. My research is aimed at identifying the role ion channels play in the basal and estrogen-stimulated proliferation of MCF-7 cells, an estrogen receptor-positive breast cancer cell line. Using pharmacological blockers and a radioactive thymidine incorporation assay I have identified two K^+ channels, KCNH1 (hEAG1) and KCNN4 (hIK4), which contribute to the proliferation of breast cancer cells. The mechanism by which K^+ channels regulate the progression through the cell cycle is not completely understood. It is believed that K^+ channel activity governs the resting membrane potential, which in turn regulates the influx of Ca^{2+} through Ca^{2+} permeable channels. Using pharmacological blockers and molecular tools I have identified numerous Ca^{2+} channels from the TRP family as well as T-type Ca^{2+} channels that contribute to the proliferation of MCF-7 cells. Using radiolabeled Ca^{2+} uptake I have identified TRPM8 as a potential contributor to Ca^{2+} entry into breast cancer cells. Using this method I will also investigate whether blocking K^+ channels can modulate Ca^{2+} entry through these and other pathways. Furthermore, recent research has shown that K^+ and Ca^{2+} channels may play a role in the mitogenic effect of certain hormones. Using quantitative PCR I have found that picomolar concentrations of estrogen increase the mRNA expression of hEAG1, suggesting that increased hEAG1 expression may be important for estrogen-stimulated proliferation. At concentrations that inhibit MCF-7 proliferation hEAG1 blockers do not inhibit the mitogenic effect of estrogen, suggesting that hEAG1 contributes to basal proliferation but not estrogen-stimulated proliferation. Interestingly, Ca^{2+} channel blockade inhibits estrogen-stimulated proliferation, suggesting that Ca^{2+} influx may be critically important for this effect of estrogen.

Young Children's Morning Cortisol Secretion Predicts the Development of Depressive Symptoms in Middle Childhood

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Disturbances in typical HPA functioning have been associated with various psychopathologies, including depression. Recent findings suggest that altered HPA functioning, as demonstrated by elevated morning cortisol secretion, may play a causal role in the development of adolescent depressive symptoms (Goodyer et al., 2000; Halligan et al., 2007).

The current study examined the longitudinal association between 44 children's diurnal cortisol at 3-5 years of age and depressive symptoms six years later. At Time 1, mothers reported on child internalizing symptoms and assisted in collecting child saliva samples over one waking day. Saliva was later assayed for cortisol. Six years later (Time 2), mother and teacher reported on child internalizing symptoms. Elevated morning cortisol secretion predicted symptoms of internalizing behavior six years later, above the association between cortisol and preschool internalizing symptoms ($F(2,22)=12.07, p<0.001$). Altered morning cortisol secretion can predict subsequent depressive symptoms in younger, less at-risk individuals than previously identified.

The Colonic Pathogen *Entamoeba histolytica* Down-Regulates Colonic Immune Responses by a TLR-dependent Mechanism

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Amebiasis is estimated to currently affect approximately 10% of the world's population; however in over 99% of cases the course of disease is completely asymptomatic and only a minority of patients develop amebic colitis and/or liver abscess. It is the third leading cause of death (100,000/yr) by a parasite. The mechanisms that allow the parasite to be a successful colonizer in the absence of a host inflammatory or immune responses are not known. We hypothesized that products secreted in the colonic lumen by *E. histolytica* trophozoites may cause tolerance induction in antigen presenting cells, through up-regulation and/or activation of inhibitory ILT2 receptor, which upon phosphorylation can block CD64 signalling and cell activation through recruitment of SHP-1, thus leading to immune suppression and facilitating parasitic colonization. In support of this, we demonstrate that proteins secreted by *E. histolytica* could persistently stimulate the ILT2 receptor leading to significant increase in its surface expression on bone marrow derived dendritic cells (BMDCs), which are accompanied by down regulation of co-stimulatory molecule expression (CD80) and rapid expansion of CD4⁺ILT2⁺ T lymphocyte population. Moreover, recognition of the secreted amebic proteins (SP) was critically dependent on TLR signalling, since induction of CD4⁺ILT2⁺ T cells was completely abrogated in co-culture with Myd88 deficient BMDCs. Because the induction of CD4⁺ILT2⁺ T cells in MyD88^{-/-} BMDC co-culture upon stimulation with SP was significantly delayed when compared to WT, but a significant expansion of tolerogenic T cells was still observed when compared to untreated controls (but not WT controls), we hypothesized that the recognition of the amebic protein responsible for this effect must occur through a TLR that is capable of both MyD88 and TRIF-dependent signalling. Since only TLR4 is capable of both TRIF and Myd88-dependent signalling, we assessed the level of surface expression of ILT2 in WT and TLR4^{-/-} BMDCs following treatment with SP. Following a 48 hour stimulation only WT, but not TLR4-deficient BMDCs significantly up-regulated ILT2 expression in response to secreted *E. histolytica* proteins. Taken together, these results provide a novel mechanism that the parasite utilizes to suppress local immune responses to facilitate colonization and survival in the gut.

The Brain Cholesterol Homeostasis Gene CYP46A1 is Post-Transcriptionally Regulated by Aberrantly Expressed miRNAs During Prion Disease

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MicroRNAs (miRNAs) are a new class of small (~22 nt long), non-coding, gene-regulatory RNA molecules. miRNAs play a central role in growth, development and disease by post-transcriptionally silencing the expression of protein-coding mRNA molecule(s) through sequence specific complementary hybridization to the 3'UTR. To date, hundreds of miRNAs have been identified in vertebrate genomes with each miRNA possessing potentially hundreds of targets. It is suspected that ~30% of the human genome is under post-transcriptional regulation by miRNAs and estimates are expected to go substantially higher. Gene regulation by miRNAs is believed to be only rivalled by that of transcription factors.

Recent studies performed in our lab have identified several miRNAs that are aberrantly expressed in the brains of mice inoculated with prions in comparison to control mice at the terminal stage of the disease. This mode of regulation triggers considerable interest in the analysis of potential cellular targets of these molecules. A gene that is bioinformatically predicted to be a target of several of the up-regulated miRNAs is 24S-hydroxylase (Cyp46A1). Cyp46a1 is the principal brain cholesterol homeostasis gene and its expression is known to be altered in a variety of neurodegenerative diseases. Interestingly, this gene has been shown to be consistently down regulated in gene expression studies performed on several mouse models of prion disease. Therefore, in this study, we aimed to provide experimental evidence for the post-transcriptional regulation of Cyp46a1 by the aberrantly expressed miRNA(s) during prion pathogenesis.

To verify our predictions, we cloned the 3'-UTR of Cyp46a1 into a reporter vector and challenged it with the predicted miRNAs in a representative cell line. Titration with increasing concentrations of the predicted miRNAs revealed an inverse relationship between the abundance of miRNAs and the degree of target gene expression.

Taken together, the results suggest that aberrant expression of specific miRNAs may contribute to dysfunctional gene expression seen in the prion disease process. In this study, we provide a specific example with Cyp46a1, although hundreds or even thousands of genes have the potential to be de-regulated by these miRNAs.

Identification and Tissue Specific Expression Pattern of the Different Transcriptional Isoforms of *Dlc-1* Tumor Suppressor Gene in Mouse

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Objectives: The Deleted in liver cancer 1 (*Dlc-1*) gene encodes a Rho GTPase activating protein (RhoGAP) that increases the hydrolysis of active GTP bound form of Rho GTPase to an inactive GDP bound form. This gene is found inactivated in many human cancers including liver, breast prostate and lung. The gene has been reported to be alternatively spliced and three transcriptional isoforms have been reported in human however, it is not known whether similar splicing pattern also exists in mice. Since we have knockout mice for this gene, it is important to know all the splice variants. The objective of the present study was to identify and determine the tissue specific expression patterns of the different transcriptional isoforms of *Dlc-1* gene in the mouse.

Methodology: Our methodology involved the use of Rapid Amplification of 3' cDNA End (3'RACE) PCR to identify the different transcriptional isoforms and Real Time PCR quantification to determine the tissue specific expression pattern of the transcriptional isoforms.

Results: In the work, presented here, we report the presence of three spliced transcripts of *Dlc-1* gene arising as a result of alternative splicing and exon bypass. We also report the presence of a novel 7 exons containing transcript arising from a putative promoter, which is approximately 400kb upstream of exon 2 of the *Dlc-1* gene, which shares exons 2 and 3 with the *Dlc-1* gene. These transcriptional isoforms are expressed at different levels in diverse tissues however, the shortest alternative splice form is the major transcript for this gene. A *Dlc-1* knockout mouse previously made in the lab using a gene trapped embryonic stem cell line showed down regulation of the shortest transcript and embryonic lethality.

Conclusion: The complex splicing pattern of *Dlc-1* and the relative abundance of all these transcriptional isoforms in mouse indicate that the alternative transcriptional isoforms may have different biological functions.

Tobacco Smoke as a Modulator of Matrix Metalloproteinase (MMPs) Activity

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Introduction: Matrix metalloproteinases (MMPs) are a family of 26 cell-membrane bound or secreted, inducible, zinc-dependent proteolytic enzymes. They have been divided into six groups depending on their multi-domain structures and substrate specificities. They process or degrade a huge repertoire of extracellular molecules and thus play pivotal roles in a myriad of physiological, pathological and developmental processes. Preliminary evidence suggests that tobacco smoke exposure may alter the MMP functions and these changes may be related to tobacco-induced diseases. The objective of our study was to examine and compare the effects of cigarette smoke on the release of MMPs by the highly resilient and rapidly turning over isolated periodontal ligament (PDL) fibroblasts and the fetal developing lung cells.

Methods: Cells were isolated following protocols established in our laboratory and exposed *in vitro* to increasing concentrations of cigarette smoke extract (CSE) prepared by a modification of Carp and Janoff's method (*Carp and Janoff, 1978*) using 2R1 research cigarettes from Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington Kentucky, USA. The conditioned media were collected and applied to 10% polyacrylamide-gels impregnated with MMP-specific substrate (Gelatin A, Sigma Aldrich), employing a highly sensitive quantitative technique, gel zymography. This reveals specific molecular masses as well as clear bands of substrate degradation corresponding to MMP activity. Immunosorbent Enzyme-Linked Assays (ELISA Kits, R&D Systems) were performed to further identify and quantitate total MMP-secretion. CSE-induced cellular and morphological changes were assessed by phase contrast microscopic imaging, while cellular proliferation and viability were examined by formazan and crystal violet assays.

Results: Our results showed that cigarette smoke extract altered MMP secretion, cellular viability and proliferation in a concentration-dependent manner.

Conclusions: Our studies suggest that CSE may alter the PDL and lung fibroblasts viability and proliferation and may modulate their MMP secretion, which in turn may involve uncontrolled over-destruction or loss of activity. Both outcomes are equally hazardous as the periodontium necessitates MMPs continuous involvement to maintain its resiliency, high turnover rate, rapid remodeling capacity and easy adaptability to the

innumerable constantly changing local conditions and fetal lung development involves repetitive dichotomous branching of the duct system, which is also dependent on tissue degradation and remodeling on a large scale and hence proteases activity (MMPs). Thus changes in the local conditions may impact on the extracellular matrix formation, renewal and turnover rates, remodeling capacity and developmental interactions at the cellular level.

Severe Inhibition of Glutamate Uptake by PARP-1-Induced ATP Depletion in Astrocytes

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Introduction: The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) is activated in neurons and astrocytes in response to oxidative DNA damage, and leads to cell death in cerebral ischemia. Thus, reduction of PARP-1 activity could be a promising therapeutic target for stroke. While we have some information about the mechanism of neuron death induced by neuronal PARP-1, the mechanisms by which PARP-1 activation leads to death of neurons in mixed cell populations are unclear. Since PARP-1 activation leads to nicotinic adenine dinucleotide (NAD⁺) and ATP depletion, we hypothesized that active glutamate uptake in astrocytes is compromised in conditions of elevated PARP-1 activity.

Methods: To test this, we measured NAD⁺ and ATP levels, and glutamate uptake capacity in cultured neonatal mouse astrocytes following PARP-1 activation by the DNA alkylating agent, 1-methyl-3-nitro-1-nitroguanosine (MNNG).

Results: Significant astrocyte death developed beginning 6 hours after MNNG exposure (100 μ M for 30 minutes), so we limited our measurements to 4 hours after MNNG treatment, when astrocyte death was not significantly observed. The level of ATP decreased with time, diminishing by ~40% at 3 hours and ~90% at 4 hours after MNNG exposure. The depletion of NAD⁺ preceded declining cellular ATP content, as NAD⁺ levels were reduced by ~30% by an hour. In contrast, the depletion of ATP was not observed at this time point. Glutamate uptake capacity in astrocyte cultures was reduced in a manner that correlated with the degree of ATP depletion, declining by ~90% by 4 hours after MNNG exposure. Bioenergetic depletion and reductions in glutamate uptake were not observed in PARP-1^{-/-} astrocyte cultures. Upon MNNG post-treatment, the survival was greatly improved for neurons that were plated on PARP-1^{-/-} astrocyte cultures.

Conclusion: Our overall results suggest that PARP-1 activation in astrocytes contributes to bioenergetic depletion, elevated glutamate levels, and increased risk of glutamate excitotoxicity in neurons.

Inactivation of HLTF Caused Telomere Dysfunction, Leading to Genomic Instability in Tumor Cells

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Introduction: The helicase-like transcription factor (HLTF) belongs to the SWI/SNF family of chromatin-remodeling factors. HLTF has recently been demonstrated to be inactivated by hypermethylation in more than 40% human colon, gastric and uterine cancers, implicating it could function as a tumor suppressor in tumorigenesis.

Methods: To determine the role of HLTF *in vivo*, we created HLTF knockout mice in which HLTF expression was fully disrupted. We further used Q-FISH and SKY to understand the role of HLTF in genomic stability.

Results: We found that although HLTF^{-/-} mice are postnatal viable and did not develop any abnormalities, HLTF deficiency significantly increase tumor incidences on either p53 null or Apc^{+/-} background. Increased formation of intestinal adenocarcinoma and colorectal carcinomas in HLTF/Apc double mutant mice strongly suggested that HLTF could be an important factor involved in the transition of adenoma to carcinomas. By characterizing HLTF/p53 double mutant tumor cells using cytogenetic approaches, we found that these tumor cells, as compared to p53 null tumors, had significant high incidence of chromosomal instabilities, such as chromosomal breaks, trisomies and Robertsonian fusions with telomere-free ends which is a genetic hallmarker for telomere dysfunction. Such telomere dysfunction was also presented in cultured HLTF^{-/-} embryonic stem (ES) cells, further suggesting that HLTF could be required for telomere maintenance.

Conclusion: HLTF increases the tumor incidence on p53 null and Apc^{+/-} background, suggesting its role in promoting cancer formation. Our Q-FISH data shows that HLTF induces telomere dysfunction and thus leads to genomic instability, which could be the main contribution of HLTF DNA helicase in tumorigenesis.

Farnesyltransferase Inhibitor Prevents Oxidized LDL Induced Oxidative Stress and Mitochondrial Dysfunction in Cultured Vascular Endothelial Cells

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Introduction: Coronary artery disease (CAD) is the major cause of morbidity and mortality in the Western society. Elevated plasma low density lipoprotein (LDL) cholesterol is a major modifiable risk factor for CAD. Our previous studies showed that reactive oxygen species (ROS) generation is increased in vascular endothelial cells (EC) exposed to oxidized LDL (ox-LDL). ROS is implicated in oxidative stress, endothelial dysfunction and CAD. Mitochondria are one of the main sources of intracellular ROS production. Previous study in our laboratory demonstrated that ox-LDL affected the activity of mitochondrial respiratory chain. Small G-protein, H-Ras has been implicated in oxidative stress. Farnesyltransferase inhibitor, FTI-277 is an inhibitor of Ras farnesylation. The present study investigated the effect of FTI-277 on ox-LDL induced mitochondrial dysfunction in cultured vascular EC.

Methods: Cultured human umbilical vein endothelial cells (HUVEC) or porcine aortic endothelial cells (PAEC), Western blotting, OROBOROS oxygraph and ROS assay.

Results: Stimulation with ox-LDL reduced the oxygen consumption of mitochondrial complex I, II and IV in response to their substrates in PAEC compared to control. Ox-LDL treatment significantly increased the release of hydrogen peroxide (H_2O_2) from PAEC and HUVEC following 2 h of incubation. FTI-277 inhibited ox-LDL induced increase in the abundance of H-Ras in HUVEC. Treatment with FTI-277 prevented mitochondrial complexes impairment and reduced ROS generation in EC induced by ox-LDL.

Conclusion: The results indicate that the inhibition of farnesyltransferase prevents the ox-LDL induced oxidative stress with concomitant alleviation of mitochondrial dysfunction, possibly through the inhibition of H-Ras activation.

Endogenous High Molecular Weight FGF-2 is Increased in Apoptotic, Compared to Non-Apoptotic, Cardiac (Myo)Fibroblasts from Valvular Cardiomyopathy Patients.

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Introduction: FGF-2 is a multifunctional and ubiquitous protein, accumulated by the human cells as high molecular weight (hi) FGF-2 (22-34 kDa), or low molecular weight (lo, 18 kDa) FGF-2 versions, by differential translation. Overexpression experiments in human HEK293 cells, or neonatal rat cardiac myocytes have shown that increases in nuclear hi-FGF (but not lo-FGF-2) caused chromatin compaction and apoptotic cell death. It is not known if endogenous human hi-FGF-2 plays a similar role.

Methods: As a first step in addressing this question, we investigated hi-FGF-2 expression and subcellular localization in human fibroblasts grown from atrial tissue obtained from valvular cardiomyopathy patients during surgery. To this end, we produced and characterized an affinity purified polyclonal antibody preparation which recognizes only the human hi-FGF-2 isoforms by immunofluorescence or immunoprecipitation.

Results: Western blotting demonstrated robust expression of both hi- and lo-FGF-2 isoforms in lysates from human atrial tissue and atria-derived human fibroblasts. Relative expression of hi-FGF-2 was higher in human adult-derived compared to immature cardiac fibroblasts, or rat neonatal cardiomyocytes/fibroblasts. Immunofluorescence-based detection, furthermore, showed that while all human fibroblasts showed some immunoreactivity, a small fraction of cells stained strongly for hi-FGF-2. The same population of cells presented an apoptotic phenotype, indicated by condensed chromatin, and TUNEL staining.

Conclusion: Our data are consistent with the notion that endogenous hi-FGF-2, unlike lo-FGF-2, contributes to apoptotic cell death of primary human cells. Targeting translational regulation of FGF-2 isoforms therefore provides a new strategy for regulating cell survival and cell death.

Collagen I Expression Induced by TGFb1 is Markedly Suppressed by Simvastatin in Primary Human Airway Mesenchymal Cells

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Introduction: Airway remodeling is a hallmark of asthma and is characterized by excessive deposition of extracellular matrix proteins, such as collagen I, in the airway wall (subepithelial fibrosis). Airway smooth muscle (ASM) cells and (myo)fibroblasts have emerged as key contributors to this process. Transforming growth factor (TGF) b1 is a regulatory cytokine which has been implicated in the onset of several facets of airway remodeling, including fibrosis. Recently, it has become apparent that statin use is associated with improved lung function. Statins inhibit 3-hydroxy-3-methylglutaryl (HMG)-Coenzyme A (CoA) reductase and have pleiotropic effects, including inhibition of ASM proliferation. They can also indirectly suppress Rho kinase signaling by interfering with the geranylgeranylation of RhoA. In the present study we investigated the effects of simvastatin on TGFb1-induced collagen I expression, using primary cultures of patient-matched human airway fibroblasts (HAF) and ASM cells.

Methods and Results: Western blot analysis was performed using whole cell lysates from HAF and human ASM cultures stimulated with TGFb1 (0-5 ng/ml) for 48 h in the presence and absence of simvastatin (10 µM) or Rho kinase inhibitors (Y-27632 (1 µM), or H-1152P (0.1 µM)). TGFb1 dose-dependently induced collagen I protein accumulation in both ASM and HAF cultures, with surprisingly the ASM being more sensitive to TGFb1. Notably, maximum collagen I expression, induced with 2.5 ng/ml TGFb1, was markedly reduced by all inhibitors. In HAF collagen I was suppressed 25% or ~50% by Rho kinase inhibitors or simvastatin, respectively. For ASM cells, each inhibitor reduced TGFb1-induced collagen I protein by ~50%. The suppressive effects of each inhibitor on collagen I expression were also confirmed at the mRNA level using quantitative RT-PCR.

Conclusion: Collectively, these results indicate that in a manner similar to Rho kinase inhibitors, simvastatin inhibits TGFb1-induced collagen I expression in human ASM cells and HAF. In addition to the recognized ability of statins to reduce airway mesenchymal cell proliferation, the current findings demonstrate their potential to suppress airway wall fibrosis.

Decreased Ghrelin Is Associated with Increased Caloric Intake in Exercising Women with Hypothalamic Amenorrhea: Preliminary Data from an RCT to Reverse Exercise-Associated Menstrual Disturbances

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Introduction: Exercise-associated menstrual disturbances (EAMD), such as amenorrhea, are observed secondary to a chronic energy deficiency indicated by suppressed resting energy expenditure (REE) and total triiodothyronine (TT3), and elevated peptide YY and ghrelin. We report data from an ongoing 12 month randomized controlled trial to determine the effectiveness of increased caloric intake to reverse an energy deficiency and cause the resumption of menses.

Methods: Subjects were categorized by menstrual and ovulatory status into either an ovulatory control group (OvCon, n=13), EAMD + calories group (EAMD+Cal, n= 5), or an EAMD control group (n= 2). Subjects in the EAMD+Cal group were required to increase daily caloric intake (20-30%) above baseline energy needs. REE, dietary energy intake, energy expenditure, body composition, fasting ghrelin, TT₃, and daily urinary ovarian steroids were assessed during the study. Statistical analysis includes t-tests to determine baseline differences, repeated measures ANOVA to determine longitudinal differences between baseline and intervention, and correlations to identify significant associations.

Results: Presented are preliminary data for 13 OvCon and 5 EAMD+Cal participants who have completed 2 of 12 months of intervention. Groups were similar in age (25.1±1.6 y), weight (58.7±1.9 kg) and exercise training (605±143 min/week). The EAMD+Cal group and were advised to consume 536±69 kcals/day above baseline energy status and experienced a mean increase in body weight of 0.4±2.5kg. A significant time x group interaction was observed for change in caloric intake (p=0.020), body fat percentage (p=0.024), and ghrelin (p=0.040). Body fat was increased and ghrelin was decreased over time in the EAMD+Cal compared to the OvCon group. No changes were observed in REE, TT₃ or body weight. Decreases in ghrelin were associated with a higher caloric prescription (-r=0.18, p=0.006). Increased caloric intake was associated with 3/5 and 4/5 subjects resuming menses within 4 and 6 months, respectively.

Conclusion: Our preliminary results suggest that increased caloric intake and related body fat gain were associated with the reduction of ghrelin and the resumption of menses in exercising amenorrheic women.

Dystrophin Deficiency Reduces PI3K/Akt Signaling and Contractile Phenotype Expression in Airway Smooth Muscle Cells

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Introduction: Dystrophin, an intracellular subunit of the dystrophin-glycoprotein complex (DGC), is linked to the cortical actin cytoskeleton and in skeletal muscle provides mechanical support to the plasma membrane. We recently reported that expression of the DGC is a marker for phenotype maturation of human airway smooth muscle (ASM). Though in some dystrophies, such as Duchene muscular dystrophy marked by the absence of dystrophin, effects on skeletal muscle differentiation and maintenance are well described, little is known about how smooth muscle function and phenotype might be affected. We tested the hypothesis that the absence of dystrophin in ASM suppresses phenotype maturation and signaling via phosphatidylinositol-3-kinase (PI3K)-Akt1-mTOR, which we have shown is required for the accumulation of contractile proteins.

Methods and Results: We used primary cultured ASM cells (N=3) from Golden Retriever dogs (GRMD), which exhibit muscular dystrophy due to lack of dystrophin expression, and from healthy control animals. Protein lysates were obtained from subconfluent, serum-fed cultures (exhibit a “proliferative” phenotype) and from confluent cultures subjected to prolonged serum deprivation (exhibit a “contractile” phenotype). Immunoblotting and immunocytochemistry confirmed that dystrophin, which accumulates in control ASM cultures during serum deprivation, was undetectable in GRMD cultures. Notably, in GRMD cell cultures subjected to 7-days serum deprivation the accumulation of contractile phenotype markers, smooth muscle myosin heavy chain (smMHC) and calponin was reduced by $39\pm 7\%$ and $35\pm 6\%$ ($p < 0.05$) compared to ASM cultures from control animals. Moreover, accumulation of β -dystroglycan (β -DG), a DGC subunit, and caveolin-1 (a DGC-associated protein) was reduced by 2-to-4 fold in GRMD cultures. Interestingly, immunocytochemistry also suggested that the distribution of β -DG and caveolin-1, which typically appear in longitudinal plasma membrane arrays adjacent to actin stress fibers in contractile myocytes, appeared diffusely throughout the cells. Immunoblot analysis also revealed a suppression of 2.5-fold in Akt1 phosphorylation at Thr-308, a marker for activation of PI3K signaling activity.

Conclusion: The data shows that lack of dystrophin is associated with a concomitant suppression ASM cell phenotype maturation and activation of PI3K signaling, an essential event for smooth muscle differentiation. Our results provide first time evidence that the DGC and its link to intracellular actin via dystrophin could play an important role signaling events that regulate phenotype plasticity of smooth muscle cells.

The Role of Dlc-2 in Ceramide Signalling to PGP Synthase

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Introduction: Previous work in our laboratory used retrovirus promoter trap mutagenesis to develop cell lines with resistance to the chemotherapeutic drug etoposide. One cell line developed, the E91 cell line, has the retrovirus inserted in the Deleted in liver cancer two (Dlc-2) gene, which is a tumor-suppressor gene with Rho GTPase-activating protein (RhoGAP) activity. The E91 cell line was also observed to have a reduction in phosphatidylglycerolphosphate (PGP) synthase induction after ceramide treatment. This study is being conducted to understand the mechanism for ceramide's control of the PGP synthase gene and how Rho signalling negatively regulates this control.

Objectives: The specific aims of this project are to learn how PGP synthase is transcriptionally regulated after ceramide treatment, to learn if PGP synthase mRNA stability is changed after ceramide treatment, and to learn if post-translational modifications of PGP synthase enzyme are occurring after ceramide treatment.

Methods: To measure mRNA levels, I am using Real-Time Reverse Transcription-Polymerase Chain Reactions (RT-PCR). To be able to study the PGP synthase enzyme, I am developing an antibody against the protein using phage display and Western blots.

Results: Real-Time RT-PCR data shows a rapid 16-17 fold increase in PGP synthase mRNA after ceramide treatment. Cells with defects in the Dlc-2 RhoGAP gene show negative regulation of the PGP synthase mRNA levels after ceramide treatment.

Conclusion: This study, of the E91 cell line and the function of Dlc-2, is important due to the cell line's drug resistance after etoposide and ceramide treatment. This project will increase our understanding of why this cell line is more resistant to drugs, and thus, a greater understanding of how cancer cells with this gene mutated may respond to chemotherapy will be provided.

Migration, Social Capital, and Vulnerability and Risk for HIV/AIDS: A Study of Rajasthani Migrants in Mumbai and Ahmedabad, India

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Introduction: At the global level, there is enough evidence to suggest that migration and HIV/AIDS are correlated but how migration affects HIV is not very well understood. We know that not all migration leads to vulnerability to HIV risk and within a single migrant community, migrants have differential vulnerability to HIV risk. This study, as part of my PhD, was undertaken to understand the relationship of migration and HIV and role social capital plays as a mediating mechanism.

Methods: A cross-sectional survey was conducted among migrant workers in Mumbai and Ahmedabad. In total, 1600 questionnaires were filled in one-to-one setting.

Results: 95% migrants in Mumbai and 90% in Ahmedabad reported that they had heard of condoms; 80% migrants in Mumbai and 73% in Ahmedabad had heard of STDs; and 96% in Mumbai and approximately 87% in Ahmedabad had heard of HIV/AIDS. However, only 35% in Mumbai and 51% migrants in Ahmedabad could tell two or more correct symptoms of STDs; and 63.6% in Mumbai and 56.2% in Ahmedabad could tell two correct ways to prevent HIV. Only 8.5% in Mumbai and 10.6% in Ahmedabad reported regular condom use. 10% in Mumbai and 17% in Ahmedabad reported sex with a CSW in last 12 months. 8% in Mumbai and 10% in Ahmedabad reported ever having symptoms of STDs. Factors associated with sex with a CSW or having STD include: Being in the age-group of 25-35 years; being away from the wife (53% in Mumbai and 78% in Ahmedabad); duration of stay (higher among those who have been for more than 5 years in Mumbai and more than 3 years in Ahmedabad); and easy availability of sex in the city (47% in Mumbai and 73% in Ahmedabad). Peer pressure and alcohol consumption don't seem to be the major reasons. Lack of trust within the community (low bonding social capital), high trust in the neighborhood (high bridging social capital) and medium or high trust in the city people (bridging social capital) seem to be associated with higher proportion of people who had sex with CSWs or had ever had STDs in Mumbai. In Ahmedabad, low trust in community member, low trust in neighborhood and low trust in the city people seem to be associated with higher proportions of people who had sex with CSWs or had ever had STDs. In Mumbai, those reporting always

voting, always participating in associations have higher proportions of reporting sex with CSWs and STDs. In Ahmedabad, both reporting STD and sex with CSWs have higher proportions for those who 'sometimes' participated in the activities.

Conclusions: There seems to be a relationship between migration induced social situations and behaviours and high risk behaviour. This is a work in progress. Further regression analysis will give a better insight into unique effects of different factors.

Mechanisms Underlying Differences Between I/R Hearts Perfused at Constant Flow or Constant Pressure

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Introduction: Studies using isolated hearts perfused at constant flow (CF) and constant pressure (CP) have shown differences in contractile response to ischemia reperfusion (I/R). This study examined mechanisms underlying this differential response of hearts perfused at CF and CP to I/R.

Methods: Contractile parameters were assessed in hearts subjected to I/R in the presence or absence of L-arginine (LA), or L-NAME (LN) treatment. Sarcolemmal (SL) Na⁺-K⁺ ATPase activity, sarcoplasmic reticulum (SR) Ca²⁺ uptake and endothelial function were also measured in these hearts.

Results: I/R induced a 70±5% decrease in cardiac contractility at CF compared with 40±5% decrease at CP. This was associated with greater reduction in SL Na⁺-K⁺ ATPase activity and SR Ca²⁺ uptake at CF than CP. These changes were consistent with a four-fold increase in calpain activity, decrease in NO content of coronary perfusate and reduced NO mediated endothelium dependent vasorelaxation and constriction at CF as compared to CP. LA treatment attenuated cardiac dysfunction observed in hearts at CF.

Conclusion: Our results suggest that differences in response of hearts perfused at CF and CP to I/R could partly be due to flow mediated variations in shear stress, which causes more endothelial dysfunction and depression in subcellular enzyme activity at CF than CP. These differences in endothelial dysfunction and subcellular enzyme activity could be in part due to a greater increase in calpain activity and decrease in endothelium derived NO at CF than CP.

Protective Polymorphisms in the IRF-1 Gene in Highly Exposed Uninfected Kenyan Sex Workers Correlate with Decreased Exon 7 Skipping of IRF-1 mRNA

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Objectives: To characterize the mechanism by which three IRF-1 polymorphisms associated with the resistance to HIV-1 infection affect the expression of IRF-1 regulated genes.

Methods: Exon skipping and the functional impact of IRF-1 polymorphisms on expression of IRF-1 regulated genes are being analysed using the Affymetrix Human Exon 1.0 ST microarray. Quantitative RT-PCR was used to measure IRF-1 mRNA levels, as well as to confirm the microarray results. Analysis of protein levels, protein stability and protein phosphorylation will be done using Western blotting.

Results: Data indicate a link between IRF-1 polymorphisms and skipping in exon 7. Exon 7 is adjacent to the microsatellite IRF-1 polymorphism and it is part of the protein degradation domain. Decreased exon 7 skipping observed in women with protective IRF-1 polymorphisms seems to lead to decreased protein stability and potentially accounts for the decreased IRF-1 protein levels observed in earlier studies (Ball. *et al.*). Further functional analysis of IRF-1 polymorphisms and HIV resistance is underway.

Conclusion: IRF-1 protective polymorphisms are correlated with a decrease in exon 7 skipping leading to a decrease in IRF-1 protein stability. It is important to fully characterize the effect of IRF-1 polymorphisms as this will further the understanding of mechanisms of HIV resistance and guide the development of novel strategies for HIV prevention and treatment.

Phosphorylation of Estrogen Receptor α at Serine 118 and Serine 167 are Associated with a Better Response to Endocrine Therapy in ER α Positive Breast Cancer Patients

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Introduction: ER α activity is regulated by phosphorylation and important phosphorylation sites are serines 104, 106, 118 and 167. Phosphorylation at serine 118 (P-S118-ER α) and serine 167 (P-S167-ER α) can be regulated by both ligand independent as well as ligand dependent factors leading to speculation that altered phosphorylation of these sites may play a role in resistance to endocrine therapy for breast cancer.

Methods: Expression of ER α phosphorylated at these sites was investigated by immunohistochemistry (IHC) in human breast tumors *in vivo*.

Results: P-S104,6-ER α was correlated with P-S118-ER α ($r=0.30, P<0.0001$), P-S167-ER α ($r=0.14, P=0.025$) and inversely with tumor size ($r=-0.16, P=0.012$). P-S118-ER α was significantly correlated with progesterone receptor (PgR) ($r=0.22, P<0.0001$ for PgR IHC and PgR ligand LBA), while an inverse correlation was seen between P-S118-ER α and tumor size ($r=-0.15, P=0.0064$). P-S167-ER α was correlated with PgR LBA ($r=0.17, P=0.0006$), P-S118-ER α ($r=0.29, P<0.0001$) and inversely with tumor size ($r=-0.20, P<0.0001$). P-S118-ER α was associated with a better response to tamoxifen therapy and with markers of good prognosis in 336 primary ER α positive breast biopsies. Patients with P-S118-ER α positive tumours had a longer relapse free survival (RFS), compared to those whose tumours were P-S118-ER α negative ($P=0.044$). Furthermore, a trend to longer RFS was also observed in 381 biopsies of P-S167-ER α patients ($P=0.055$).

Conclusions: These data demonstrate for the first time detection of multiple phosphorylated ER α forms in breast cancer and raise the possibility that expanding ER status to include phosphorylation status of ER α may improve prediction of clinical outcome to endocrine therapy in breast cancer.

Cortical and Behavioural Adaptations Associated With Inphase Versus Antiphase Bimanual Movement Training Using the Wrists

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Introduction: Emerging evidence suggests that bilateral movement training (BMT) may be an effective strategy for enhancing sensorimotor recovery following stroke. However, the specific neural mechanisms that underlie this are not clear, nor are the effects of specific types of bimanual movements. Using EEG, our previous work (Smith & Staines, *Brain Res*, 2006,1071:165-174) reported that a single session of BMT (~ 40 mins), incorporating both inphase and antiphase wrist flexion / extension movements, induced cortical and behavioural adaptations associated with a unimanual right wrist flexion task. It was unclear if the exhibited cortical modulations were induced primarily by the in-phase or antiphase BMT.

Methods: The present study used the movement-related potential (MRP), an ERP extracted from the EEG time-locked to the onset of a cued movement (-2s to +1s), to measure cortical activity modulations during a unimanual right wrist flexion task, induced by inphase versus antiphase BMT.

Results: The early MRP component of the movement-related potential significantly increased in amplitude for a unimanual right wrist flexion task in response to inphase BMT versus antiphase BMT or repetitive unimanual movement (control). There was a strong correlation found between the changes in amplitude of the early MRP and RT for a one-handed right wrist flexion task following inphase BMT.

Conclusion: Inphase BMT has transfer effects to a unimanual right wrist flexion task. This training method may be a more effective rehabilitative strategy for some stroke patients versus antiphase BMT; however, this will be confirmed in future studies.

Consistency Between Clinical Diagnosis, Self-Reported Pain, and Vestibular Sensitivity in Provoked Vestibulodynia

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Objectives: Provoked vestibulodynia (PVD) is a common cause of dyspareunia (i.e., pain during intercourse). Its diagnosis is based upon self-reported dyspareunia and pain during a diagnostic examination. To date, little research has examined associations between subjective pain ratings and clinical diagnosis. The current study examined the relationship between pain ratings during the diagnostic examination (the cotton-swab test), self-reported pain symptoms, and vestibular pain thresholds assessed via quantitative sensory testing (QST).

Methods: This study is ongoing; to date, participants are 25 women with PVD and 31 controls who reported gynecological history, including any vulvar pain symptoms, during a telephone interview. Women were subsequently scheduled for a standardized gynecological examination that included the cotton-swab test, and a laboratory appointment that assessed vestibular thresholds using QST. Pain intensity ratings were collected during the cotton-swab test and QST.

Results: The cotton-swab test confirmed 89.3% of self-reported PVD cases and 100% of control cases. Pain ratings during this test distinguished between women with PVD and controls, with PVD women reporting significantly higher pain ratings ($t = -8.53, p < .05$). During QST, pain detection threshold was associated with pain ratings during the cotton-swab test, $r = -.63, p < .05$, indicating that increased pain sensitivity during QST related to higher pain ratings during the examination.

Conclusion: The QST results are consistent with previous research examining localized vestibular sensitivity in women with PVD. The cotton-swab examination correlated with both patient self-report and QST findings, indicating that this test provides a quick and useful tool for the accurate diagnosis of PVD.

***Hoxa2* Plays a Direct role in Regulating Murine Palatogenesis via Repressing Downstream Targets**

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Introduction: Cleft palate is one of the most common congenital birth defects in humans. The regulation of palate development in vertebrates involves a complex network of signaling pathways. *Hoxa2* null murine embryos exhibit a high penetrance of cleft palate (up to 81%). It has previously been proposed that this cleft palate is a secondary defect due to altered tongue musculature. We have investigated whether *Hoxa2* is expressed within the developing palate and determined if it played a direct role in palate development.

Methods: Quantitative real-time RT-PCR was used to examine the expression of *Hoxa2* and its putative downstream targets (*Lef1*, *Msx1*, *Ptx1* and *Six2*) in the developing palate (E12.5-E15.5) of wild-type and *Hoxa2* null embryos. Protein expression was also examined using immunohistochemical analysis in wild-type and *Hoxa2* null embryos throughout palate development (E12.5-E15.5).

Results: *Hoxa2* was found to be expressed in the developing murine palate at both the mRNA and protein levels, with the highest expression observed early in development (E12.5 and E13.5). The function of *Hoxa2* in the palate appears to be by acting as a transcriptional repressor, with all four targets (*Lef1*, *Msx1*, *Ptx1* and *Six2*) showing increased expression early in palate development in *Hoxa2* null palates compared to age matched wild-type controls.

Conclusions: *Hoxa2* has been shown for the first time to be expressed within the developing palate where it appears to be acting directly in the developing palate by acting as a transcriptional repressor.

Induction of Oxidative Stress, Neuronal Loss and Parkinsonism in Rats by Paraquat: Neuroprotection by Water-Soluble CoQ₁₀

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Parkinson's disease (PD) is a progressive neurodegenerative disease caused by loss of dopamine producing neurons in the substantia nigra pars compacta and their projections to other areas of the brain. Present studies correlate exposure to environmental toxins such as herbicide paraquat (PQ) to an increased incidence of PD. Increased oxidative stress resulting from mitochondrial dysfunction has been implicated upon exposure to paraquat. Previous findings from our laboratory have indicated that PQ causes oxidative stress and apoptosis in differentiated human neuroblastoma cells. Most important is the fact that pre-treatment with water soluble Coenzyme Q₁₀ (WS-CoQ₁₀) protects cells against PQ toxicity. The present study was designed to investigate the *in vivo* effects of PQ and the neuroprotective properties of WS-CoQ₁₀. We have successfully established a model of PD in Long Evans hooded male rats by injecting them with PQ. Both behavioral and biochemical parameters were studied to assess the toxicity caused by PQ as well as the neuroprotective effects of WS-CoQ₁₀. Results point to the indication of motor balance dysfunction in the rats as observed by a drastically reduced tendency to turn around and walk backwards on the rotorod following PQ injections. Oxidative stress markers such as lipid peroxidation and GSH measurements revealed that PQ induced oxidative stress, while reduced ATP levels indicated mitochondrial dysfunction. The loss of dopamine producing neurons and PD-like symptoms in the injected rats was illustrated by immunohistochemistry. Significantly, neuroprotection and improvement in these PD-like symptoms were observed in rats fed with WS-CoQ₁₀. Altogether, we present a novel model of sporadic PD caused by PQ and report an encouraging prospect for the inhibition of progressive loss of neurons by the unique formulation of WS-CoQ₁₀.

Antibodies against Intestinal CD163 Positive Macrophages in a Patient with Type 1 Diabetes and Celiac Disease

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Introduction: Recent studies have described abnormalities in the gastrointestinal tract of animal models and humans with type 1 diabetes (T1D). Oral exposure to dietary antigens typically leads to the induction of tolerance in the mucosal immune system. Our group has described the case of a highly wheat sensitive patient with both T1D and celiac disease (CD) who displayed antibody and T cell reactivity to wheat proteins, including a homologue of wheat storage globulin-1 (Glb1). In this study, we purified Glb1 antibodies from this patient to identify possible autoimmune targets in the gastrointestinal tract.

Methods: Batch affinity chromatography: Recombinant Glb1 was used to enrich Glb1-specific antibodies from plasma of the patient by batch affinity chromatography. ELISA: Presence of Glb1 antibodies in the enriched sample was confirmed by ELISA. Immunohistochemistry: Bouin's fixed intestinal sections from 11-100 day old Wistar Furth rats and human peripheral monocyte sections were immunohistochemically stained with the Glb1 antibody preparation.

Results: The enriched Glb1 antibody preparation labeled a subset of cells in the lamina propria on intestinal sections from rats that were ≥ 30 d. Binding was not blocked by rGlb1 and was therefore attributable to non-Glb1-specific antibodies that were co-purified during the enrichment process. This labeling predominantly co-localized with cells expressing CD163, a marker of mature tissue-resident macrophages. A subset of human peripheral monocytes was also labeled, suggesting that these anti-macrophage antibodies could represent novel T1D- and/or CD-related autoantibodies.

Conclusion: These results suggest that intestinal macrophages are targeted by autoantibodies in this patient. Resident intestinal macrophages are thought to be important for maintaining immune tolerance in the gut. This study raises the novel possibility that an autoimmune response against this cell population is present in some patients with T1D and/or CD.

Cardiac Mitochondria-Based Signal Transduction is Activated by FGF-2 and Prevents Calcium-Overload Induced Damage: Role of Mitochondrial PKC and Connexin43

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Introduction: Mitochondria make life or death decisions for cardiomyocytes. Injurious stimuli such as calcium overload and oxidative stress promote mitochondrial membrane permeability transition resulting in opening of a pore (mPTP), release of cytochrome C and cell death. Conversely, cardioprotective agents prevent mPTP opening by effecting translocation of protective signals, as well as by activating mitochondria-based signals. FGF-2 is a potent cytokine, acting both as a para-/auto-crine but also intra-crine agent. Here we investigated the ability of FGF-2 to activate mitochondria-based, direct protective signals, and protect isolated cardiac mitochondria from calcium-overload induced mPTP pore opening.

Methods: Mitochondria were isolated from adult rat heart homogenates using differential centrifugation and percoll gradient ultracentrifugation. Mitochondrial suspensions were exposed to increasing concentrations of calcium in the presence or absence of FGF-2 (25 ng/ml), and in the presence or absence of peptide inhibitors of PKC ϵ . Opening of mPTP was assessed by measuring mitochondrial swelling (light scattering, OD₅₄₀), and cytochrome C release. We also examined mitochondrial Connexin43 (mCx43), for its phosphorylation at PKC target sites, serines(S) 262 and 368.

Results: FGF-2, or PMA, caused significant resistance to calcium-induced mPTP opening while eliciting a potent stimulation of mCx43 phosphorylation at S262 and S368. FGF-2 also protected mCx43 from degradation associated with calcium overload. The effects of FGF-2 on either mitochondrial resistance to injury or mCx43 phosphorylation were completely prevented by specific PKC ϵ inhibitor peptides, but not the inactive control peptides.

Conclusion: This is the first time that cardiac mitochondria have been shown to possess a growth factor-responsive mechanism that promotes resistance to calcium overload-induced injury, requires PKC ϵ , and, likely, mCx43 phosphorylation at S262 and S368.

Disparate Induction and Maintenance of Effector- and Central- Phenotype CD8⁺ T Cells by Recombinant Live Vectors, *Listeria monocytogenes* and *Salmonella Typhimurium* Differentially Modulates Efficacy Against Tumours

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Introduction: Memory CD8⁺ T cells are a heterogeneous population segregating into tissue homing effectors and lymphoid homing central phenotype cells. We addressed whether the type of vaccine can influence cancer elimination by affecting quality rather than quantity of the CD8⁺ T cell response.

We have 1) compared the quality of the CD8⁺ T cells and 2) tumor protection in a mouse model generated by two ovalbumin (OVA) expressing vectors *Listeria monocytogenes* (LM) and *Salmonella typhimurium* (ST) that divergently generate central and effector memory CD8⁺ T cells respectively.

Methods: B6129F1 mice were vaccinated with LM-OVA or ST-OVA (i.v. 10³), and the CD8⁺ T cell response was monitored by flow-cytometry. Central and effector OVA₂₅₇₋₂₆₄-tetramer-specific CD8⁺ cells were identified by activation (CD44^{high}) and phenotypic (CD62L and IL-7R α) markers. Functionality was determined by an *in vivo* cytolytic assay and intracellular cytokine staining (IL-2 and IFN γ). Homeostatic proliferation was measured by loss of CFSE both *in vitro* and *in vivo*. Protection against murine solid melanoma tumour (B16-OVA) was evaluated.

Results: 1) Both LM-OVA and ST-OVA induced strong, functional OVA-specific CD8⁺ T cells that expressed IFN- γ and killed targets specifically *in vivo*. However, CD8⁺ T cells generated against LM-OVA (but not ST-OVA), expressed IL-2 which correlated to profound homeostatic and antigen-induced proliferation of the memory cells generated by the former. 2) Only LM-OVA vaccinated mice were protected against a challenge with B16-OVA tumors. Additionally the adoptive transfer of memory CD8⁺ T cells induced by LM-OVA infection to recipient mice conferred tumor protection.

Conclusion: Therefore, the strong proliferative capabilities rather than quantity or immediate functional ability of memory CD8⁺ T cells influences tumor vaccine efficacy. Live vectors such as LM that promote self renewing central memory CD8⁺ T cells constitute superior adjuvants for cancer vaccines.

Identification of Homing Markers in the Female Genital Tract Using the iTRAQ Approach

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Objectives: The female genital tract (FGT) relies heavily on the trafficking of immune cells for immune control of infections, yet little is known about its mechanism. Studies of this site have been plagued with technical issues that limit researchers' ability to isolate adequate cell numbers for comprehensive analysis. The identification of systemic cells destined for the FGT could overcome this obstacle providing us with novel techniques for studying the mucosal immune system. Here, we propose a new method for the identification of genital tract homing markers allowing a better understanding of genital tract immunity.

Methods: A relatively new mass spectrometer technology called isobaric tagged relative and absolute quantitation (iTRAQ) has emerged allowing for the comparison and quantification of multiple samples within a single mass spectrometer (MS) experiment. This technique will allow us to analyze the expression of membrane proteins on immune cells from the systemic compartment compared to those expressed in lymphocytes isolated from the FGT.

Results: Membrane purification and fractionation protocols were established and verified by Western blot with antibodies specific to membrane and non-membrane proteins. Membrane preparations were performed on several PBMC dilutions providing us protein concentrations for projected cervical mononuclear cell isolations. Also, optimal separation techniques were determined for MS analysis comparing SDS-PAGE and two-dimensional liquid chromatography.

Conclusion: This novel method may aid in identification of mucosal homing markers for the FGT. Understanding how immune cells traffic to the genital tract will contribute to the design of new vaccines or therapies that may elicit protection from HIV infections. A comprehensive understanding of genital tract immunity is essential and will shed further light on the pathology of FGT infections such as HIV.

Nucleoside Diphosphate Kinase from *Mycobacterium tuberculosis* as a Potential Virulence Factor Involved in Phagosome Maturation Arrest Through Inactivation of Rab7

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Introduction: *Mycobacterium tuberculosis* (Mtb), the causative agent in tuberculosis (TB), kills two million people each year. This disease poses an immediate and ongoing health concern especially in recent times where extensively drug resistant Mtb has been isolated in several countries. One main feature of Mtb pathogenesis is the ability to persist within vesicles called phagosomes that resist acidification and fusion with lysosomes by mechanisms that are still unclear. Rab7 is a small GTPase that was shown to play a central role in controlling the fusion of late endosomes/phagosomes with lysosomes. Rab7 function is mediated in epithelial cell models by the Rab7 effector RILP (Rab7-interacting lysosomal protein). The objective of this study was to examine whether mycobacteria interfere with Rab7 function and if so how this happens?

Methods: To study the Rab7-RILP interaction, a model system was established using RAW 264.7 cells (murine macrophage cell-line). The infection model used *M. bovis* BCG (TB vaccine) as a surrogate pathogen. This is feasible because BCG mimics many features of virulent Mtb, including phagosome maturation arrest. Through both *in vitro* assays and *in vivo* confocal microscopy, our objective was to demonstrate the relevance of Rab7-RILP interaction in macrophages and its subsequent disruption by mycobacterial infection. Ultimately, the goal was to identify mycobacterial factor(s) responsible for the observed disruption of Rab7-RILP interaction.

Results: Macrophage co-transfection with Rab7 and RILP revealed that Rab7-RILP interaction occurs in cells ingesting latex beads. Infection with live but not killed BCG inhibited RILP recruitment despite Rab7 acquisition by the phagosome. Further investigation using immobilized RILP to pull down active Rab7 (GTP-bound form) from cell lysates demonstrated that inactive Rab7 (GDP-bound form) predominates in macrophages infected with live BCG. In addition, cell-free system experiments demonstrated that BCG culture supernatant contains a factor that catalyzes the GTP/GDP switch on recombinant Rab7 molecules. Further studies identified ndkA (nucleoside diphosphate kinase) of Mtb as a potential virulence factor responsible for the inactivation of Rab7 and the subsequent disruption of phagolysosome fusion pathway.

Conclusion: These findings demonstrate that live mycobacteria express a Rab7 deactivating factor leading to abortion of RILP-mediated fusion with lysosomes. Ongoing studies focus on the validation of ndkA as virulence factor and ultimately an attractive drug target.

Boundary Dispute in the Developing Forebrain - DLX versus PAX Genes

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Objectives: The forebrain is required for complex functions of the vertebrate brain, and defects in human forebrain development can lead to severe neurodevelopmental disorders, such as Autism and Epilepsy. The extracellular matrix protein Tenascin-C (Ten-C) gene promoter contains candidate binding sites for proteins containing homeodomains, paired domains and paired class homeodomains. *Dlx2* and *Pax6* genes may control the expression of Ten-C at the striatal/neocortical boundary, thereby affecting neuronal migration during forebrain development. Our objective is to understand the gene regulatory network between the transcription factors, DLX1/DLX2 and PAX6, and the candidate target gene Ten-C.

Methods: To determine Ten-C expression in mutant mice, we performed immunohistochemistry (IHC). To study the protein-DNA interactions of DLX1/DLX2 and PAX6 and the Ten-C promoter, we carried out chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift (EMSA) assays. To study the functional consequences of DLX1/2 and PAX6 Ten-C promoter interactions, we performed luciferase gene reporter assays.

Results: TEN-C protein expression is unaffected in the *Dlx1/Dlx2* double knockout mouse, whereas it is not detected in the forebrains of the *Pax6* homozygous null mouse. Both DLX2 and PAX6 bind to regions of the Ten-C promoter *in vitro* and *in vivo* and transactivate expression of a Ten-C reporter gene construct *in vitro*. Of interest, using ChIP-reChIP assays, both DLX2 and PAX6 bind to a small region of the Ten-C promoter *in vivo* and DLX2 and PAX6 form protein-protein complexes *in vitro* and *in situ*.

Conclusion: It is unclear why DLX2 transactivates Ten-C expression *in vitro* yet loss of *Dlx1/Dlx2* function does not affect Ten-C expression at the striatal/neocortical boundary *in vivo*. It is possible that PAX6 and DLX2 compete for binding to the Ten-C promoter. Further characterization of the interactions between these transcription factors and their target genes will improve our understanding of forebrain development and help elucidate the mechanisms underlying human neurodevelopmental disorders.

Interferon-beta, for the Treatment of Multiple Sclerosis, Produced in High Yields from Macroporous Microcarrier Cultures Under Hypothermic Conditions

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Introduction: Recombinant human β -Interferon (IFN- β) is used for the treatment of relapsing and remitting multiple sclerosis. IFN- β is a 166 amino acid glycoprotein produced naturally in human cells in response to viral infection or exposure to other biologics. There are 2 recombinant forms of β -IFN available clinically: IFN- β -1a and IFN- β -1b. IFN- β -1a is expressed in CHO cells and is a glycosylated protein similar to that found in humans. The glycosylation profile of IFN- β from mammalian cells appears to be fairly robust with respect to changes of culture conditions. However, the greatest challenge for the production of IFN- β is a tendency for intramolecular aggregation because of the hydrophobic nature of the peptide backbone.

Methods: Macroporous microcarriers such as Cytopore entrap cells in a mesh network allowing growth to high cell concentrations in a protected environment from high shear forces within a pseudo-suspension stirred culture. In an attempt to maximize production of IFN- β , microcarrier cultures were subjected to a low temperature regime. Low temperature culture conditions (32°C) have been shown previously to enhance cell specific productivity in suspension cultures although at reduced cell growth rates. These conditions can be optimized by a timely shift from physiological to hypothermic conditions during the culture run in order to maximize volumetric protein production. In the case of IFN- β production the lower temperature has the added advantage of stabilizing the product and reducing intramolecular aggregation.

Results: Using a biphasic temperature-shift regime from 37°C to 32°C the volumetric production of IFN- β was enhanced 4.2-fold compared to a single temperature suspension culture in a controlled bench-top bioreactor. Furthermore the degree of intramolecular aggregation of IFN- β was reduced significantly (59%) compared to control cultures, largely due to the lower temperature but also partially due to the presence of microcarriers.

Conclusions: These results indicate that the hypothermic conditions in a Cytopore culture had a combined and possibly synergistic effect of increasing volumetric production of the recombinant protein.

Reduced Myocardial Uptake and Function in Arg302Gln PRKAG2 Mutant Mice as Measured by FDG PET and Echocardiography

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Background: AMP-kinase (AMPK) is a heterotrimeric serine-threonine kinase with both catalytic (α) and regulatory subunits (β and γ) augmenting myocardial glucose and fatty acid metabolism during increased metabolic demand. Mutations in the γ 2 subunit of AMPK have been identified in humans to cause an autosomal dominant disease termed PRKAG2. This disease results in excessive cardiac glycogen deposition and development of ventricular pre-excitation, progressive conduction system disease and cardiac hypertrophy. The pathological mechanism is currently being investigated using the transgenic Arg302Gln mutant (TGmut) and non-transgenic (NTG) mouse model of the human PRKAG2 disease.

Objectives: This study aims to evaluate alterations in glucose metabolism and cardiac function in TG-mut and NTG mice as measured by 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG) uptake with small animal PET and echocardiography.

Methods: TGmut mice expressing the mutant AMPK cardiac gene and NTG 1yr-old mice were investigated using echocardiography and PET. Echocardiography was conducted with VisualSonics Vevo 770 (45 MHz). Dynamic 60min acquisitions with FDG (0.8-2.4mCi, 150 μ L, iv) were obtained with Siemens InveonTM. Blood glucose levels were measured at the time of FDG injection. The fractional rate of radiotracer uptake (K) is derived from PATLAK analysis. The rate of myocardial glucose uptake (rMGU) by the left ventricle is calculated as (K/LC) x blood glucose. LC (lump constant) corrects for the differences in the transport and phosphorylation of FDG and glucose.

Results: A significant decrease in ejection fraction (EF) was found in the TG-mut (n=5) (51.0 ± 17.4) compared to the NTG mice (n=4) (84.1 ± 7.8). The mean rMGU in the TG-mut (n=5) (3.6 ± 1.9) was significantly lower than NTG (n=2) (7.06 ± 0.99) (T-test p value 0.01). No difference in the mean plasma glucose levels was observed between the groups.

Conclusions: Reduced myocardial glucose uptake and function in TG-mut mice are indicative of the severity in disease state observed at 1 year. These results suggest that alterations in the AMPK activity caused by the γ 2 subunit mutation decreases myocardial glucose transporter or hexokinase activity. Future work will include serial imaging from one month to one year, and *in vitro* assays to determine enzyme activity, glucose transporter expression and assess tissue glycogen deposition.

Stable Silencing of SHP-1 Phosphatase in Mature Murine NK Resulted in Loss of Cell Viability and Impaired NK Functions

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Natural killer (NK) cells are a subset of lymphocytes important in the initial defence against invading microorganisms, viruses and transformed cells. They express two groups of receptors on the cell surface, activation and inhibitory. The status of NK activity depends on the balance of signals generated from both groups of receptors. Inhibitory receptors recognize self-MHC and non-MHC molecules giving them an important role in the prevention of NK-mediated autoimmunity. Inhibitory receptors signal through immunoreceptor tyrosine based inhibitory motifs (ITIM), and recruit phosphatases such as SHP-1 to inhibit NK cell activation. Over-expression of a catalytically inactive form of SHP-1 in human and murine NK results in decreased MHC-Class I mediated inhibition of cytotoxicity. NK cells of the Motheaten mice (*me*) and viable motheaten mice (*me^v*) (that have a complete or partial loss of SHP-1 enzymatic activity respectively) are hyporesponsive. However, it remains to be determined whether the effects associated with a loss of SHP-1 function in the transgenic mice represented a summation of direct NK defects in its differentiation pathway, and/or a defect in mature NK cell function. We previously described an *in vitro* engineering protocol to modulate protein expression levels (and functions) at primary NK precursors, resting, and cytokine-activated NK cell levels. We will use these platforms to test the hypothesis that the same receptor signaling in NKP, immature and mature NK cells will have differential effects on NK functional development. In this current study, we used our established lentiviral transduction system to deliver shRNA-mediated shutdown of SHP-1 in murine IL-2 activated NK cells. We demonstrated that SHP-1 silenced activated NK cells are impaired in IL-2-induced cell proliferation, more susceptible to apoptosis and necrosis, and less cytotoxic towards YAC-1 targets. Interestingly, SHP-1 silenced NK showed spontaneous increase in degranulation in culture. This study demonstrated an essential role of SHP-1 in mature NK, and suggested that SHP-1 may play a role in regulating self-tolerance and autoimmunity in mature NK.

Identification of New Markers for Human Lung Adenocarcinoma Using Proteomic Techniques and Transgenic Mouse Model

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Introduction: Lung cancer is the leading cause of cancer deaths in North America and worldwide. The prognosis is generally poor, with an overall 5-year survival rate for patients receiving treatment of only 14%. The high mortality rate of this disease is due to the fact that the majority of lung cancers are diagnosed at advanced stages when the options for treatment are mostly palliative. New techniques and screening markers for early diagnosis must be developed. One of the approaches for identification of new cancer biomarkers is a direct analysis of serum proteins from cancer patients. However, the complexity and variability of serum proteins in different patients make data interpretation difficult. To overcome these disadvantages, transgenic murine models can be used.

Methods: We recently generated a transgenic mouse overexpressing calreticulin in the endothelial cells (ENCRT mice). The main phenotype of ENCRT mice was an increased incidence of lung cancer as compared to non transgenic CD1 background mice. These tumors possess a striking histopathological similarity to human conditions and frequently metastasize to the liver, spleen and lymph nodes. The objective of this study is to use this mouse model to identify early biomarkers of pulmonary lung adenocarcinoma. In our study we are using proteomic analysis (DIGE, MALDI-TOF) of serum collected from wild type and transgenic animals in order to correlate the changes in the serum proteins with the disease progression.

Results and Discussion: Preliminary two dimensional electrophoresis and sample processing methods were performed in order to assay intrinsic limitations and resolution of the techniques for the analysis of the mouse plasma proteome. Five plasma samples from ENCRT mice and two samples from non-transgenic mice were combined in equal amounts to provide two plasma pools (transgenic and non-transgenic). All animals were age and sex matched. Albumin and other abundant proteins were depleted from plasma using the Na/Cl method. Samples were precipitated and then solubilized in 8M urea, 4% CHAPS buffer. The first dimension separation was carried out with Immobiline Drystrips (17 cm, pH 3-10, linear gradient, Bio-Rad) using an Ettan IPGphor unit (Amersham Biosciences) at 8000V. The second dimension separation was performed on

a 12% PAG with the Protean II XL system (Bio-Rad) followed by colloidal coomassie G250 staining. Image acquisition was done with a GS-800 calibrated imaging densitometer (Bio-Rad). Spot detection and image matching were performed with Delta 2D software (Decodon Inc.). More than 400 spots were detected in each gel. The arrows indicate spots whose expression differed significantly, by more than two fold, between plasma samples obtained from ENCRT mice and non-transgenic mice. Albumin depletion significantly enhanced detection of the proteins in the plasma as compared to unfractionated sample. In gel digestion and sample preparation for MS analysis was probed on lung tissue obtained from ENCRT and non transgenic mice. The results of this study will validate the usefulness of the ENCRT transgenic mice as a model for human lung adenocarcinoma. The proteins identified in this project may be used as part of a screening panel to identify patients with early stages of lung cancer.

Development and Characterization of Neutralizing Anti-Ricin Chimeric Monoclonal Antibodies

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Introduction: This project is concerned with the development of neutralizing mouse-human chimeric monoclonal antibodies (mAbs) to ricin toxin. Chimeric mAbs are composed of human derived antibody constant domains genetically fused to known protective mouse binding domains (V-domains). This creates highly specific Abs with therapeutic potential, while potentially lessening the host immune response against foreign Abs. Furthermore, this will create a human serological standard for ricin serology. Ricin is a Type II ribosome inactivating protein (RIP), and by definition is composed of two structurally and functionally different domains (A-chain and B-chain) connected by a disulfide bond. The B-chain is a galactose binding lectin, and is responsible for allowing entry of the toxic A-chain into mammalian cells. Ricin is a Category B Biothreat Agent (CDC) that has been discovered in many terrorist plots across the globe over the past four decades.

Methods: Traditional monoclonal antibody technology has been used to create several mAbs targeting either the A-chain or B-chain. These murine mAbs will be characterized based on a series of immunological testing, including target binding specificity, affinity (using BIAcore 2000) and epitope mapping. The murine variable domains from the hybridomas will be sequenced from complementary DNA, and inserted into a eukaryotic expression vector containing the human constant Ab domains as a scaffold. Human 293 cells will be transfected with the vector and the chimeric mAbs produced will be characterized using similar immunological testing as previous.

Results: The murine anti-ricin mAbs were specific for either the ricin A- or B-chain. Binding affinities of the murine mAbs was in the nM (10^{-9}) range. Western immunoblotting indicates that the mAbs exhibit a linear binding conformation, and PepScan epitope mapping of the murine mAbs is currently being accomplished. Antibody variable domains were sequenced and ligated into the pIgG-8 vector for human expression. Expression in 293 cells is underway.

Conclusions: Research is still ongoing.

Post-Transcriptional Events Regulate Cell Death by Apoptosis: Impacts on the Fate of Tumour Cells

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HuR is an mRNA-binding protein that is known to regulate gene expression post-transcriptionally. HuR performs this function by influencing the localization, stability, and translation of certain fragile mRNAs involved in growth, survival and cell cycle regulation, and many cancers have shown to have increased levels of HuR as well as some of its mRNA targets. HuR also plays a role in cell death, dependent on the presence of pp32/PHAPI, a known partner of HuR and an activator of the apoptosome. Recently, we showed that following severe stress-treatment, HuR is cleaved by caspases-3/7 at Asp266, and blocking this cleavage delays cell death. Here, we investigate how the cleavage of HuR affects its RNA-binding properties, and aim to characterize the regulation of this cleavage.

By performing immunoprecipitation and cDNA microarray experiments, we have found that the messages with which HuR associates also change following severe stress. For the same lethal stress treatments that cause cleavage, HuR loses association with mRNAs for anti-apoptotic factors, such as survivin and prothymosin α , whereas it remains associated with the mRNA of the apoptotic factor caspase 9. These data suggest that the cleavage of HuR represents a role-reversal for the protein that can alternate between survival and death of a cell. Interestingly, the well-characterized stress kinase PKR can use a number of downstream signaling pathways to also direct a cell to either survive or die following stress. This shared property of HuR and PKR has directed us to investigate if PKR is involved in the cleavage, or role-reversal of HuR.

We have found that PKR is required for the cleavage of HuR, though this does not depend on the phosphorylation of the translation initiation factor eIF2 α . Since PKR affects several other downstream pathways, we are now proceeding to test their importance on HuR cleavage. By fully exposing the pathway leading to HuR cleavage, we will gain a greater understanding of how this cleavage is regulated. This will identify players that may be targeted in cancer therapy to increase the amount of apoptosis-promoting HuR cleavage.

MeCP2 Regulation of Myelin Gene Expression

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Objective: To investigate myelin specific gene expression levels in the brain of a MeCP2^{null} transgenic mouse.
Materials and Methods: *Quantitative RT-PCR*-Total RNA was isolated from brains of female MeCP2^{null} transgenic mice (Shahbazian, M. D. et al. Hum. Mol. Genet. 2002, 11, 115-124) and expression levels compared to age-matched wild-type controls. Gene expression in different areas of the brain was performed using a SYBRGreen RT-PCR kit and analysed using an ABI7500 real-time thermal cycler. For each sample in each run, there were 7 replicates. RNA levels were compared to the housekeeping gene, GAPDH. *Cell Isolation* -Mixed glial cultures were prepared from P0 rat pup cortices as previously described (Frost, E. et al. Dev Neurosci 1996, 18, 266-73.) OL progenitors (OP) were shaken free of the underlying astrocyte layer and microglial contamination was removed as previously described (Milner, R. et al. Development 1994, 120, 3497-506.) The astrocyte monolayer was shaken for further 24 hours to remove all remaining OP and microglia prior to use. *Western Blot* -Cells were lysed in Laemmli buffer (Laemmli, U. K. Nature 1970, 227, 680-5) and boiled for 4 minutes prior to loading on an 8% polyacrylamide gel. Proteins were transferred to PVDH membranes, and probed with polyclonal anti-MeCP2 antibody. Bands were visualised using HRP conjugated secondary antibody, enhanced with ECL reagent, and exposed to X-Ray film. *Immunohistochemistry (IHC)*-OP were cultured for 1-7 days *in vitro*, and fixed in 10% formalin. MeCP2 expression was visualised using a polyclonal anti-MECP2 antibody with FITC conjugated secondary antibody. Images were captured using Image Pro 7.0 via an inverted Olympus IX51 fluorescent microscope with RETIGA 2000RV monochrome camera attached to an imaging station.

Results: Both astrocytes and OP express MeCP2 protein, as assessed by WB and IHC. Further, in the MeCP2^{null} mouse, the expression of the myelin-specific genes, myelin basic protein (MBP) and myelin associated glycoprotein (MAG) genes are significantly up-regulated (by 2.38 and 4.59 fold respectively), while proteolipid protein (PLP) is down-regulated to 0.45X the level in control brain .

Conclusion: The majority of cases of classical RTT syndrome are caused by mutations in the MeCP2 gene (Bienvenu, T. et al. Hum Mol Genet 2000, 9, 1377-84.) The current research on molecular mechanisms underlying RTT focuses on neurons, while glial cells are widely considered to not express MeCP2. We show

convincingly that OLs express MeCP2. The role of MeCP2 in OL is yet left to be elucidated. The OLs also express various myelin proteins like MBP, MAG and PLP before becoming mature myelinating OLs. These myelin associated proteins are structural components of myelin sheath. Our qRT-PCR results show significant dysregulation of these genes in the MeCP2^{null} mouse. Such a result showing that the expression patterns of myelin genes are altered in the MeCP2^{null} mice supports to our hypothesis that myelination is abnormal in the RTT.

The Polycomb Group Protein PCL2 is Required for the Pluripotency of Embryonic Stem Cells

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PCL2 (polycomb-like 2) is a highly conserved polycomb group protein identified in a genome-wide screen for novel regulators of self-renewal and pluripotency (Walker et al., Cell Stem Cell 1(1), 2007). It is highly expressed in undifferentiated embryonic stem cells (ESCs) and immediately down-regulated upon both the removal of LIF and the addition of retinoic acid (RA). Co-immunoprecipitation studies show that PCL2 is associated with the PRC2 complex, which is responsible for tri-methylation of lysine 27 on histone 3. To study the function of *Pcl2* in ESCs, we generated stable shRNA knockdown ESC lines which expressed 15-30% of *Pcl2* compared to the shRNA mismatch-control ESC line. Single-cell immunofluorescence analysis reveals that OCT4 protein levels are heightened in undifferentiated knockdowns and remain heightened even after 72 hours in -LIF and -BMP4 conditions. Supporting this data, transcripts levels of *Oct4*, *Nanog* and *Sox2* are all increased by 2-fold. Microarray data reveals that expression of differentiation markers are reduced by as much as 7-fold. The down-regulation of early mesoderm markers is especially apparent, suggesting that *Pcl2* may have a specific role in mesoderm formation. Markers of undifferentiated ESCs as well as genes involved in chromatin remodeling, DNA damage response and cell cycle are up-regulated. In colony forming assays, knockdown cells form undifferentiated, alkaline phosphatase (ALP) positive colonies (80-95%) at a much greater efficiency than mismatch controls (40%). Knockdown cells are unable to differentiate into neural precursor cells, or form mature embryoid bodies (EBs). Even following 25 days in culture, trypsinized EBs replated in +LIF conditions readily form ALP positive colonies. qPCR analysis of differentiating EBs reveals that expression of self-renewal markers is prolonged, accompanied by a two day delay in peak expression of key markers of development. Thus, while it appears that *Pcl2* is dispensable for self-renewal, it is critically required for the initiation and timing of commitment to differentiation.

Patched1 Deficient Medulloblastomas Contain Rare Nestin+Sox2+ Tumour Initiating Cells

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Human brain cancer growth is sustained by relatively rare, therapy resistant cancer stem cells (CSC) both *in vitro* and *in vivo*. Although mouse tumours recapitulate many features of human cancer it is controversial whether these cancer models comprise a CSC hierarchy. Identifying mouse models in which CSCs drive tumour growth is critical for validating their relevance to human cancer, studying the mechanisms that govern CSC survival, and developing novel therapies. Here we report that medulloblastomas (MB) arising in Patched1 deficient (Ptc1^{+/-}) mice contain rare Nestin+Sox2+ cells that reside at the apex of the tumor hierarchy. In contrast, the granule cell precursor (GCP) marker Math1, thought to identify the MB cell of origin, is expressed throughout the tumor hierarchy. Using a novel culture system, Nestin+Sox2+ cells can be greatly enriched *in vitro*, demonstrate long term proliferative potential and multilineage differentiation capacity, and retain activated Hedgehog (Hh) and Notch signaling. The frequency of Nestin+Sox2+ cells in primary tumours clearly correlates with disease incidence and severity, suggesting that cancer aggressiveness is associated with the size of the CSC population. Importantly, orthotopic transplantation of Nestin+Sox2+ cells results in the formation of heterogeneous tumours that phenocopy the original medulloblastoma. Definitive identification of cancer stem cells requires prospective identification and *in vivo* tumorigenesis from freshly dissociated tumours. Therefore, we are fluorescent activated cell sorting (FACS) established Nestin+Sox2+ cell lines and freshly dissociated tumours to identify the cancer stem cell subpopulation. We have observed that the mouse neural stem cell markers CD15 and CD44 enrich for the most clonogenic subpopulation of Ptc1^{+/-} MB *in vitro*, and preliminarily *in vivo* experiments. These observations are consistent with a rare cell with stem cell properties and phenotype driving tumour growth and suggest that Ptc1 deficient MB retain the stem cell organization seen in the human disease. Moreover, our study identifies a new cellular target for brain cancer drug discovery whose chemical interrogation has already yielded promising results *in vitro*.

Relationships Among Measures of Ankle Power and Functional Performance in Mobility-Impaired Older Women

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Introduction: Results of cross-sectional studies indicate that power decreases at a greater rate than strength with age and is more closely associated with functional decline. Although the ankle muscles are important for mobility, most power studies in older adults have focused on the hip and knee musculature. The purpose of this study was to investigate the relationships among measures of ankle power and functional performance in mobility-impaired older women.

Methods: Forty-four older women (75.6 ± 4.3 years of age) with mobility limitation defined as an inability to walk one mile took part in the study. Ankle dorsiflexion (DF) and plantarflexion (PF) peak power was measured on a Biodex dynamometer at $90^\circ/s$ and average DF and PF acceleration capabilities were measured using the isotonic mode. The Short Physical Performance Battery (SPPB), gait speed and a stair climb test were used to determine lower extremity performance.

Results: Separate adjusted multivariate regression models demonstrated that ankle PF power per kilogram body weight (/kg bw) explained the most variance in each of the performance measures ($R^2 = 0.40$ for SPPB score, $R^2 = 0.35$ for gait speed, $R^2 = 0.44$ for stair climb power/kg bw, $p < 0.01$ for all independent variables and models). PF acceleration contributed to models predicting SPPB score and stair climb power/kg bw ($R^2 = 0.36$ and $R^2 = 0.30$ respectively, $p < 0.05$). DF power/kg bw contributed to models predicting gait speed and stair climb power/kg bw ($R^2 = 0.31$ and $R^2 = 0.26$ respectively, $p < 0.05$).

Conclusion: In mobility-limited older women, measures of ankle power and acceleration are significantly related to functional performance scores, with PF capabilities having a greater influence than DF.

Persuasive Strategies at the Crossroads of Knowledge and Action: A Rhetorical Analysis of Clinical Practice Guidelines for the Management of Hypertension

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Background: Clinical practice guidelines are a valuable resource for clinicians who must navigate continually-advancing, proliferating, and often conflicting scientific evidence in order to maintain expertise. However, the genre has demonstrated limited success in translating between scientific knowledge and clinical practice. Comparative analyses of guidelines have focused on content, methodology, and bias. Little attention has been paid to the persuasive strategies used within guidelines to convince practitioners to adopt recommended practices. This study sought to describe rhetorical strategies used in current guidelines for the management of hypertension. Hypertension has been a hub of activity both in the creation of guidelines and in the study of the knowledge translation process.

Methods: Seven hypertension guidelines published internationally between 2003 and 2006 were analyzed using rhetorical genre theory (which understands genres as regularized strategies for achieving recurrent social actions) and classical Aristotelian rhetoric (which categorizes kinds of persuasive appeals).

Results: There is an evident trend toward the production of guidelines in multiple versions varying in length and format. This strategy is used to manage, and in effect to divide, the genre's multiple audiences (generalist, specialist) and purposes (to provide recommendations, present evidence, and incite change). Guidelines differed markedly in their ethos (appeals to the character of the speaker or writer) and pathos (appeals to the emotions of an audience), revealing disparate conceptions of scientific knowledge, clinical practice, and their interrelationship.

Conclusion: Guidelines use a range of persuasive strategies that can be described using the analytical resources of rhetorical theory. This line of research has promise for improving guideline design; revealing cultural values and tensions; and developing critical appraisal skills attentive to both the content and social uses of evidence.

Effects of VSV Δ G/ZEBOV GP on NK Cell Killing

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Introduction: *Zaire ebolavirus* (ZEBOV) causes haemorrhagic fever with a case fatality rate of 90%. Our lab has developed a replication-competent recombinant virus vaccine (VSV Δ G/ZEBOV GP) by replacing the glycoprotein (GP) of Vesicular Stomatitis virus (VSV) with that of ZEBOV. Recent experiments with this vaccine suggested that the innate immune system plays an important role in protection until the adaptive immune response is mounted. We were, therefore, interested to characterize the interaction between NK cells, a prominent cell type in innate immunity, and targets cells infected with VSV Δ G/ZEBOV GP.

Methods: A human lymphoblast cell line (K562) was either incubated in media or infected with VSV Δ G/ZEBOV GP or VSV at different MOIs for 24 h, then incubated with or without a human NK cell line (NK-92MI) at different E:T ratios. At 4 h after NK cell addition, cell samples and supernatants were harvested and the percentage of target cell killing by NK cells determined by FACS analysis.

Results: Following infection with either virus, a higher percentage of K562 target cells were killed by NK-92MI cells than target cells that had been incubated in media or that had not been incubated with NK cells. Varying the amount of virus infecting the target cells also changes the percentage of killing the NK cells can carry out.

Conclusions: Infection with VSV Δ G/ZEBOV GP causes target cells to express the ZEBOV GP on the cell surface. In addition, it has been demonstrated here that NK cell killing is increased when the target cells are virally infected compared to uninfected target cells. Studies to determine if soluble cellular protein(s), such as cytokines, released from either the target cells and/or the NK cells play a role in this increased killing rate are currently on going.

Diltiazem and Silymarin Enhance Hepatocyte Viability

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Introduction: To investigate the hepatoprotective role of d-diltiazem, silymarin and their combination in Chang and PLC hepatocytes. Hepatoprotection was assessed by analyzing the drugs' antioxidant activity, cell viability and inhibitory potency to the pro-apoptotic protein Bax.

Methods: Hepatocytes (Chang and PLC) were treated with d-diltiazem (2.5 or 10 μ M) and/or silymarin (10 or 1000 μ g/L) for 24 hrs at 37°C followed by half hour incubation with H₂O₂ (400 μ M) to initiate oxidative stress. The dichlorofluorescein (DCF) assay was used to assess the extent of reactive oxidative species (ROS) in the liver cells. The effect of silymarin and d-diltiazem on the cellular proliferation was determined by the MTT assay. ATP changes by d-diltiazem and silymarin were measured using bioluminescent assay. Western blot and RT-PCR were used to determine Bax expression.

Results: Compared to control, d-diltiazem and silymarin statistically attenuated DCF fluorescences and reduced Bax expression while promoted cell viability and mitochondrial ATP content. Combining diltiazem with silymarin further protected hepatocytes compared to use of individual drugs.

Conclusions: Diltiazem and silymarin are associated with significant hepatoprotective properties. We conclude that the combination of silymarin and d-diltiazem provides further antioxidant protective effects than d-diltiazem alone in hepatocytes.

SRA/SRAP Modulates the Expression of ER β in Breast Cancer Cells.

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Objective: The Steroid receptor co-activator (SRA) has been implicated in estrogen receptor signaling pathway. Its expression is altered during breast tumorigenesis and its molecular role in underscoring these events has been suggested. The SRA gene encodes both functional RNA and protein (SRAP) products, making it a unique member amongst the growing population of steroid receptor co-regulators. We have recently reported that many SRA transcripts still contain intron-1 sequences. Retention of this intron inhibits the coding potential of these RNAs but does not alter RNAs functions. Using splice-switching-oligonucleotides and real-time PCR, we observed that increasing intron-1 retention led to an increase in ER β expression in T5 cells. SRA RNA and/or SRAP might therefore regulate the expression of ER β .

Methods and Results: In order to investigate the respective action of SRA RNA or protein, we used different constructs leading respectively to SRA RNA or SRAP. We observed that SRA RNA has no impact on the expression of ER β . On the other hand SRAP can decrease the expression of total ER β in mRNA level by using radioactive TP-PCR and real-time PCR. In addition, SRAP down-regulates the ER β protein expression detected by western blot analysis after 48 hours transfection.

Conclusion: Altogether these results suggest SRAP rather than SRA RNA could regulate the expression of ER β .

Hypersensitive Site III is a Candidate 'Entry' Point for Elk-1 Recruitment of Activator Pit-1 Binding and Remodeling the Human Growth Hormone Locus.

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Introduction: The organization of the five pituitary and placental growth hormone/chorionic somatomammotropin (GH/CS) genes in the human locus is peculiar to primates. Based on gene transfer studies *in vitro* and *in vivo*, pituitary-specific expression of human GH requires direct binding of pituitary Pit-1 to sequences marked by a hypersensitive site (HS) region (HSI/II) about 14.6 kilobases (kb) upstream of the human GH-N gene. What is less clear is how HSI/II becomes available for Pit-1 binding thereby triggering GH-N gene expression. Our hypothesis is that another region (HSIII) located further upstream is an entry point into the GH/CS locus control region (LCR).

Methods: In the absence of human pre-somatotrophs and given inherent difficulties associated with non primate or transgenic systems, we have used human embryonic kidney 293 (HEK293) cells overexpressing wild type and mutant Pit-1 proteins as a model system to gain insight into this process.

Conclusions: (i) The addition of functional Pit-1 to these cells resulted in a significant increase in histone H4 hyperacetylation and RNA polymerase II (pol II) tracking (DNA accessibility) extending from HSIII, even in the absence of a Pit-1 DNA element at HSIII and GH expression. (ii) Both RNA pol II tracking and histone H4 hyperacetylation in this area was dependent on the presence of the POU homeodomain of Pit-1, required for low-affinity, non-specific DNA binding. (iii) The POU homeodomain-dependent action of Pit-1 appears to be mediated by association with the transcription factor Elk-1, a member of the ETS family member, and its DNA element, which was shown previously to support enhancer activity in both pituitary and placental cells. In brief, these data are consistent with HSIII as a 'window' or initial entry point to the GH/CS LCR. This is mediated, at least in the pituitary, through a larger and/or less tissue restricted (ETS) family of transcription factors, but one that is capable of tissue-specific interactions, including with pituitary-specific Pit-1.

Connexin 43 New Marker for Adipogenesis

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Obesity is a major health problem around the world since it is associated with other diseases such as glucose intolerance, insulin resistance, dyslipidemia, and hypertension. Furthermore, metabolic syndrome, a combination of obesity with these other disorders promotes the development of cardiovascular disease, the leading cause of death worldwide. Adipose tissue forms shortly after birth as a result of proliferation and differentiation of preadipocyte cells, which originate from stromal vascular cells. Adipogenesis is characterized by changes in gene expression and shape that result in their conversion from a fibroblastic to a round morphology.

Connexin-43 (Cx43) is a membrane phosphoprotein expressed by numerous cell types. In addition to forming gap junctions and mediating direct inter-cellular communication, Cx43 is increasingly recognized for its potential to influence intra-cellular signaling, gene expression, differentiation and growth. Its role in adipogenesis is unknown.

Azarnia et al. (1985) reported that adipocytes do not have functional gap junctions whereas pre-adipocytes do. In addition, Yanagiya et al. (2007) showed that the levels of Cx43 change during adipogenesis. They also reported that Cx43 is necessary for adipogenesis. We have been using 3T3-L1 pre-adipocytes which can be induced to differentiate into adipocytes with a cocktail of insulin, dexamethasone and methylisobutylxanthine. Our studies have confirmed that Cx43 is down-regulated in adipogenesis. In extending this work we have established that phosphorylation of Cx43 is a marker for early-stage adipocyte differentiation, prior to expression of adipokines and formation of lipid droplets. While the subcellular localization of Cx43 did not change when examined by western blotting after fractionation, microscopy showed differences in the staining pattern. Based on this results, we hypothesize that phosphorylation of Cx43 by protein kinases is required for adipogenesis. A description of the mechanism which drives this process will be presented.

Tie2 Driven Over-Expression of Calreticulin Induces Lung Adenocarcinoma

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Introduction: Calreticulin (CRT) is a multifunctional Ca^{2+} dependent chaperone protein, localized to the endoplasmic reticulum and plays many biological roles. In addition to its critical role in cardiovascular development, CRT has been reported to be important in cell migration, apoptosis, and cancer cell growth. Furthermore, several proteomic studies conducted on different human cancer tissue and cell lines have shown an increase in the expression of CRT as compared to normal cells. Therefore, the aim of this study was to investigate the *in vivo* role of CRT in the development of cancer.

Methods: We generated a transgenic mouse over-expressing CRT under the control of Tie2 promoter (referred to as *Tie2-CRT*) which is active in both endothelial cells and hematopoietic stem cells. The phenotype of these mice was characterized using histological and immunohistological (IHC) techniques.

Results: The early phenotype of *Tie2-CRT* mice is localized in hemorrhagic lesions in different tissues, accompanied by increased vessel fragility. With age, the main phenotype of these mice is development of spontaneous lung adenocarcinoma (over 90%). Our histological studies show that lung tumour in the *Tie2-CRT* mice is spontaneously arising from the peripheral airway preceded by sequences of morphological changes including mild congestion, accumulation of inflammatory cells and atypical adenomatous hyperplasia in peripheral airway cells. As the lesions progressed, they formed small and peripheral tumour with a glandular and papillary structure resembling human pulmonary adenocarcinoma. IHC staining revealed that the tumour cells are CRT⁺, CD31⁻, Clara cell negative and surfactant protein-C positive, suggesting that these tumours are of alveolar origin (not endothelial origin).

Conclusion: Over-expression of CRT under the control of Tie2 promoter increases the incidence of lung tumour in the mice. This is the first report on a direct link between the expression of CRT and the development of lung tumour.

Generation of Two *in vitro* Models to Study the Role of the Claudin 1 Tight Junction Gene in Breast Cancer Progression

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Introduction: The role of the claudin (cldn) family of tight junction proteins in breast tumorigenesis is not known but an absence of, or defects in tight junctions have been associated with development of the neo-plastic phenotype.

Methods: To begin to delineate a direct functional role for claudin 1, a member of this family, in breast cancer we generated a knockdown claudin 1 siRNA *in vitro* model, in the T47D human breast cancer (HBC) cell line. We have also generated a second *in vitro* model in which the tetracycline inducible system was used to overexpress claudin 1 in MDA-MB231 HBC cell line. Cells were then evaluated for proliferation, invasiveness, apoptosis and anchorage independence.

Results: Cldn 1 gene knockdown in T47D was confirmed by western blot analysis. Claudin 1 protein expression was significantly down regulated at 72 hours post transfection whereas the expression of other tight junction proteins (E-cadherin, occludin and ZO-1) was unaltered, highlighting the specificity of the claudin 1 siRNAs. We have also generated 45 stably transfected clones that overexpress claudin 1 at different levels following 24 hr doxycycline induction. The effect of cldn 1 expression on proliferation, invasion, apoptosis and aggressiveness are presented.

Conclusion: We have successfully established two *in vitro* models to investigate the effect of alterations in cldn 1 gene expression on human breast cancer tumor progression and metastasis. These models will allow us to examine whether cldn 1 has a direct effect on breast cancer progression. Identification of genetic changes that permit metastasis may provide new insights for therapeutic targets to facilitate more effective patient management and impact on reducing mortality rates.

Speaking of Safety: Young Workers Experiences

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Introduction: Youth employment is normative, formative and - all too often - dangerous. It is normative in that young workers (i.e., those aged 15–24 years) now participate in the paid labor force at an unprecedented rate (Loughlin & Barling, 1999; 2001). These early employment experiences have substantial formative and developmental implications for individuals (for a review see Frone, 1999) and their employing organizations (Kelloway & Barling, 1999). However, youth employment is also dangerous. Although there are many large surveys of safety outcomes among young workers, comparatively few studies have asked young workers' about their experiences of workplace safety. The purpose of this study was to explore young workers' experiences of workplace safety and their perceptions of safety messages in the workplace and the media.

Methods: Participants were selected according to a system of purposive sampling for variation (Silverman, 2000) and individual interviews were conducted concerning workplace safety. A phenomenological frame was used to interpret the data to capture and identify the personal experiences and interpretations from the perspective of the respondents themselves (Patton, 1990). Through grounded theorizing, we were able to dwell upon the interviews themselves and not attach any presuppositions based upon prior theory, research or biases

Results: Participants did not identify working safely as part of a successful job performance, reported receiving minimal safety training and did not talk about safety issues at work. Participants recognized some jobs, but not their own, as being risky. The lack of recognition of safety issues is particularly important because over half of the participants reported being injured at work. Coworkers also glorified injuries, presenting them as a sign of experience in their industry.

Conclusion: The most striking observation is how little health and safety intruded into the work experiences of young workers. Although participants reported experiencing injuries and near-miss incidents, they minimized these occurrences and attributed such events to carelessness. Supervisors and coworkers also viewed injuries as a normal and expected part of the job. The challenge for marketing safety is making the message of safety relevant to young workers.

Human Rhinovirus-16 induced Expression of IP-10 in Human Airway Epithelial Cells is Negatively Regulated by the ERK 1/2 MAPK Signaling Pathway Through Transcriptional Effects

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Introduction: Human rhinovirus (HRV) is associated with exacerbations of asthma and chronic obstructive pulmonary disease and with increased generation of proinflammatory chemokines, such as IP-10 (CXCL10), in an NF- κ B-dependent manner. We have shown that HRV-16 induced gene transcription of IP-10 in HAE is selectively enhanced upon inhibition of the extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) pathway, which includes the ERK1/2 and ERK5 pathways. This suggests a role for the ERK pathways in the negative regulation of HRV-16 induced IP-10 expression. We also have shown that the enhancement of HRV-16 induced IP-10 gene transcription is dependent upon the interferon-stimulated response element (ISRE), κ B1, and κ B2 recognition sites in the promoter.

Objective: To determine the transcription factors involved in the ERK-mediated enhancement of HRV-16 induced IP-10 expression and also to determine if this enhancement is mediated through the ERK1/2 or ERK5 pathway.

Methods: BEAS-2B cells were pre-incubated (1h) with either ERK pathway inhibitor PD98059 (10 μ M) or U0126 (3 μ M), then exposed to HRV-16. Nuclear extracts (5 μ g) were incubated (1h) with ³²P-labeled IP-10 κ B1, κ B2, or ISRE oligonucleotides (25bp) to determine transcription factor binding. To determine the identity of these transcription factors, nuclear extracts were pre-incubated (2h) with separate antibodies before incubation with ³²P-labeled oligonucleotides. Whole cell lysates (15 μ g) collected from HRV-16 infected cells pre-incubated with PD98059 or U0126 at various concentrations were run on SDS/PAGE and immunoblotted for phospho-ERK1/2 and ERK5.

Results: Inhibition of the ERK pathway(s) does not enhance HRV-16 induced binding of the NF- κ B isoforms p65 or p50 on κ B1 or κ B2. In contrast, there is enhanced binding of interferon regulatory factor (IRF-1) on the ISRE. The inhibitors, PD98059 and U0126, abrogate HRV-16 induced phosphorylation of ERK1/2, but not ERK5.

Conclusions: Inhibition of the ERK1/2 pathway enhances HRV-16 induced IP-10 expression, in part, through an enhancement in the binding of IRF-1.

Identification of Stress-Responsive TLS Nucleolar Relocalization Motifs

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Introduction and Objective: TLS is a proto-oncogene found translocated in human leukemia and sarcoma. We have discovered that TLS is essential for the maintenance of genomic stability and functions as a DNA damage response protein. The mechanisms for the role of TLS in DNA damage response remain unknown. However, we have found a minimum relocalization domain in the N-terminus of TLS protein that is responsible for the relocalization of TLS to the nucleolus after transcriptional inhibition—a stress response associated with the prevention of DNA damage. Furthermore, we have identified a consensus sequence, SQ·SQ·YGQQ, within the domain that is highly conserved in all mammalian species and may identify regulatory elements that control the dynamic relocalization. Our objective is to identify whether the SQ·SQ·YGQQ consensus sequence is responsible for the relocalization of TLS after transcriptional inhibition.

Methods: In order to test whether SQ·SQ·YGQQ consensus sequence will lead to the relocalization of enhanced green fluorescence protein (EGFP) which does not relocalize by itself after transcriptional inhibition, single SQ·SQ·YGQQ consensus sequence and triple repeat of SQ·SQ·YGQQ consensus sequence were inserted into pEGFP-C1 vectors. The pEGFP-SQ·SQ·YGQQ constructs and empty pEGFP-C1 vectors were then transiently transfected into primary MEFs. 48 hours later, primary MEFs were treated with Actinomycin D for 1 hour. After fixation and mounted to glass slides, the MEFs were observed under fluorescence microscope.

Results: For all the constructs, the untreated cells do not show relocalization. After Actinomycin D treatment, EGFP alone still does not show relocalization, while pEGFP-single SQ·SQ·YGQQ constructs show relocalization in 77.2% of cells observed, among which 0.6% were strong relocalization. Furthermore, pEGFP-SQ·SQ·YGQQ triple repeat constructs show relocalization in 96% of cells observed, among which 15% were strong relocalization.

Conclusion: The SQ·SQ·YGQQ consensus sequence alone is sufficient to confer the dynamic relocalization of the EGFP constructs. Current research is focused on whether SQ motif or YGQQ motif in the consensus sequence regulates the relocalization of the constructs.

Quantitative Expression Levels of CD40 on Mature DC Determine a Critical Threshold that Defines its Immunogenic/Tolerogenic Properties

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Dendritic cells (DC) are professional antigen presenting cells (APC) that initiate and regulate immune responses. Phenotypic analysis of the surface expression of co-stimulatory molecules has been used routinely in defining immature and mature DC, terms that are often used interchangeably with tolerogenic and immunogenic DC respectively. The increasing number of co-stimulatory molecules identified to date highlights the importance and complexity of co-stimulatory signals in defining DC functions. We hypothesized that the expression levels of co-stimulatory molecule(s) on DC are pivotal in determining the different functional properties of DC to tolerize, activate or polarize antigen-specific Th1/Th2 immune responses *in vivo*. We established the uses of replication-incompetent lentiviral vectors in expressing transgenes or shRNAs to induce stable gene silencing in primary bone marrow-derived DC. Using different shRNA sequences that specifically target different regions of CD40 mRNA, we were able to generate modified DC that express low (CD40^{low}) or medium level (CD40^{med}) of CD40 on the surfaces of the LPS-stimulated DC. We demonstrated that down-regulation of CD40 suppressed naïve OVA-specific T cell proliferation in the CD4 transgenic DO11.10 T cells *in vitro*. The impairment in priming T cell proliferation correlated with the extent of CD40 down-regulation. In a normal (non-transgenic) animal, the precursor frequency of naïve T cells that will respond to OVA Ag is low when compared to the DO.11 TCR transgenic animal. We therefore evaluated the ability of the CD40^{med} and CD40^{low} DC to prime polyclonal OVA-specific immune responses physiologically in naïve animals *in vivo*. We found that both of the CD40^{med} and CD40^{low} were impaired in priming polyclonal OVA-specific T cells *in vivo*. Interestingly, only the CD40^{low} DC (but not the CD40^{med} DC) demonstrated immunological tolerance upon a rechallenge of active immunization of the WT DC pulsed with OVA protein antigen *in vivo*. We observed a marked suppression of OVA-specific CD8 T cell proliferation response in the rechallenged CD40^{low} DC-immunized mice. Our preliminary data supported the notion that expression level of CD40 is a critical regulator of immunity versus tolerance because the CD40^{low} DC were able to exert its tolerogenic ability *in vivo* despite LPS upregulated expression of other costimulatory molecules (such as CD80/CD86) on the cell surface. Adaptation of this system to develop human tolerogenic DC might allow us to modify Ag-specific immune responses without the need for myeloablation or broad immunosuppression.

Bam32/DAPP1 Deficiency Impairs BCR-Mediated Antigen Presentation and Causes Premature Germinal Center Dissolution

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The PH domain adaptor protein Bam32/DAPP1 was isolated from human tonsil germinal centers (GC); however, the role of Bam32 in the GC response is unknown. *In vitro*, Bam32-deficient B cells are competent in proliferation, chemotaxis, isotype switch and plasma cell differentiation in response to signals present in GCs, but have a specific impairment in BCR-mediated antigen presentation and formation of polarized conjugates with antigen-specific T cells. We found that GC responses elicited by sheep red blood cells or protein Ag are initially comparable in wild-type and Bam32^{-/-} mice; however, in the latter case premature dissolution of GCs was observed. Consistent with the lack of GC persistence, Bam32^{-/-} mice showed impaired affinity maturation. *In vivo* studies indicated that Bam32^{-/-} GC B cells proliferated well, but they appeared to be more apoptotic, which may be caused by the lack of GC B cell-T cell interaction within GC. Correspondingly, increased IgM and decreased IgG1 surface expression were found on those GC B cells. Adoptive transfer experiments confirmed that intrinsic defect of Bam32^{-/-} B cells led to premature dissolution of GCs, accompanied by increased apoptosis. These data indicate that Bam32 links PI 3-kinase signaling to B cell Ag presentation function within GC to optimize cell survival-based affinity selection.

New Features of Atypical Antipsychotic Drug Quetiapine: Regulating Oligodendrocyte Development and Regeneration, Preventing Demyelination Induced by Cuprizone

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Introduction: Schizophrenia is a brain disease featured with alterations in oligodendrocytes (OLs) indicated by recent human studies employing new magnetic resonance imaging techniques and micro-array analysis. However, there is little information available regarding effects of antipsychotic drugs on OLs.

Methods: We studied the effects of quetiapine (QTP, a new atypical antipsychotic) both on demyelination model induced by cuprizone in the brain of C57BL/6J mouse and on the rat neural stem cell culture. To induce the demyelination, the mice were given cuprizone (CPZ) mixed in their diet for 5 weeks, which induces a consistent and evident demyelination and dramatic decreases in myelin producing OLs in brain white matter.

Results: In response to demyelination, microglia and astrocytes were activated in the demyelinated sites where adult OL precursor cells dramatically increased. However, all these pathological changes were prevented or alleviated in the mice co-administered with CPZ and QTP. Recovery occurred automatically in demyelinated sites during a two-week period following the withdrawal of CPZ. This recovery was promoted by QTP treatment, indicated by more completed remyelination and a higher ratio of myelin producing OLs over OL precursor cells in demyelinated sites, as compared to vehicle-treated mice. To understand the mechanism underlying the effects of quetiapine on oligodendrocytes, quetiapine was administered to the rat embryonic neural stem cell culture. Quetiapine was found to increase the proliferation of neural progenitors and promote the neural progenitors differentiate into oligodendrocyte lineage through extracellular signal-related kinases.

Conclusion: These findings suggest a novel neural mechanism of antipsychotic action of QTP, which provides the new insights regarding antipsychotics and helps to establish a role for oligodendrocytes in schizophrenia. These could be invaluable for understanding the pathogenesis and potential therapy of schizophrenia.

Contribution of the C-Terminal Catalytic Core Domain of HIV-1 Integrase to its Chromatin Tethering and Viral Replication

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Introduction: HIV-1 integrase (IN) is a key viral enzyme required for integration step. In addition, this viral protein has also been demonstrated to function in HIV-1 cDNA tethering to host chromatin, a prerequisite step for HIV-1 integration. However, the mechanism of IN chromatin tethering is not fully understood. Several cellular proteins, such as lens epithelium-derived growth factor (LEDGF/p75) and Integrase interactor 1 (Ini1), have been put forward as important partners for HIV integration. It has been shown that LEDGF/p75 directly interacts with IN and may function as a tethering factor for HIV IN to the chromosome. In this study, we have performed mutagenic, functional and biochemical analyses to investigate the contribution of different regions of IN to its chromatin binding ability. Moreover, the involvement of cellular proteins LEDGF/p75 and Ini1 in IN chromatin tethering has been studied by testing their interactions with newly identified IN chromatin-binding defective mutants.

Methods and Results: We have established a cell-based chromatin binding assay to identify the critical amino acid(s) and/or regions(s) in HIV-1 IN responsible for this biological activity. The results revealed that several IN mutants, located in a C-terminal catalytic core domain of HIV-1 IN encompassing amino acids V165 to I200, lost their binding ability to cellular chromatin, suggesting that this region of HIV-1 IN is necessary for IN tethering to host chromatin. By a co-immunoprecipitation method, we demonstrated that these chromatin-binding defective IN mutants failed to interact with host protein LEDGF/p75, while remaining the binding ability to Ini1, a component of the chromatin remodeling hSWI/SNF complex. The effect of the IN chromatin-binding defective mutants on HIV-1 replication was also evaluated by using a VSV-G pseudotyped HIV single cycle replication. Results showed that all chromatin-binding defective IN mutant viruses were replication deficient, and their defects can be, at least in part, complemented by the presence of an IN class I catalytic mutant.

Conclusions: Taken together, this study provides evidence indicating that the region between V165 to I200 of IN is involved in the interaction with host protein LEDGF/p75, and is important for both IN binding to host chromatin and HIV-1 replication. Thus, it presents a promising target for therapeutic intervention against IN chromatin tethering in HIV-1 infection.

Patterns of Expression of Oligodendrocyte Specific Proteins During Development of the Mouse Brain

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Objective: To map the expression patterns of platelet derived growth factor-A (PDGF-A), and its receptor PDGF Receptor-alpha (PDGFR alpha), during development of the embryonic mouse brain.

Introduction: Oligodendrocytes (OL) produce myelin, which is essential for normal conductance of nerve impulses throughout the CNS. OL originate in the germinal matrix (GM) of the developing CNS. A variety of factors regulate the OL progenitor cells (OPs) in the developing CNS, such as extracellular matrix (ECM) proteins, soluble factors and axonal cues. As the brain develops, OPs migrate away from the GM to populate white matter tracts such as the corpus callosum. OPs migrate and proliferate in response to growth factors, including platelet-derived growth factor A (PDGF-A), and fibroblast growth factor-2 (FGF2). PDGF-A is essential for the development of myelin in the brain, and its receptor, PDGFR alpha, is commonly used as a marker of OP.

Materials and Methods: We collected and sectioned brains of embryonic mice from days 11.5 to 20.5. Dissected heads were fixed in 10% buffered formalin for 36 hours at 4°C. Tissue was then cryopreserved in 30% sucrose prior to embedding in OCT cryocompound, and stored at -80°C until sectioned. Brains were sectioned at 12µm from olfactory bulb to brain stem. Serial sections were stored at -80°C until stained. Immunohistochemical analysis of PDGF-A and PDGFR alpha was performed as previously described.

Results: We show that PDGFR alpha (a marker of OPs) immunoreactivity is seen only in the GM at the earliest stage of brain development studied (E11.5). As the brain develops, PDGFR alpha immunoreactivity is seen at increasingly larger distances from the GM. Whereas PDGF-A can be seen consistently everywhere from E11.5 to E19.5.

Conclusion: PDGF-A is known to be a potent mitogen for OP and an important regulator of OP migration. Early hypotheses suggested a chemotactic gradient of growth factors such as PDGF. However, our findings do not support such a gradient. Indeed, PDGF-A immunoreactivity is seen throughout the developing mouse brain, whereas PDGFR alpha immunoreactivity is restricted to the putative location of the migrating OPs.

Jab1 and Estrogen Receptor α (ER α) in human breast cancer.

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Introduction: Jab1 (Jun activation domain-binding protein 1) may have a role in the development and progression of breast cancer. It is a coactivator of the gene-regulatory AP-1 proteins (Jun/Fos-oncogenes) involved in cell proliferation control. Jab1 (also known as CSN5) is present in CSN (COP9 signalosome). Results from different groups suggest that CSN is involved in the degradation of several proteins. Specially is reported that CSN5/Jab1 increases hormone-induced ER α degradation.

Methods: Immunohistochemistry, Immunoblot analysis, Confocal/Immunofluorescence Microscopy, Coimmunoprecipitation, siRNA

Results: Interestingly, in a recent study with ER α +ve breast tissues, examined by immunohistochemistry, a significant positive correlation between Jab1 and ER α expression was found. To investigate potential mechanisms underlying this relationship, we determined Jab1 expression in breast cancer cells after estrogen (E2) and anti-E2 treatment by western blot. Exposure of cells to 4-hydroxy-tamoxifen (4-HT) resulted in little up-regulation of Jab1 after 24h, and increased expression of ER α (\geq 24 h). Strong down-regulation of ER α due to treatment with ICI 182,780 was found with no significant change in Jab1 expression. E2 treatment caused down-regulation of ER α , but no significant change in Jab1 expression. Further, cellular distribution of Jab1 (detected by immunofluorescence microscopy) was not changed by E2 or anti-E2 treatment. Interaction of ER α and Jab1 was demonstrated by coimmunoprecipitation (Co-IP), especially under 4-HT incubation. Curcumin pre-treatment increased the portion of Co-IPed ER α with Jab1. In addition, using siRNA to knock-down Jab1, significant down-regulation of ER α was also observed (P = 0.049).

Conclusion: Strong correlation between Jab1 and ER α expression occurs in breast tumors in vivo. There is no short-term regulation of Jab1 expression under E2 or anti-E2 treatment, and cellular distribution of Jab1 is not changed. However, Jab1 and ER α may interact directly or within a complex, and this may be influenced by ligand. Transient knock-down of Jab1 resulted in a small decrease in ER α expression, suggesting that longer term knockdown may decrease ER α steady-state levels further.

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